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Renal, Cardiac and Osteo – protective effects of *beta* – sitosterol glycoside in hypertensive rats

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Abstract

Phytosterols are now popularly used as nutraceuticals for preventing, managing and retarding the progression of chronic diseases. Although several experimental studies have shown that β – sitosterol possess cardioprotective and hepatoprotective effects in different chronic diseased animal model, there is insufficient information on the biological effects of its conjugated form. This study therefore investigated the renoprotective, cardioprotective and osteoprotective effects of β – sitosterol glycoside (BSSG) in Cadmium Chloride ($CdCl_2$) – induced hypertensive rats.

Renal damage was induced by $CdCl_2$ and the hypertensive groups treated with lisinopril (standard drug) at low dosage and BSSG at both low and high dosage. Serum urea and creatinine were quantified using appropriate standard methods to ascertain the integrity of the glomeruli and determine estimated Glomerular Filtration Rate (eGFR) while serum sodium, potassium, chloride, carbonate levels were assayed for spectrophotometrically to determine the extent of tubular damage and serum osmolarity. Serum alkaline phosphatase (ALP) and calcium levels were measured as markers of bone demineralisation. The effect on lipid profile was also determined, while atherogenic and coronary heart indices were calculated to determine the degree of predisposition to cardiovascular diseases. β – sitosterol glycoside in a concentration dependent manner significantly ($p = .05$) reduces renal damage markers (serum creatinine, blood urea nitrogen (BUN) and serum electrolytes (sodium, potassium, chloride and bicarbonate ions)), bone demineralisation markers (alkaline phosphatase and serum calcium ion) and markers of risk of atherosclerosis (total cholesterol, triacylglyceride and LDL - cholesterol). The results indicate that β – sitosterol glycoside elicits biological activities similar to its unconjugated form.

Keywords: Cadmium Chloride, hypertensive, renal damage, β – sitosterol glycoside, estimated Glomerular Filtration Rate (eGFR)

1. Introduction

Kidney Disease (KD) is worsen by the deteriorating effects of serum calcium and phosphate overload, atherosclerosis, vascular calcification and hypertension which resulted from progressive renal damage (Henry 1990; Moe *et al.,* 2007; Kovesdy and Kalantar – Zadeh, 2007, Ouchi and Orimo, 1990). Renal damage plays a primary role in the generation and progression of essential hypertension (Moe *et al.,* 2007). Sabanayagam *et al.,* 2013 showed an inverse continous relationship between renal function and hypertension in an epidemiological survey.

The use of drugs in the management of renal damage and associated complications has been extensively studied and documented. Recent advances have shown that nutritional and phytochemical therapy of renal damage and its complications yields better outcome. Phytosterols, which are naturally found in vegetable oils, nuts, seeds and grains have been investigated for their biochemical roles in chronic diseases. β–sitosterol showed cardioprotective and hepatoprotective effects in $CdCl_2$ – induced hypertensive rats (Olaiya *et al.,* 2013: Olaiya *et al.,* 2014). Its anti – atherogenic and antioxidative properties have also been reported (Awad *et al.,* 2001). However, there is sparcity of data in the literature on the effects of its glycoside on renal damage and associated complications like hypercalcemia and risk of atherosclerosis in hypertensive rat model. Although, it has been suggested that conjugation of β – sitosterol might be important in their bioactivity (Olaiya *et al.,* 2013) as esterification of β – sitosterol increases their solubility in the fat phase of margarines and enhances their desirable bioactivity (Katan *et al.,* 2003; Spilburg *et al.,* 2003, its amphipathic structure raises questions about the degree of solubility in intestinal bile salt micelles and reactivity with pancreatic enzymes (Olaiya *et al.,* 2013). Therefore, this study was conducted to investigate the effects of β – sitosterol glycoside (BSSG), an active phytosterol abundant in *Ficus asperifolia*, on renal damage, hyperphosphatasemia, atherosclerosis and coronary heart disease indices in Cadmium Chloride – induced Chronic Kidney Disease (CKD) male Albino rats.

2. Materials and Methods

Plant material, extraction and chemical analysis: β – sitosterol glycoside was isolated from fresh leaves of *Ficus asperifolia* according to the method described previously by Olaiya *et al.,* 2013.

