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L-arginine Exposure Improves Renal Function Markers of Metabolic Syndrome in Female Rats

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ABSTRACT

Female gender is an independent risk factor for the development of metabolic syndrome (MES) (a cluster of features indicating metabolic disorders), that is associated with kidney damage, insulin resistance and a significant reduction in Nitric Oxide (NO), a major metabolite of L-arginine (ARG). This study aimed to ascertain the effect of ARG on selected markers of MES related to kidney damage in female Wistar albino rats. Two groups of rats were given 3 mL kg^{-1} body weight (b.wt.) of distilled water, DW and 60 mg kg^{-1} b.wt. of ARG, respectively as control and treated groups. Exposing the female rats to ARG caused a significant decrease ($p<0.01$) in the concentration of urea (6.34±0.23 mg/100 mL), creatinine (4.41±0.50 mg/100 mL) and albumin (14.30±0.15 mg/100 mL) in rats' serum. It decreased ($p<0.01$) creatinine clearance (1.78±0.27 mL min^{-1}) but elicited a significant increase ($p<0.01$) in the albumin:creatinine ratio (3.27±0.32) of the rats. Improved kidney histology as indicated by lots of renal corpuscles, was observed in the ARG-fed group while correlation analysis showed that urea correlated positively ($r = 0.01$) with creatinine, albumin and creatinine clearance, but negatively ($r = 0.01$) with Albumin: Creatinine ratio. The study suggests that L-arginine ingestion could improve these renal function markers and perhaps, metabolic syndrome related to kidney dysfunction, in female Wistar rats. The effect could be concerted and significant as indicated by the histomorpholgy and correlation results. Thus, with the abundance of ARG in nuts, including walnut, cashew nut, ground nut and even coconut, the implication of this study in the prevention and management of MES in, especially female, animals is noteworthy hence, deserve follow up, probably in humans.

Key words: Renal function, metabolic syndrome, albumin, L-arginine, urea

INTRODUCTION

Metabolic syndrome (MES) is a cluster of cardiovascular risk factors that is characterized by obesity, atherogenic dyslipidemia, insulin resistance and hypertension (Gallagher *et al*., 2010; Mahajan *et al*., 2010). It is not a disease entity, but a cluster of medical disorders in an individual that could predispose animals to further health challenges. These include type 2 diabetes mellitus (Azhar, 2010), cancer (Siddiqui, 2011; Rosato *et al*., 2011; Capasso *et al*., 2011), obstructive sleep apnea (Mugnai, 2010) and Kidney damage (Mathur, 2010). Globally, the prevalence of MES has increased dramatically (Bakoma *et al*., 2011) with possible huge economic implications.

In particular, the kidney is vital in the maintenance of homeostasis through excretion of catabolites, including urea and creatinine (Uboh *et al*., 2011; Atangwho *et al*., 2007) and elevated concentration of these catabolites in the plasma or serum indicates compromised renal function

(Appel *et al*., 2003; Zanna *et al*., 2008) and possibly incidence of MES. Furthermore, the association of a significant reduction in NO with the pathophysiology of MES (Garlichs *et al*., 2000) suggested that L-arginine (ARG), a major precursor in the synthesis of NO (Ezeanyika and Egbuonu, 2011), may improve MES in animals.

Nitric oxide, NO, is a vasodilator molecule that plays an important role in the regulation of cardiovascular function (McGrowder and Brown, 2007). Lokhande *et al*. (2006) reported that abnormal concentration of NO could lead to pathological conditions, including hypertension and diabetes. A decrease in ARG availability resulted in the reduction of the biological activity of NO (Harisa, 2011) and in the conversion of NO into peroxynitrites that could mediate cell damage (Subratty *et al*., 2007). However, ARG supplementation resulted in the decrease of the atherogenic index in hypercholesterolaemic rats (Harisa, 2011) and weight loss in experimental animal (Sepehri *et al*., 2006), suggesting possible benefit of ARG supplementation on MES since increase in weight and atherogenic index are major features of MES (Gallagher *et al*., 2010).

