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RESEARCH ARTICLE

INCREASED SERUM ZINC, COPPER AND SELENIUM IN ALBINO RATS FED WITH *MORINGA OLEIFERA* LEAF EXTRACT

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ABSTRACT

Certain degree of the world's population is probably deficient in one or more essential elements and at increased risk of nutritional disorders. Availability of dietary supplement of these elements may prevent some of these nutritional disorders. This study was designed to access serum levels of zinc, copper and selenium in albino rats fed with *Moringa oleifera* leaf extract. The research was carried out in the animal house of Ladoke Akintola University of Technology, Osogbo. Seventy healthy male and female rats (40 tests and 30 controls) were selected into the study. Test rats were fed with diet fortified with extracts of *Moringa oleifera* leaf for a period of five weeks while the control rats were fed with normal diet. Serum levels of zinc, copper and selenium were determined after six weeks of the experiment using Atomic Absorption Spectrophotometer. Statistical analysis was done using SPSS software package (version 17). The mean serum levels of zinc (99.41±31.59 Vs 66.05±33.24), copper (14.45±6.87 Vs 7.82±4.33) and selenium (17.30±7.33 Vs 9.73±5.25) were significantly higher in the test group when compared with control group, the mean differences were statistically significant ($p<0.05$). There was a significant strong positive correlation between zinc and copper($r = 0.786^{**}$), copper and selenium ($r = 0.931^{**}$) and zinc and selenium ($r = 0.671^{**}$) ($p<0.01$). In conclusion, this study establishes a strong relationship between *Moringa oleifera* leaf feeding and increase in the levels of serum zinc, copper and selenium.

INTRODUCTION

Humans require some essential trace elements to be optimally functional as expected. However, majority of people are deficient in one or more essential trace elements making them to be at increased risk of nutritional disorders. Addressing these is a challenge for the researchers to look into dietary supplement to prevent any form of nutritional disorders that could arise (Broadly *et al*, 2010). Malnutrition is a common disorder in Africa population affecting mainly some groups of people such as children, elderly and those living in low socioeconomic areas. Nutrients are most importantly classified as macro and micro nutrients. Malnutrition may result from either inadequacy or excessive presence of one or more micronutrients like trace elements and vitamins (Gibson *et al*, 2010). These inorganic compounds called trace elements are essential for proper growth and development as well as physiologic functions of humans and animals. Despite the important biologic functions of these elements, they are needed only in minute quantity (recommended dietary allowance (RDA)) (Marletta *et al.*, 2003). The roles of these trace elements in humans can never be over discussed. It helps in immune development and adequate help in provision of anti-oxidant defense mechanism (Sikler *et al.*, 2003; Daren *et al.*, 2005; Zimowska *et al.*, 2001). Essential trace elements include zinc, copper, selenium, cobalt etc, but this study has been based on the importance of zinc, copper and selenium in nutrition especially their function as cofactors to enzymes, antioxidants and vitamins (Chaturved *et al.*, 2012). Deficiencies may occur when there is a decreased absorption or excessive excretion or ineffective utilization of trace elements. Mal-absorption syndrome has been accounted for Zinc and copper deficiencies especially in the phase of associated conditions like chronic diarrhea, ileostomy, inflammatory bowel disease, alcoholic cirrhosis, and burns (Goodmana *et al.*, 2006). Important sources of trace elements are vegetable plants and animal/poultry sources. Vegetables play an important role as source of nutrients to humans especially in our environment. The diet of an average African is vegetable based prepared in various forms. These vegetables have to be available in both dry and wet seasons. However, most plants dry up in dry season; this is then an advantage

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Moringa oleifera has over some other plants (Gueye *et al.*, 2007). *Moringa oleifera* is a nutritious vegetable tree. It is a softwood tree that is slender with drooping branches and in full leaf during and at the end of dry season (Adebayo *et al.*, 2011; Sarwatt *et al.*, 2002). It is grown for dietary purposes but of resent it has been advocated for medicinal and industrial uses (Fadiyimu *et al.*, 2011). The awareness of the tree is becoming generally known to treat some disease conditions and people usually recommend it for its nutritional value despite the inability of the available research to fully explain its nutritional contents like trace elements in various parts of the world. This study is also to look at to what extent does *Moringa oleifera* leaf may increase trace elements in rats bearing in mind that *Moringa oleifera* grown in different parts of Africa has been shown to have difference contents and quantity of nutritional parameters (Tsaknis *et al.*, 1994). This may be due to different minerals in the soils as well as different weather conditions.

