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Effect of Copper, Manganese and Zinc With Antioxidant Vitamins on Pulse Rate and Lipid Profile of Salt-Loaded Albino Rats.

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ABSTRACT: Hypertension and dyslipidemia are associated with oxidative stress and are major causes of cardiovascular diseases amounting to 30% of global death rate. The effect of antioxidants supplementation on pulse rate and lipid profile in salt-loaded albino rats were investigated using a randomized control study with 30 albino rats divided into 5 experimental groups of 6 rats each. Groups 1 and 2 were normal untreated and salt-induced untreated respectively. Groups 3-5 were treated with Vitamins (A, C and E) with Cu, Mn and Zn respectively. Hypertension and dyslipidemia were induced using Salt-loading method (8% NaCl) for a period of five (5) weeks where Group 1 received normal rat feed and Groups 2-5 received salt-loaded diet. The heart rate of the rats was measured before and after the salt loading and dyslipidaemia was assessed at the end of the experiment. The results indicated that salt loading induced significant increase ($p<0.05$) in pulse rate, total cholesterol (TC) and LDL-cholesterol. It also induced significant ($p<0.05$) decrease in HDL-cholesterol and no change in Angiotensin converting enzyme activity. Treatment with antioxidants caused significant ($p<0.05$) decrease in pulse rate, TC and LDL-cholesterol and significant increase ($p<0.05$) in HDL-cholesterol even in the presence of the salt. There is no significant difference between treatments with the various elements. The results of the current study may therefore suggest that antioxidants supplementations may delay or reverse the onset of dyslipidaemia and hypertension associated with high salt diets in rats.
Key words: Pulse rate, Salt-loading, Dyslipidemia and Antioxidants.

INTRODUCTION

Cardiovascular disease refers to disease conditions that affect cardiovascular system such as atherosclerosis (Maton *et al.*, 1993). Cardiovascular diseases include coronary heart disease, cerebrovascular disease, hypertension, peripheral artery disease, rheumatic heart disease and congenital heart disease (Kumar *et al*., 2004).

About half the populations with essential hypertension are NaCl sensitive (Weinberger, 1996). There is also accumulating evidence that in animal experiments, salt loading produces hypertension and cardiovascular diseases (Frisbee *et al.,* 1999). Hypertensive with increased aldosterone activity (primary aldosteronism) where hypertension is critically salt- dependant has been reported (Bravo *et al*., 1986). Activation of sympathetic nervous system as well as abnormalities in ions transport has also been linked to Na overload-induced hypertension. Chronic Na overload has been reported to increase the levels of intracellular Na. This results in decreased activity of Na/Ca exchanger, thereby increasing $[Ca^{2+}]_i$ with an increased in vascular tone (Krishnamuri and Shyamola, 2001). A rise in blood pressure has been reported to influence the vascular endothelial function (Paniagua *et al.,* 2000).

Furthermore, salt loading results to over expression of $P47^{phox}$ and $g91^{phox}$ component of NADPH oxidase (generator of superoxide anion) and reduced expression of superoxide dismutase (quencher/ scavenger of superoxide anion) given rise to oxidative stress (Christoper, 2004). Strong experimental evidence indicates that, increased oxidative stress and associated oxidative damages are mediators of renovascular injury in cardiovascular pathologies. Increased production of superoxide anion and hydrogen peroxide, reduced nitric oxide (vasodilator) synthesis and bioactivity, and reduced bioavailability of antioxidants resulting in cardiovascular diseases. (Touyz, 2004)

Many risk factors are associated with cardiovascular diseases, one of which is abnormal level of serum cholesterol. High blood cholesterol has been shown to be a leading cause of cardiovascular disease. The most possible mechanism by which high level of LDL-C causes cardiovascular diseases is; high level of LDL-C may directly impair endothelial cell function through increased production of free radicals that deactivate nitric oxide (the major endothelial-relaxing factor), LDL accumulate within the intima at the site of increased endothelial permeability. The chemical changes of lipid induced by free radicals generated in macrophages or endothelial cells in the arterial wall yield oxidized (modified) LDL-C which is ingested by macrophages through scavengers receptor distinct from LDL receptor, thus forming form cells. Oxidized LDL also increases monocytes accumulation in lesion and stimulates release of growth factors and cytokins. Growth factors stimulate migration and proliferation of smooth muscle cells from the media into the intima there by converting fatty streak into a mature fibrofatty atheroma and contribute to the progressive growth of atherosclerotic lesion, the underlying cause of cardiovascular disease (Murry *et al*., 1993, Kumar *et al.,* 2004,).