Thus, the present study aimed to ascertain the effect of ARG on markers of MES related to kidney damage through the specific objectives of studying the effect of ARG on the concentration of urea, creatinine and albumin as well as creatinine clearance, Albumin:creatinine ratio and histomorphology of the kidney, using female rats as model. The choice of female rats in this study derived from report that the female gender is an independent risk factor for incidence of MES (Ravikiran *et al*., 2010) and that the prevalence of MES is higher in the females (Mangat *et al*., 2010; Kilic *et al*., 2010; Titty *et al*., 2008) where it could result to polycystic ovary syndrome (Mathur, 2010) that worsens infertility. ARG is abundant in nuts, including walnut, cashew nut, groundnut and even coconut, thus possible benefit of ARG on MES may be easily harnessed in dietary food choice and nutraceutical formulation for the prevention and management of MES in animals.

MATERIALS AND METHODS

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

Concentration determination/justification: The test concentration, ARG (60 mg kg^{-1} b.wt.) was calculated and adjusted based on the WHO reported daily ARG oral intake (Marshal, 1994) and the concentration used in earlier studies (Alexander *et al*., 2004; Egbuonu *et al*., 2010a-c).

Experimental design

Animals and treatment: Procurement of female weanling Wistar rats used in this study was from the animal house of the Faculty of Biological Sciences University of Nigeria, Nsukka. The rats weighed 60-80 g. The animal study was according to International guidelines for the care and use of laboratory animals in Biomedical Research (World Medical Association, American Physiological Society, 2002). The animal study was carried out between August/September, 2010.

The rats acclimatized for a week and immediately thereafter were randomized into two groups (based on their body weight) with sample size of eight rats each. Group B rats were exposed to ARG (60 mg kg^{-1} b.wt.) whereas Group A rats were given Distilled Water (DW) (3 mg kg^{-1} b.wt.), corresponding to the volume of vehicle. Exposure route was by oral intubation, which was consecutive for 28 days.

The rats, housed in a well-ventilated stainless steel cages at room temperature (28±2°C) and tropical humid condition, were maintained under standard natural illumination of twelve hours of light alternating with twelve hours of darkness (i.e., a normal daylight/dark cycle). In compliance with the ethical guidelines for treating laboratory animals, the rats were allowed unrestricted access to tap water and standard rat chow (Grand Cereals and Oil Mills Limited, Jos, Nigeria) for the experimental period.

Sample collection and preparation: The animals were fasted overnight before sacrifice after 28 days. Collection of the respective blood samples of animals was by ophthalmic venous plexus or retro orbital sinus venipuncture. This involved inserting a sterile capillary tube into the medial canthus of the eye of the rat to puncture the retro-bulbar plexus resulting in out flow of blood into clean non-anticoagulated glass tube for serum tests.

Centrifugation of clotted blood at 3000 rpm for 10 minutes yielded the serum. Thereafter, the serum (aspirated separately into stoppered polystyrene tubes) was stored in a deep freezer for subsequent use in determining the selected biochemical markers of metabolic syndrome. Kidney specimen promptly excised from the sacrificed rats for histology were fixed in 10% formaldehyde buffered saline (formal saline) until used.

Parameters determined

Creatinine clearance: Creatinine clearance was calculated using Cockroft formula (Demirovic *et al*., 2009; Hjelmesaeth *et al*., 2010), but with adjustment for size and assumption of uniform age:

$$\text{Creatinine clearance (mL min}^{-1}) = (140\text{-age}) \times \text{LBW} \times \text{Serum creatine} \times 1.23 \times 0.85 \text{ (for female)}$$

Serum urea concentration: The determination of serum urea concentration was by the method of Alexander and Grifith (1992). In this method, conversion of ammonia (obtained from the hydrolysis of urea to ammonia and carbon dioxide in a reaction catalyzed by urease) in the presence of sodium nitroferricyanide-phenol and hypochlorite reagents yields indophenol blue that was measured colorimetrically at 625 nm.

Serum creatinine concentration: The determination of serum creatinine concentration was by the method of Wilding and Kennedy (1977) based on the Jaffe alkaline picrate reaction that forms reddish alkaline solution of sodium picrate that was measured colorimetrically at 500 nm.

Serum albumin concentration: This was estimated by the Bromocresol green (BCG) method as described by Ochei and Kolhatkar (2008). This method is based on the principle that under acidic conditions, serum albumin binds specifically with bromocresol green to form a green coloured complex that was measured colorimetriclly at 640 nm.

Calculation of the diagnostic ratio: The diagnostic ratio (albumin: Creatinine ratio) were calculated from the corresponding parameters obtained in this study.

