



Journal of Medical Sciences

ISSN 1682-4474

science
alert

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JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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Influence of L-arginine on the Heart Histology and Function Markers of Metabolic Syndrome in Female Wistar Albino Rats

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High blood pressure (a condition associated with vascular constriction) is a major feature of metabolic syndrome (MES). MES, a constellation of metabolic disorders, is prevalently higher in females and was associated with a reduced concentration of a vasodilator molecule, Nitric Oxide (NO). L-arginine (ARG), a precursor of NO may improve MES, warranting this study. Two groups (n = 8) of female Wistar albino rats were (per orally for twenty eight days) exposed to a single dose of 60 mg kg⁻¹ b.wt. of ARG and 3 mL kg⁻¹ b.wt. of distilled water, DW, respectively as treated and control groups. Significant differences in means were separated by student's t-test (p<0.05; p<0.01) and results expressed as Mean±Standard deviation. ARG exposure caused a significant reduction (p<0.01) in sodium ion (Na⁺) concentration (136.42±1.66 mmol L⁻¹; 6.54%), but a non-significant decrease (p>0.05) in potassium ion (K⁺) concentration (4.54±0.66 mmol L⁻¹; 14.01%) in the rats' serum, suggesting improved/reduced blood pressure. ARG treatment in the rats had a significant increase (p<0.01) in Alkaline Phosphatase (ALP) activity (8.30±0.23 IU L⁻¹; 196.43%) in the rats' serum, indicating adverse influence on high metabolic organs, including the brain. Sodium ion had a significant negative correlation (r = 0.01) with potassium ion, whereas the heart histomorphology revealed degenerations in the ARG-fed rats, apparently confirming the observations and suggestions thereto. Thus, ARG may improve blood pressure in the rats, perhaps at the expense of compromised heart function and histology of the rats. These may be pointing to a new arginine phenomenon, hence warrant follow up.

Key words: Alkaline phosphatase, heart histology, L-arginine, metabolic syndrome, potassium, sodium ions

INTRODUCTION

High blood pressure (a condition associated with vascular constriction) is a major feature of metabolic syndrome (MES). It was associated with a reduced concentration of nitric oxide, NO (a vasodilator molecule) and characterized by a cluster of cardiovascular risk factors, including high blood pressure (Deedwania and Gupta, 2006; Gallagher *et al.*, 2010) and increased health challenges (Pelucchi *et al.*, 2010; Siddiqui, 2011). Metabolic syndrome scourge is pandemic (Gotto *et al.*, 2006; Grundy, 2008) and is prevalent in children (Pedrosa *et al.*, 2011). The syndrome afflicts about 20-30% of the adult population the world over (Grundy, 2008; Chaabo *et al.*, 2010), including Nigeria (30.7%) as reported by Ijeh *et al.* (2010). The increasing prevalence of MES (Bakoma *et al.*, 2011) may be higher among the female gender, with huge implications.

Garlichs *et al.* (2000) had associated reduced NO concentration with the pathophysiology of MES. NO regulated cardiovascular function (McGrowder and Brown, 2007) and at abnormal concentration, had elicited pathological condition (Lokhande *et al.*, 2006) in animals. A reduction in the concentration of ARG affected the biological activity of NO (Subratty *et al.*, 2007; Harisa, 2011), suggesting exogenous supply of ARG may influence the activity of NO in animals. Indeed, possible role of ARG in metabolic processes that could improve MES features in animals was suggested (Sepelri *et al.*, 2006; Van Waardenburg *et al.*, 2007; Ezeanyika and Egbuonu, 2011; Harisa, 2011) and appear to be supported by recent studies (Egbuonu and Ezeanyika, 2012a, b; Egbuonu and Ezeanyika, 2013).

Since high blood pressure is a major feature of MES (Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, 2001 (NCEP) (USA); Gallagher *et al.*, 2010), this study aimed to ascertain the effect of ARG on the heart histology and function markers of MES in female rats. Objectives set to achieve the stated aim include the study of the effect of ARG on serum alkaline phosphatase activity, sodium ion and potassium ion concentrations as well as on the heart histology of female Wistar albino rats. In particular, potassium ion contributes to the optimal functioning of the cells and the organs and its deficiency (hypokalaemia) was associated with cardiac dysfunction (Bush, 1991) and high blood pressure (Siani *et al.*, 1987; Strazzullo *et al.*, 1990). On the other hand, elevated sodium ion concentration (hypernatraemia) indicated high blood pressure (Jaitovich and Bertorello, 2010).