MATERIALS AND METHODS

Study Site

The research was carried out in the animal house of Ladoke Akintola University of Technology (LAUTECH), College of Health Sciences, Mercyland Campus, Dada Estate, Osogbo. The animal house is a well ventilated, clear and clean environment, with no overgrown bushes that might predispose the animals to dangers from dangerous reptiles.

Leaf Identification and Preparation

Moringa oleifera plant gotten from a local farm in Osogbo, Osun State, Nigeria was identified at the herberium of the Obafemi Awolowo University (OAU), Ile-Ife, Nigeria. The leaves were harvested from several trees at early flowering stage. Branches were cut from the *Moringa oleifera* trees, spread out and dried under the shades for a period of 3-4 days. The dried leaves were grounded to powder which was stored in nylon bags during the entire period of the study. The fresh leaves were washed, grounded to solutions and refrigerated during the entire period of the study.

Animal Selection

Animals used for this study were healthy males and female albino rats weighing between 100-150g. A total of 70 albino rats were used for the experiment. The animals were randomly divided into two groups (group1-test and group2-control). The test group consists of 40 albino rats while the control group consists of 30 albino rats. The control animals and test animals were treated under the same conditions. They were nursed in two different cages. Cage 1, rectangular in shape with dimension 165cm by 80cm in length and width (floor area of 13,200cm^2) housed test animals. Cage 2 also rectangular in shape with dimension 165cm by 55cm (floor area of 9,075cm^2) housed the control animals. The floor areas of cages are made of plastic material and the majority of the body is made up of wire mesh giving animal minimal illumination for proper monitoring. The rats were housed in a temperature and humidity controlled room with a 24 hours light/dark cycle.

The rats were acclimatized for a period of 1 week during which they were given access to their normal feed (growers mash) and deionized water. After acclimatization, the control animals (group 2) continued on normal diet and drinking water for a period of 5weeks, while to the test animals (group 1), dried *Moringa oleifera* leaf powder were included in their normal feed (growers mash) for a period of 5weeks. To the animals in the test group, 15% of the dried *Moringa oleifera* leaf powder was included to their diet, while the *Moringa oleifera* leaf solution was added to their drinking water at 50:50 ratios. They were fed this supplemented diet starting from the 2nd week up to the 6th week.

This study was approved by Ladoke Akintola University of Technology, Osogbo ethical committee for laboratory animals, and the rats were maintained in accordance with the guidelines of the University for the Care and use of Laboratory Animals.
Blood Sample Collection and Preservation. At the end of the 6th week, the rats (both the control and test groups) were sacrificed; blood was collected from the carotid artery into a screw caped plain acid washed bottle. The blood samples were centrifuged at 3500rpm for 5minutes at room temperature to separate the serum. The serum samples were dispensed into a new, well labeled screw caped plain bottle and stored at -20^{0}C for a period of two weeks before analysis.

Serum trace elements (selenium, zinc and copper) were determined using Atomic Absorption Spectrophotometer serial number SN 115863752DJ from Beckman Laboratory, Germany. Control sera were run with each batch of analysis.

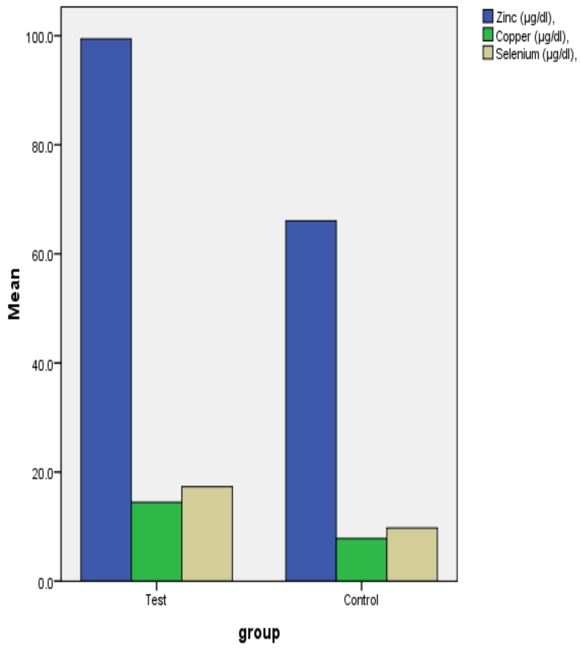

Figure: A Bar Chart Showing the Pattern of Increase in Zinc, Copper and Selenium Levels in Test and Control Rats