Organ histology: Organ specimen (kidney) promptly excised from the sacrificed rats for histological examination were fixed in 10% formaldehyde buffered saline (formal saline) until used

as reported (Egbuonu *et al.*, 2010c). In brief, 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. Thereafter, drying of the sections was performed on a hot plate maintained at a temperature of 2-3°C above the melting point of the paraffin followed by staining and mounting of the sections using haematoxylin and eosin.

Statistical analysis: Analysis of data to determine the significant differences in means was by Student's t-test, 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 $p<0.01$. Furthermore, correlation of the results for possible association among the studied parameters was by Pearson's bivariate method ($r = 0.01$).

RESULTS

Serum urea concentration: The results of this study on serum urea concentration as presented in Fig. 1 show that serum urea concentration decreased in rats treated with ARG (6.34±0.23 mg/100 mL) when compared with the control (10.52±0.60 mg/100 mL). The observation (representing a decrease of 39.73% in ARG-treated group) was statistically significant ($p<0.01$).

Serum creatinine concentration: When compared with the control (9.07±0.44 mg/100 mL), the serum creatinine concentration was significantly ($p<0.01$) reduced in ARG-treated group (4.41±0.50 mg/100 mL) (Fig. 2). The present observation shows a decrease of 51.38% in the ARG-treated group relative to the control.

Creatinine clearance: The results of the present study as presented in Fig. 3 reveal that the creatinine clearance decreased in rats exposed to ARG (1.78±0.27 mL min^{-1}) in contrast with the control (3.73±0.23 mL min^{-1}). The observed reduction in creatinine clearance (representing a decrease of 52.28% relative to the control) was statistically significant ($p<0.01$).