MATERIALS AND METHODS

Chemicals: The chemicals (analytical grade) used in this study were products of reputable companies based in Europe and America.

Concentration determination/justification: Based on the WHO reported daily ARG oral intake (Marshall, 1994) and the concentration used in earlier studies (Alexander *et al.*, 2004; Egbuonu *et al.*, 2010a-c), the concentration of ARG used in this study was calculated and adjusted to 60 mg kg⁻¹ b.wt.

Animals and treatment: The female Wistar rats (60-80 g) used in this study were procured from the animal house of the Faculty of Biological Sciences University of Nigeria. The animal study was according to International guidelines for the care and use of laboratory animals in Biomedical Research (CCAC, 1985; WMA/APS, 2002). The animal study was conducted between August and September, 2010.

The rats were acclimatized for a week and randomized into two groups (sample size of eight rats each) based on their body weight. Group B rats were exposed to ARG (60 mg kg⁻¹ b.wt.) whereas Group A rats were given Distilled Water (DW) (3 mL kg⁻¹ b.wt.). The rats were exposed orally for 28 consecutive days.

The rats were kept in a well-ventilated stainless steel cages at room temperature (28±2°C) and tropical humid condition. They were maintained under twelve hours of light alternating with twelve hours of darkness. The rats were allowed free access to tap water and standard rat chow (Grand Cereals and Oil Mills Limited, Jos, Nigeria) throughout the experimental period.

Sample collection and preparation: After 28 days oral intubation, the animals were fasted overnight and sacrificed on day 29. The respective blood sample of the animals was collected by ophthalmic venous plexus or retro orbital sinus venipuncture, using sterile capillary tube to direct blood into clean non-anticoagulated glass tubes.

Clotted blood in each tube was centrifuged (at 3000 rpm for 10 min) to yield the serum. The respective serum was aspirated separately into stoppered polystyrene tube and stored in a deep freezer for subsequent use in determining the biochemical markers of MES related to heart function. Organ specimen (heart) excised from the sacrificed rats for histology were fixed in 10% formaldehyde buffered saline (formal saline) until used.

Our choice for using female rats in this study was based on the recent listing of female gender as an independent risk factor for the development of MES (Ravikiran *et al.*, 2010) and reports that MES was prevalently higher in females (Mangat *et al.*, 2010; Kilic *et al.*, 2010).

Parameters determined

Serum alkaline phosphatase (ALP) activity: The serum alkaline phosphatase (ALP) activity was assayed by the method of Walter and Schutt (1974). This is based on the principle that alkaline phosphatase could hydrolyze colorless phosphate esters of various alcohols and phenols yielding p-nitrophenol as the yellow nitrophenolate ion in alkaline solution that was measured colorimetrically at 405 nm.

Photometric estimation of sodium (Na⁺) and potassium (K⁺) ions concentrations: The estimation of sodium and potassium ions in serum was with flame emission photometer. This is based on the principle that passing a liquid sample through a nebuliser (atomizer) could excite the atoms which on falling back to the ground state, emit light of characteristic wavelength, color and intensity. On passing through a suitable filter, a photosensitive detector measures the emitted light as the amount (concentration) of the atom (metallic ion) present.

Organ histology: Organ specimen (heart) for histological examination was promptly excised from the sacrificed rats and fixed in 10% formaldehyde buffered saline (formal saline) as reported by Egbuonu *et al.* (2010c). Briefly, after dehydration (in graded levels (70-100%) of alcohol), clearing (in xylene impregnated with paraffin wax) and sectioning (at 5 microns thickness using rotary microtome) the sections were floated on a water bath maintained at a temperature of 2-3°C below melting point of the paraffin wax. The sections were dried on a hot plate maintained at a temperature of 2-3°C above the melting point of the paraffin and stained. Then, the sections were mounted, using haematoxylin and eosin.

Statistical analysis: Data were analyzed by Student's t-test to determine the significant differences in means, using the Statistical Package for the Social Sciences (SPSS) for Windows version 16.0 (SPSS Inc., Chicago, IL., USA). Results were expressed as mean and standard deviation (Mean±SD) of eight rats per group at significance levels of p<0.05 and p<0.01. Furthermore, correlation of the results for possible association among the studied parameters was by Pearson's bivariate methods (r = 0.01).

RESULTS

Serum alkaline phosphatase (ALP) activity: Results of this study reveal that rats exposed to ARG had a significant (p<0.01) increase in their serum ALP activity (8.30±0.23 IU L⁻¹) compared with those exposed to DW (2.80±0.10 IU L⁻¹). This is an increase of over one-fold (196.43%) in ARG-fed group relative to the control (Fig. 1).