Cardiovascular diseases are associated with low serum level of antioxidants. This give rise to excess free radicals species (oxidative stress) which attack many macromolecules including enzymes, membrane lipids, DNA, or any nearby molecule causing a cascade of chain reaction resulting in cellular damage and many diseases (Piece *et al*., 2004). It is the objective of this work to study the effect of antioxidants supplementation on pulse rate, lipid profile and oxidative stress in salt-loaded albino rats with a view to understanding whether or not the supplementation will reduce the risks of cardiovascular diseases.

METHOD

Procurement of the experimental rats: A total of thirty (30) male and female albino rats of about eight (8) weeks old were procured from Usmanu Danfodiyo University animal farm. The rats were acclimatized to the caging environment for two (2) weeks before commencing the experiment.

Induction of hypertension and dyslipidemia using Salt loading.

A total of 30 rats were randomized into 5 experimental groups. The first group received normal commercially available diet while the remaining groups received salt loaded diet (8% NaCl) for a period of 5 weeks according to then method of Hiromitsu, *et al.,* (2006). Body weight and pulse rate were recorded throughout the experimental period.

Treatment with antioxidants (vitamin A, vitamin C, vitamin E, Cu, Mn and Zn)

The 4 salt loaded groups were maintained with or without antioxidants. 3-5 groups received either of Cu (4.5mg/kg body weight), Mn (20mg/kg body weight) and Zn (23mg/kg body weight) in addition to vitamin A (7.16mg/kg body weight), vitamin E (13.17mg/kg body weight) and vitamin C (16.67mg/kg body weight) orally for a period of 3 weeks. Body weight and heart rate were also recorded. The rats were slaughtered under anesthesia using chloroform vapour and blood sample collected

Estimation of serum lipoprotein cholesterol

Total cholesterol (TC) level was estimated by enzymatic method as described Allain *et al.,* (1974). High Density Lipoprotein cholesterol (HDL-C) level was estimated using method reported by Burstein *et al*. (1970) while Triacylglycerol (TAG) level was estimated by the method of Trinder (1992). Low Density Lipoprotein cholesterol (LDL-C) and Very Low Density Lipoprotein cholesterol (VLDL-C) level were calculated using the formulae; HDL+TAG/5 and TAG/5 respectively. (Friedewald *et al*., 1974)

Angiotensin converting enzyme (ACE) Assay:

Angiotensing converting enzyme (ACE) like chemotrypsin is able to degrade tossyl arginine methyl ester (TAME). Exactly 0.9ml of 0.5M TrisHCl buffer pH-7.4 was added to 0.1ml of the serum and it was mixed. 20µl of TAME was added into the mixture and absorbance was taken after 0min, 1min, 2min and 3min against blank. The difference in absorbance was determined.

Statistical analysis: The results are presented as means and standard deviations and were compared using one-way Analysis of Variance (ANOVA) and individual means were compared

using Turkeyôs Multiple Comparison Test by InStat3 software. P value < 0.05 was considered significant.

RESULTS AND DISCUSION

Body weight and pulse rate of salt loading experimental rats are presented in Table 1. From the table, body weight remained the same throughout the experiment as no significant difference was found among the groups. The pulse rate of salt loaded groups was significantly ($p<0.05$) high as compared with control. However, the trend was reversed with antioxidant supplementation. The salt loaded untreated had significant ($p<0.05$) high in pulse rate when compared with normal untreated (control). This may be an indication that salt loading induces hypertension, since blood pressure is the product of cardiac output and total peripheral resistance, and cardiac output depends on pulse rate (Kumar *et al.,* 2004). Interestingly, antioxidant supplementations in this current study reduce pulse rate in salt loaded rats, suggesting that supplementation might have reversed the trend leading to hypertension and thus, might be useful in reversing or preventing events leading to cardiovascular diseases. This might be due to increase bioavailability of antioxidants and hence decreased oxidative stress resulting to an increased bioactivity of nitric oxide and decreased oxidative damages. Cu, Mn and Zn are component of superoxide dismutase and their supplementation might have probably increased the activity of the enzyme thereby reducing the level of superoxide anion which if not quenched may lead to unavailability of nitric oxide and oxidative damages ensures (Libor and Majid, 2005).

The result for serum lipoproteins cholesterol and triacyl glycerol levels of salt loading experimental rats, showed no significant difference ($p > 0.05$) in triacyl glycerol and VLDL-C levels in all the groups as shown in Table 2. However, there is a significant difference in TC, LDL-C and HDL-C levels for salt loaded untreated when compared with normal untreated and salt loaded treated groups. The high TC and LDLC with significant ($p < 0.05$) low HDLC in salt-loaded untreated group compared with control is an indication of cardiovascular complication.