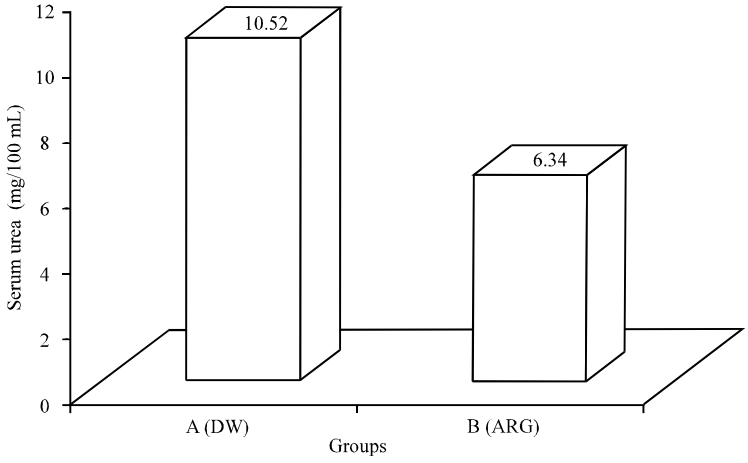

Fig. 1: Effect of DW and ARG on serum urea concentration of rats

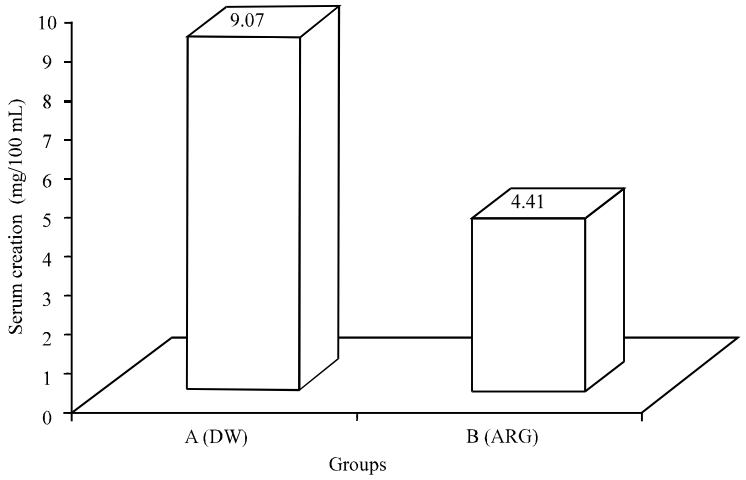

Fig. 2: Effect of DW and ARG on serum creatinine concentration of rats

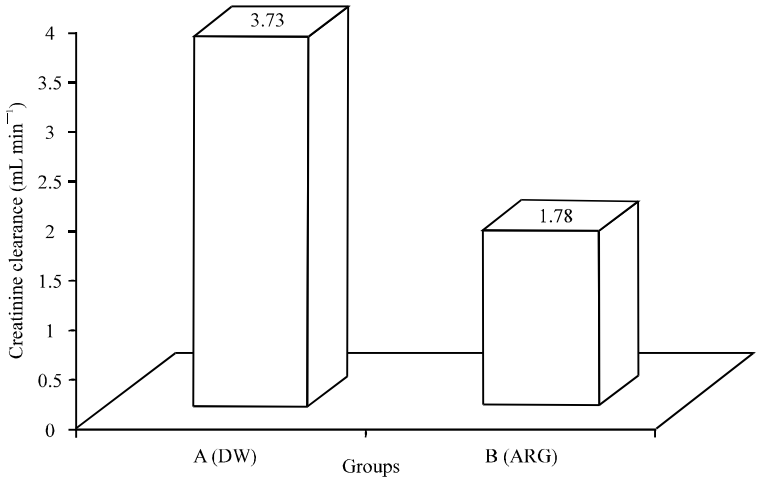

Fig. 3: Influence of DW and ARG on calculated creatinine clearance of rats

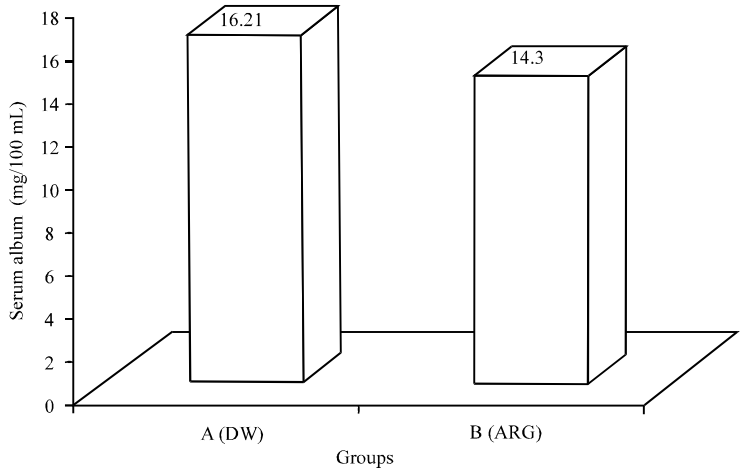

Fig. 4: Effect of DW and ARG on serum albumin concentration of rats

Serum albumin concentration: The results of this study as presented in Fig. 4 show that exposing rats to ARG elicited a significant ($p<0.01$) reduction (14.30±0.15 mg 100 mL) in serum