Animal Treatments: Twenty – five albino rats of Wistar strain weighing 200 – 250g were procured from the Central Animal House, University of Ibadan, Nigeria. They were housed at room temperature with a 12-hour light and dark cycle, acclimatized for a week, allowed free access to clean drinking water and fed on standard feed throughout the period of study. They were seperated into four different groups according to their weight, with five animals in each group. Renal damage was induced induced by 1 mg/kg body weight $CdCl_2$ for 2 weeks (Puri, 1999) and subsequently treated by lisinopril at 1.3 mg/kg bw, low dose of BSSG (LBSSG) at 1.3 mg/kg bw and high dose of BSSG (HBSSG) at 2.3 mg/kg bw in the standard drug, LBSSG and HBSSG-treated rats respectively. Treatment lasted for additional 2 weeks. The normal and hypertensive groups still had free access to clean drinking water and were given standard feed throughout the period of study.

Biochemical assay: Urea and creatinine were quantified to ascertain the integrity of the glomeruli and determine estimated Glomerular Filtration Rate (eGFR) (NKF, 2003) while serum electrolyte (sodium, potassium, chloride, carbonate) levels were assayed for to ascertain the extent of tubular damage and serum osmolarity. Serum alkaline phosphatase (ALP) and calcium levels were used as markers of bone demineralisation. The lipid profile was also assayed as a metabolic system fuction test, while atherogenic and coronary heart indices were calculated to determine the degree of predisposition to cardiovascular diseases.

Estimation of serum calcium level was done using colorimetry technique. Serum sodium and potassium ions were estimated by Flame Emission Spectrophotometry using SEAC FP 20. Serum bicarbonate levels was estimated as described by Meites and Faulkner, (1962). Estimation of Serum Chloride was assessed by the method described by Mather *et al.,* (1982). Estimation of serum triglycerides, Total Cholesterol, HDL – Cholesterol and LDL – C holesterol levels were done by standard kit methods based on CHOD-PAP colorimetric method, and the analyses carried out following the standard protocols. Serum creatinine levels were determined according to the standard kit method of Bartels *et al.,* (1972). Serum urea levels and ALP enzyme activities were measured using standard kit methods.

Statistical analysis: Data collected were expressed as mean ± SEM and analysed using analysis of variance (ANOVA). Least Significant Difference (LSD) were used as a test of significance within treatments. p =.05 indicated statistical significance unless otherwise stated.

3. Results

Serum creatinine and blood urea nitrogen (BUN) levels and estimated GFR

The increased serum creatinine and BUN levels in the hypertensive rats was reversed significantly (p =.05) in the lisinopril, LBSSG and HBSSG - treated hypertensive rats as shown in Table 1. In addition, there was a stepwise reduction in creatinine and BUN levels in the LBSSG and HBSSG - treated hypertensive rats. This was evident in the improved eGFR of the lisinopril, LBSSG and HBSSG treated hypertensive rats when compared with the hypertensive rats.

Serum electrolyte

Table 2 shows that there was a significant (p = .001) increase in sodium and potassium levels in rats with untreated hypertensive group when compared with the control (normal rats). However, a significant (p = .001) decrease in sodium levels in the lisinopril, LBSSG, HBSSG treated hypertensive rats was observed when compared with the untreated hypertensive rats. Also a significant reduction (p=.001) in the chloride levels of Lisinopril and LBSSG treated hypertensive rats was observed in comparison with the untreated hypertensive rats. There was no significant difference (p = .001) on bicarbonate levels. An increase in the urea, sodium and potassium levels in the untreated hypertensive rats suggestively increases the osmolarity of the blood which might directly affect the water retention capacity of the body by the kidney. This impaired renal function contributes to a major extent to the hypertension condition in the $CdCl_2$ treated rats. However, treatment with the phytosterol significantly restore this renal function.

Serum alkaline phosphatase activity and calcium levels

High activity and level of serum alkaline phosphatase and calcium respectively in the hypertensive rats which was significantly reversed (p =.05) in the LBSSG and HBSSG treated hypertensive rats as shown in Table 3 suggest that the β – sitosterol glycoside could suficiently reduced and minimise the extent of bone demineralisation in rats with sufficient renal damage. In addition, a stepwise reduction in serum alkaline phosphatase activity and calcium levels in the LBSSG and HBSSG treated hypertensive rats was observed.