Serum sodium ion (Na⁺) concentration: As depicted in Fig. 2, the sodium ion concentration in serum decreased in the ARG-treated rats (136.42±1.66 mmol L⁻¹) relative to control (145.97±1.22 mmol L⁻¹). The observation, representing a decrease of 6.54% was statistically significant (p<0.01).

Serum potassium ion (K⁺) concentration: The results of this study as presented in Fig. 3 show that ingesting ARG by rats non-significantly decreased (p>0.05) the serum K⁺ ion concentration (4.54±0.66 mmol L⁻¹) in comparison with the control (5.28±0.44 mmol L⁻¹). This observation represents a decrease of 14.01% relative to the control value.

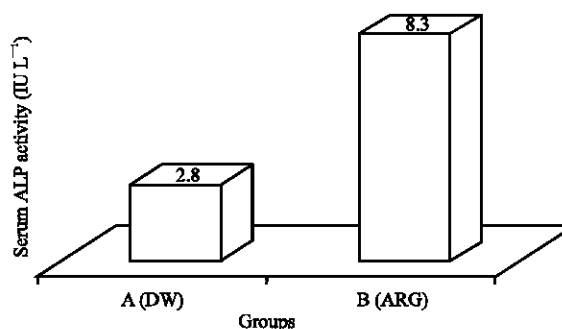


Fig. 1: Effect of DW and ARG on serum ALP activity of rats

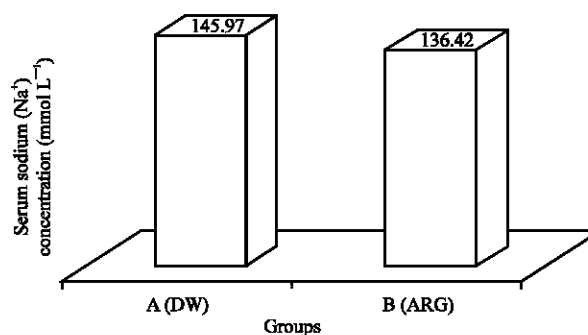


Fig. 2: Effect of DW and ARG on serum sodium ion concentration of rats

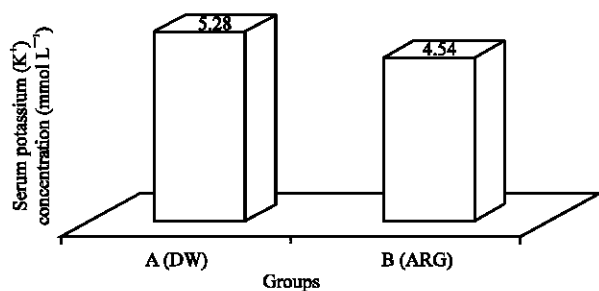


Fig. 3: Effect of DW and ARG on potassium ion concentration in serum of rats

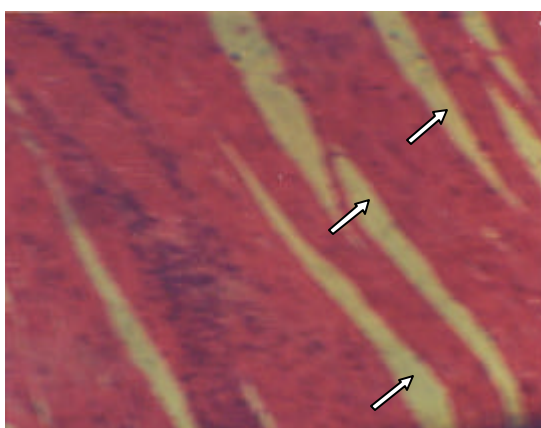


Fig. 4: Heart section of untreated, control (Group A) rats showing normal papillary muscles (arrow heads). H and E stains, ×400

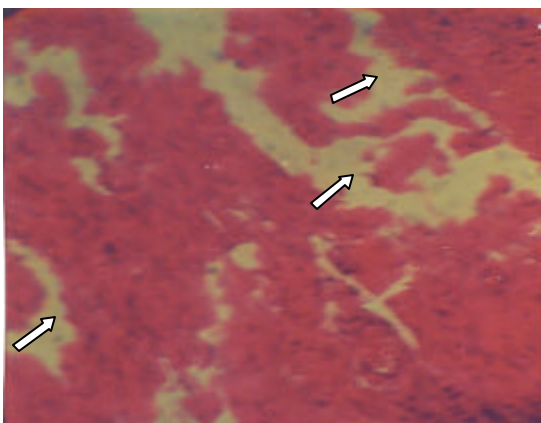


Fig. 5: Heart section of rats treated with high ARG (Group B) showing degeneration of the papillary muscles (arrow heads). H and E stains ×400