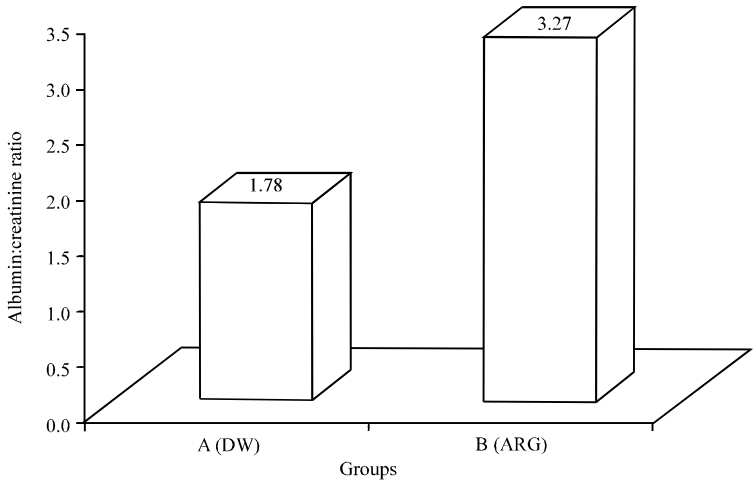

Fig. 5: Effect of DW and ARG on serum albumin:creatinine ratio of rats

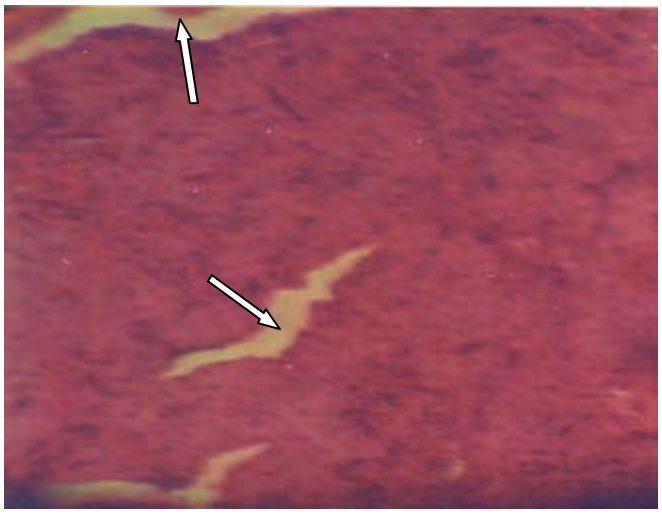

Fig. 6: Kidney section of untreated (Group A) rats showing striated ducts (arrow heads) devoid of renal corpuscles. H and E stains, x400

albumin concentration in comparison with control (16.21±1.30 mg 10/mL). This represents a decrease of 11.78% in the ARG-treated rats relative to the control.

Serum albumin:creatinine ratio: The results of the present study as shown in Fig. 5 reveal that the serum albumin:creatinine ratio of the ARG-treated rats (3.27±0.32) increased ($p<0.01$) above that of the control rats (1.78±0.14). This represents an increase of 83.71% in the ARG-treated group relative to the control.

Histomorphology of the kidney: Kidney sections of the control (Group A) rats showed striated ducts devoid of renal corpuscles (Fig. 6). Rats treated with ARG (Group B) showed improved kidney histology as indicated by lots of renal corpuscles and devoid of striated ducts (Fig. 7).

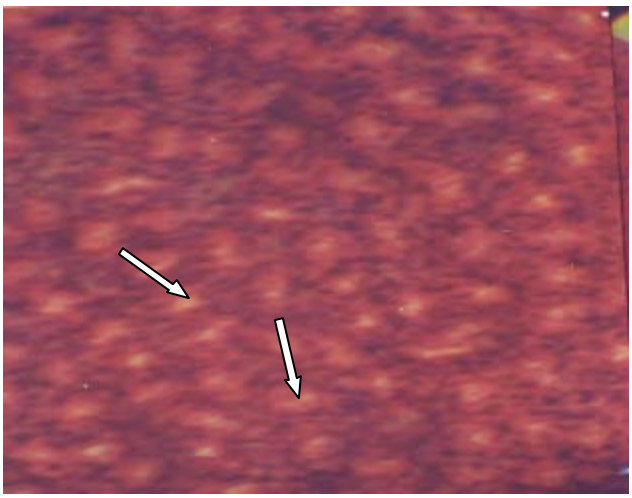

Fig. 7: Kidney section of rats treated with high ARG (Group A) showing lots of renal corpuscles (arrow heads) devoid of striated ducts. H and E stains x400

Table 1: Pearson two-tailed correlation analysis of urea, creatinine, creatinine clearance, albumin and albumin:creatinine ratio

Analysis	Urea	Creatinine	Creatinine clearance	Albumin	Albumin: creatinine ratio
Urea					
Pearson correlation	1	0.925**	0.938**	0.648**	-0.891**
Sig. (2-tailed)		0	0	0.001	0
N	24	24	24	24	24
Creatinine					
Pearson correlation	0.925**	1	0.970**	0.689**	-0.939**
Sig. (2-tailed)	0		0	0	0
N	16	16	16	16	16
Creatinine clearance					
Pearson correlation	0.938**	0.970**	1	0.737**	-0.887**
Sig. (2-tailed)	0	0		0	0
N	16	16	16	16	16
Albumin					
Pearson correlation	0.648**	0.689**	0.737**	1	-0.445*
Sig. (2-tailed)	0.001	0	0		0.029
N	24	24	24	24	24
Albumin:creatinine ratio					
Pearson correlation	-0.891**	-0.939**	-0.887**	-0.445*	1
Sig. (2-tailed)	0	0	0	0.029	0
N	16	16	16	16	16

**, *Correlation is significant at the 0.01 and at 0.05 level, respectively (2-tailed)

Correlation analysis: The results of Pearson's correlations analysis (Table 1) revealed that urea correlated positively (r = 0.01) with creatinine, albumin and creatinine clearance, but negatively (r = 0.01) with Albumin:creatinine ratio.