Statistical Analysis

Statistical analysis was done using SPSS software package (version 17). Results of comparison were reported as mean ± standard deviation using student's t-test and relationships between means of variables were determined using Pearson's correlation coefficient (r). Results were regarded as significant at $p<0.05$

RESULTS

The numbers of rat examined at the end of the experiment were 39 and 28 for test and control groups respectively. One and two rats were lost from test and control groups respectively in the course of the experiment. The cause of their death is not known as these animals were doing well until the point of death. In Table 1, the mean ± S.D of the weights of the tests and control groups (both their baseline and final weights) were analyzed and compared. The mean ± S.D of the final weights of the test group (200.62 ± 22.00)g was higher than the mean ± S.D of the baseline weights of the test group (124.70 ± 23.43)g. This difference was statistically significant ($p<0.05$). The mean ± S.D of the final weights of the control group (175.47 ± 30.24)g was higher than the mean ± S.D of the baseline weights of the control group (120.97 ± 18.71)g. This difference was statistically significant ($p<0.05$). The mean ± S.D of the baseline weights of the test group (124.70 ± 23.427)g was slightly higher than the mean ± S.D of the baseline weights of the control group (120.97 ± 18.71)g, though the mean difference was not statistically significant ($p>0.05$). The mean ± S.D of the final weights of the test group (200.62 ± 21.996)g was higher than the mean ± S.D of the final weights of the control group (175.47 ± 30.238)g, and the mean difference was statistically significant ($p<0.05$).

Table 1. Mean ± SD of Baseline and Final Weights of Rats between the Groups

Groups		N	Mean ±SD (g)	Std Error	*p*-value
Test	Baseline	40	124.70±23.43	3.794	
					<0.05
	Final	39	200.62±22.00	3.478	
Control	Baseline	30	120.97±18.71	3.416	
					<0.05
	Final	28	175.47±30.24	5.521	

As shown in Table 2, the mean serum levels of zinc (99.41±31.59 Vs 66.05±33.24), copper (14.45±6.87 Vs 7.82±4.33) and selenium (17.30±7.33 Vs 9.73±5.25) were significantly higher in the test group when compared with controls, the mean differences were statistically significant ($p<0.05$). In Table 2, Pearson's correlation between zinc, copper and selenium reveals a significantly strong positive correlation between zinc and copper($r = 0.786^{**}$), copper and selenium ($r = 0.931^{**}$) and zinc and selenium ($r = 0.671^{**}$) ($p<0.01$).

Table 2. Comparison of Zinc, Copper and Selenium Levels Between Test and Control Rats

Biochemical Parameters	Groups	N	Mean ±SD	Std Error	*p*-value
	Test	39	99.41±31.59	4.99	
Zinc (µg/dl)					<0.000
	Control	28	66.05±33.24	6.09	
	Test	39	14.45±6.87	1.086	
Copper (µg/dl)					<0.000
	Control	28	7.82±4.33	0.791	
	Test	29	17.30±7.33	1.120	
Selenium (µg/dl)					<0.000
	Control	28	9.73±5.25	0.959	

P < 0.05 is considered significant
N—Number of subjects

Table 3. 2-Tailed Pearson's Correlations Between all the Biochemical Parameters

		ZINC	COPPER	SELENIUM
ZINC	r	1	.786**	.671**
	p		.000	.000
COPPER	r	.786**	1	.931**
	p	.000		.000
SELENIUM	r	.671**	.931	1
	p	.000	.000	

** Correlation is significant at $p< 0.01$ level

DISCUSSION

The significant increase in final weight of the test animals than control animals indicates an increase in the physical growth of the test subjects compared to the control subjects. This may be as a result of ability of some of these trace elements that might present in *Moringa oleifera* leaf to improve appetite. The test animals tend to eat more than the control animals. Essential trace elements such as zinc, copper and selenium have been linked to performng important metabolic functions like acting as cofactors to antioxidants enzymes (superoxide dismutase etc) and vitamins (vitamin C etc) (Chaturved *et al.*, 2006). Deficiency of any of these essential elements may not be common but could be serious, and is mostly associated with malnutrition. The ultimate source of these trace elements is from the diet. Hence the need for a diet balanced in all or most of these essential trace elements.