Histomorphology of the heart: Heart sections of the control (Group A) showed typical histology with normal papillary muscles (Fig. 4). Sections collected from rats

Table 1: Correlations output of serum potassium ion and sodium ion

Pearson		Potassium ion	Sodium ion
Potassium ion	Pearson correlation	1	-0.592**
	Sig. (2-tailed)		0.002
	N	16	16
Sodium ion	Pearson correlation	-0.592**	1
	Sig. (2-tailed)	0.002	
	N	16	16

**Correlation is significant at the 0.01 level (2-tailed)

treated with ARG (Group B) showed moderate degeneration of the papillary muscles (Fig. 5).

Correlation of results: The results of Pearson's correlation analysis showed that sodium ion concentration had a significant negative correlation ($r = 0.01$) with potassium (Table 1).

DISCUSSION

The female gender is an independent risk factor for the development of metabolic syndrome, MES (Ravikiran *et al.*, 2010). MES, a constellation of metabolic disorders, is prevalently higher in the females. It was associated with a reduced concentration of NO (a vasodilator molecule) and characterized by a cluster of cardiovascular risk factors, including hypertension (high blood pressure) (Deedwania and Gupta, 2006; Gallagher *et al.*, 2010). Possible role of ARG in metabolic processes that could improve MES in animals was suggested (Van Waardenburg *et al.*, 2007; Ezeanyika and Egbuonu, 2011) and appear to be supported by recent studies (Egbuonu and Ezeanyika, 2012a, b; Egbuonu and Ezeanyika, 2013). High blood pressure (a condition associated with vascular constriction and heart function) is a major feature of MES. These therefore warranted this study.

ARG ingestion by rats elicited a significant increase ($p < 0.01$) in ALP activity in the rats' serum, indicating alterations of pancreatic function (Fraulob *et al.*, 2010) and other high metabolic organs, possibly the heart and brain. The ALP enzyme is present in various high metabolic organs hence is not a specific marker of toxic effect. In similar studies, we demonstrated that L-arginine exposure improved the kidney/renal function (Egbuonu and Ezeanyika, 2013) and liver function (Egbuonu *et al.*, 2013), but damaged the brain function (unpublished).

Sodium ion accumulation caused high blood pressure in animals (Jaitovich and Bertorello, 2010). ARG ingestion to the female rats decreased ($p < 0.01$) the sodium ion (Na^+) concentration, implying lowered blood pressure and possible benefit on MES in the rats. This is consistent with earlier reports of ARG-induced vasodilation

(Rang *et al.*, 2003) and reduction of hypertension or high blood pressure (Alexander *et al.*, 2004) in animals. The possible vasodilatory activity of ARG via its metabolite, NO, could enhance the increase in the wall of the blood vessels resulting in lowered blood pressure. On the other hand, ARG treated rats had a decrease in serum potassium ion (K⁺) concentration. Decreased potassium ion (hypokalaemia) resulted to neurological and cardiac dysfunctions in animals (Bush, 1991), but the observation was not statistically significant ($p > 0.05$) hence may not be treatment related.

Furthermore, correlation of the results showed that sodium ion had a significant negative correlation ($r = 0.01$) with potassium ion, suggesting overriding effect of ARG on sodium ion over potassium ion in relation to blood pressure reduction in this study. Generally, degenerations observed in the heart sections of the rats appear to indicate ARG-induced adverse influence on their heart histomorphology, apparently confirming the observation on the serum ALP activity and the suggestion thereto. Agent-induced histomorphologic alterations in organs was reported by Egbuonu *et al.* (2010c). It appears that the possible blood pressure lowering potential of ARG elicited adverse influence on the heart histology. We did neither know nor study how this could have occurred but L-arginine-induced complex effect had been reported in animals and attributed to the multiple catabolic pathways of ARG (Schriek *et al.*, 2007). 'Arginine paradox' (a phenomenon referring to the dependence of cellular NO production on exogenous L-arginine concentration despite the theoretical saturation of nitric oxide synthase enzymes with intracellular L-arginine) may be fundamental to this complexity.

CONCLUSION

In conclusion, this study suggests that exposure to ARG may improve blood pressure (a major feature of MES) in the female rats, perhaps at the expense of compromised heart histology and function of the rats. This may be pointing to a an entirely new arginine phenomenon that warrants further investigation and caution in the use of ARG against MES associated with heart function in female rats.

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