DISCUSSION

Female gender is an independent risk factor for the development of Metabolic Syndrome (MES) (Mangat *et al*., 2010; Kilic *et al*., 2010) a cluster of features indicating metabolic disorders that is associated with kidney damage, insulin resistance and a significant reduction in Nitric Oxide (NO),

a major metabolite of L-arginine (ARG) (Garlichs *et al.*, 2000). This study aimed to ascertain the effect of ARG on selected markers of MES related to kidney damage in female Wistar albino rats. The kidney is vital in the maintenance of homeostasis, including blood pH, water and electrolyte balance, through excretion of catabolites, including urea and creatinine (Uboh *et al.*, 2011) after ultrafiltration and reabsorption of desirable elements in the filtrate via urine (Ochei and Kolhatkar, 2008). Thus, elevated concentration of these catabolites in the plasma or serum indicates compromised renal function (Appel *et al.*, 2003; Zanna *et al.*, 2008; Ochei and Kolhatkar, 2008), enhanced oxidative stress in animals (D'Apolito *et al.*, 2010), hence, could indicate presence of physiological dysfunction in animals, including incidence of MES.

Generally, the decrease in the concentration of urea and creatinine observed with ARG ingestion by the rats suggest benefit on the renal function and possibly MES (Ochei and Kolhatkar, 2008). Urea and creatinine are products of protein catabolism eliminated *via* urine and their decrease in serum as observed in this study indicated efficient elimination probably by efficient renal organ and function. Thus, MES related to kidney damage may be improved in rats exposed to ARG. Consistent with these results, exposing the rats to ARG decreased albumin concentration in the rats' serum, indicating absence of renal and cardiovascular diseases as suggested by Cerasola *et al.* (2009).

Exposing the rats to ARG decreased ($p<0.01$) the creatinine clearance of the rats, suggesting a decrease in muscle mass (Ochei and Kolhatkar, 2008) that could improve MES in the rats. On the other hand, ARG ingestion by the rats caused an increase ($p<0.01$) in the serum albumin: Creatinine ratio, indicating macroalbuminuria (large amount of albumin in urine), slight kidney dysfunction (Khan, 2010) and insulin resistance (Nitta, 2010). The apparent adverse influence of ARG on this parameter (Albumin:creatinine ratio) of renal function is attributable to multiple catabolic pathways of ARG (Schriek *et al.*, 2007). On the other hand, it may be due to the possibly unrealistic value of creatinine clearance, which was not determined by gold standard method, but calculated using Cockroft formula (Demirovic *et al.*, 2009; Hjelmesaeth *et al.*, 2010) and with adjustment for size and assumption of uniform age. This is a noted limitation of this study. However, Albumin:creatinine ratio correlated negatively ($r = 0.01$) with the other markers of renal function (Table 1), suggesting that its contribution to renal dysfunction in the rats may be negligible.

Histomorphological alterations in organs were the most consistent treatment-related changes and in concert with the serum chemistry results may give a clear picture of physiological function in animals (Egbuonu *et al.*, 2010c). Agent-induced physiological and biochemical disturbances (Adeniran *et al.*, 2006), as well as alterations in liver and kidney histology (Farrag and Shalby, 2007; Egbuonu *et al.*, 2010c) have been reported. In particular, the observed changes in the histomorphology of the kidney sections of rats showed improvement in the ARG-fed rats, seemingly supporting the serum chemistry results of this study. Furthermore, Pearson's correlation analysis indicated that urea correlated positively ($r = 0.01$) with creatinine, albumin and creatinine clearance but negatively ($r = 0.01$) with Albumin: Creatinine ratio, suggesting concerted ARG-induced benefit on the renal function and possibly on MES related to renal dysfunction in the female rats.

In conclusion, the study suggests that L-arginine ingestion could improve these renal function markers and perhaps, metabolic syndrome related to kidney dysfunction, in female Wistar rats. This could be concerted and significant as indicated by the histomorpholgy and correlation results. Thus, with the abundance of ARG in nuts, including walnut, cashew nut, ground nut and even coconut, the implication of this study in the prevention and management of MES in, especially female, animals is noteworthy hence deserve follow up, probably in humans.

Strength and limitation: The validity of this study is strengthened by the strict adherence to ethics in animal studies and to standard methods. In terms of weakness, creatinine clearance was calculated rather than determined by gold standard method, hence its value (and that of Albumin:creatinine ratio) in this study may not be realistic.

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