Moringa oleifera like some other leaves (vegetable) has been said to contain various micro nutrients which are required for growth and development especially in pregnancy, infants and children, and may be widely used to prevent or ameliorate malnutrition in children and pregnant women. In this study, serum zinc, copper and selenium levels in albino rats fed with *Moringa oleifera* leaves were measured and compared with albino rats fed with normal diet. The concentration of zinc, copper and selenium were found to be significantly high in the test group compared to the control group. This finding is similar to the study of Fiazi *et al.* (1994) who pointed out that *Moringa oleifera* leaves contain some selected trace elements like zinc, copper and cobalt. This study shows significantly elevated serum zinc, copper and selenium levels which are due to the *Moringa oleifera* supplemented diet given to the test group. This proves that the *Moringa oleifera* leaves in addition to containing vitamins and minerals also contain these essential trace elements.

Zinc has catalytic, structural and regulatory roles and it is recognized for these important functions. It involves in cognitive functions and reproduction, as well as physiological growth (Sikler *et al.*, 2003; Daren *et al.*, 2005). Cell division and activation, DNA replication, RNA transcription, also require optimal function of zinc. Zinc is also forms part of superoxide dismutase (SOD). It functions as cofactor for SOD to catalyze the conversion of highly reactive superoxide species to less reactive hydrogen peroxide (Bannister *et al.*, 1987). Patients with below optimal function of this trace element may present with growth retardation, impotency, delayed sexual maturation, alopecia, and skin lesions, impaired host defence properties (Ibs *et al.*, 2003). The significant increase in the serum zinc level in the test group may also be as a result of absence of phytic acid in the Moringa leaves. Phytic acid has been said to reduce the bioavailability of zinc in plant products high in zinc, because phytic acid inhibits zinc absorption (Rosalind *et al.*, 2010). This absence of phytic acid then led to a higher bioavailabilty of zinc in the test group, resulting in a significant rise in their serum zinc level.

Copper like some other trace elements has physiological functions, and tendency to improve immune system (Zimowska *et al.*, 2001; Hunt *et al.*, 2003). It also has antioxidant effect, it forms part of ceruloplasmin and superoxide dismutase {SOD}. With these effects, patients with copper deficiency may present with anemia, immune suppression, chronic diarrhea, impaired reproductive performance (Wilians *et al.*, 1983). The significant increase in the serum copper level in the test group may also be as a result of the absence of zinc toxicity. It has been observed that excessive zinc consumption (toxic level) can lead to copper deficiency, because toxic zinc level blocks absorption of copper from the stomach and duodenum leading to copper deficiency. It could be deduced that the significant increase in serum zinc level observed in our study (test group) was not at toxic level. It may also be deduced that the level was not enough to block copper absorption hence increase in serum copper levels probably as a result of external source from *Moringa oleifera* in diet.

Trace elements like selenium also functions as co-factors. It helps in the reduction of glutathione peroxidase (an antioxidant enzyme). It also has beneficial effects on thyroid glands and in every cell that enjoys actions of thyroid hormones (Atthus *et al.,* 1991). Cancer, diabetes, tuberculosis and further suppression of the immune system in HIV/AIDS patients have all been associated with insufficient serum level of selenium (Kassu *et al.*, 2006; Vilamour *et al.*, 2008). As previously mentioned the increased serum level of selenium in our test animals may be as a result of high content of selenium in the leaf of *Moringa oleifera* consumed by these animals

These test animals were fed with dried *Moringa oleifera* leaves in their diet as well as fresh *Moringa oleifera* leaf solution in their water for 5 weeks. This shows that continuous intake of *Moringa oleifera* leaves did not lead to toxicity of any of these trace elements, but increased their levels making them considerably available in sufficient quantity for their catalytic, structural and regulatory roles in the body. This research therefore indicates that *Moringa oleifera* leaves can be used to reduce the incidence of disorders associated with trace element deficiencies that occur as a result of malnutrition.

Conclusion

This study establishes a strong relationship between *Moringa oleifera* leaf feeding and increase in the levels of serum zinc, copper and selenium. This suggests that *Moringa oleifera* leaves contain these selected trace elements, and therefore its inclusion in the diet may be advised to reduce the incidence of nutritional disorders

Recommendation

This study therefore recommends an intake of *Moringa oleifera* leaves to improve quality of life.

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Conflict of Interest: Authors have declared no conflict of interests.

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