Salt loading- induced oxidative stress through oxidation of LDL receptor leading to receptor defect and inadequate hepatic uptake of LDL (Libor and Majid, 2005). The enzyme 7Ŭ-hydroxylase responsible for cholesterol degradation is a Cu containing enzyme that is maintained at reduced state by vitamin C. Decrease vitamin C levels affect the activity of the enzyme and consequently leading to abnormal accumulation of LDL-C (Kumar *et al.,* 2004). Antioxidant supplementation was able to lower LDL-C in salt loaded rats perhaps by decreasing oxidative stress and increasing antioxidants bioavailability. The decreased oxidative stress reduces oxidative damage that stimulates the whole battery of atherosclerosis. In addition, increased antioxidants bioavailability increases the level of vitamin C, which enhances the activity of 7Ŭ-hydroxylase through maintaining Cu at reduced state. This probably lowers the level of LDL.

The Angiotensin Converting Enzyme (ACE) activity of salt loaded rats was not significantly different indicating that salt loading and antioxidant treatment might have not affected the angiotensin converting enzyme activity. This is supported by the fact that salt sensitive hypertension is associated with low renin activity (Frisbee *et al* 1999). Renin converts angiotensinogen to angiotensin I which is converted to angiotensin II by ACE. As such, if renin which produces the substrate of ACE is low, the ACE too will be low as part of enzyme regulation (Robert *et al.,* 2000).

CONCLUSION

The result of the current study demonstrated that antioxidants supplementation may delay or reverse the onset of dyslipidaemia and hypertension associated with high salt diets in rats.

Table 1: Body Weight and Pulse Rate of Salt Loading Experimental Rats

Group	Initial Group	After 4 weeks Salt loading	After treatment With antioxidant
Body weight (g)			
1	112.62±19.23	185.73±14.62	195.28±7.03
2	136.80±20.86	168.70±23.35	169.50±21.38
3	134.65±15.02	201.06±42.40	173.30±7.68
4	146.90±23.04	165.50±19.60	151.96±12.74
5	116.30±51.36	194.18±19.89	158.70±6.77
Heart Rate (Beat/min)			
1	195.46±8.21	184.43±4.07^{b}	194.85±4.54^{b}
2	188.89±3.65	207.73±8.61^{a}	240.13±3.54^{a}
3	194.90±6.45	217.59±5.92^{a}	199.40±4.43^{b}
4	186.40±15.93	211.73±4.93^{a}	199.36±3.51^{b}
5	197.77±9.11	208.90±2.35^{a}	202.70±3.46^{b}

Values are mean ± standard deviation

Values with different superscript down the columns are significantly different ($P < 0.05$).

1 - Normal untreated.

2 - Salt loaded untreated.

3 - Salt loaded treated with Cu in addition to vitamin A, C and E.

4 - Salt loaded treated with Mn in addition to vitamin A, C and E.

5 - Salt loaded treated with Zn in addition to vitamin A, C and E.

Table 2: Serum Lipoproteins Cholesterol (TC, HDLC, LDLC and VLDLC, Triacylglycerol (TAG) and levels and Angiotensin Converting Enzyme Activity (ACEA) of Salt-Loading Rats

Group	1	2	3	4	5
TC (mmol/L)	1.59±0.09^{b}	2.15±0.09^{a}	1.65±0.07^{b}	1.67±0.16^{b}	1.62±0.12^{b}
TAG (mmol/L)	0.58±0.04	0.66±0.03	0.59±0.03	0.50±0.03	0.58±0.04
HDLC (mmol/L)	0.92±0.07^{a}	0.59±0.04^{b}	0.96±0.03^{a}	0.93±0.05^{a}	0.89±0.07^{a}
LDLC (mmol/L)	0.57±0.11^{b}	1.44±0.13^{a}	0.58±0.09^{b}	0.55±0.13^{b}	0.61±0.11^{b}
VLDLC(mmol/L)	0.11±0.02	0.13±0.01	0.12±0.01	0.12±0.01	0.11±0.01
ACEA	0.006±0.004	0.003±0.001	0.004±0.002	0.003±0.001	0.003±0.001

Values are mean ± standard deviation.

Values with different superscript across the raw are significantly different ($p<0.05$).

1 - Normal untreated.

2 - Salt loaded untreated.

3 - Salt loaded treated with Cu in addition to vitamin A, C and E.

4 - Salt loaded treated with Mn in addition to vitamin A, C and E.

5 - Salt loaded treated with Zn in addition to vitamin A, C and E.

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