Lipid profile and atherosclerosis index

In Table 4, lisinopril, LBSSG and HBSSG significantly reduced (p = .05) the total cholesterol, triacylglycerides, HDL – cholesterol and LDL – cholesterol in the treated hypertensive rats when compared with the untreated

hypertensive rats. A progressive reduction was observed in the LBSSG and HBSSG treated hypertensive rats. This significant reduction in the lipid profile was evident in a stepwise reduction ($p = .05$) in the risk of formation of atherosclerotic plague in the treated hypertensive rats.

4. Discussion

Creatinine clearance test levels which has become the most popular test for measuring the eGFR is extremely useful in determinig the capacity of the glomerular to adequately perform the function of ultrafiltration in the kidney. An increase in plasma or serum creatinine levels implies impaired glomerular function. Although urea clearance test is not a measure of the glomerular filtration rate, it is an index of of overall renal function. The reduction in creatinine and BUN levels in the β – sitosterol glycoside treated hypertensive rats signifies that it could ameliorate renal damage and probably restore overall renal functions. In addition, serum electrolyte tests is commonly used as a indicator of the osmoregulatory function of the kidney. Also an increase in the serum concentration of these electrolytes signifies an increase in the osmolarity of the body fluids (Agada and Briade, 2009) and by implication the kidney sets up a system that leads to high blood pressure which if untreated results in essential hypertension and hyperthropy of the heart tissue.

A reduction in some of the electrolytes in the β – sitosterol glycoside treated hypertensive rats implies that this might be a major pathway in which it mitigate hypertension in the hypertensive rat model. Moreover, a concentration dependent increase in the protective effects of β – sitosterol glycoside in the hypertensive treated rats shows that it has little or no toxic effect on the wellness of the rats.

A build – up of cholesterol in blood vessel walls leads to atherosclerosis which increases in pathological conditions like hypertension (Shanmuganayagam *et al.,* 2007; Olaiya *et al.,* 2013). Increased level of total cholesterol, triacylglycerides and LDL – cholesterol speed up atherosclerosis and are major risk factors of cadiovascular diseases (NCEP, 2002). Also, an intense formation of atherosclerotic plagues predisposes an individual hypertension. Atherosclerosis index gives a clue to the predisposition of an individual to formation of atherosclerotic plague and risk of coronary heart diseases. A reduction in the lipid profile and atherosclerosis index by β – sitosterol glycoside suggests that this might be another pathway although not a major one in which it reduces the predisposition of an individual to hypertension.

A major event that characterises kidney diseases is bone demineralisation. Several factors are involved in bone demineralisation and low bone density in individuals with kidney disease. However, hyperphosphatasemia (increase in alkaline phosphatase active) and hypercalcemia (serum calcium overload) are indicators of bone demineralisation (Catapano, 1997). They are also factors in the calcification of atherosclerotic plagues (Kovesdy and Kalantar-Zadeh, 2008). Alkaline phosphatase catalyses the degradation of pyrophosphate (an inhibitor of calcification and bone demineralisation) (Kovesdy and Kalantar-Zadeh, 2008) thereby leading to an overload of phosphate in the blood. This overload of blood phosphate and calcium leads to the formation of hydroxylapetite which is deposited on the atherosclerotic plague and other smooth muscle cells. Uncontrolled formation and deposition of hydroxylapetite accelerate hypertension and cardiac hyperthrophy (Ouchi and Orimo, 1990). β – sitosterol glycoside reduces the activities of alkaline phosphatase and calcium levels in the hypertensive treated rats. Antihypercholesterolemic potentials of phytosterols and plant extracts had been investigated (Olaiya and Omolekan, 2013; Omolekan and Olaiya, 2013; Olaiya *et al.,* 2013: Olaiya *et al.,* 2014). In hypercholesterolemic patients, phytosterols showed 44 – 45% decrease in cholesterol (Nature life, 2005).

The results in the present study indicate that β – sitosterol glycoside elicit renoprotective, cardioprotective and osteoprotective activities irrespective of their conjugation to a glucose moiety. However, molecular studies are needed to further understand the mechanism by which β – sitosterol glycoside elicit these activities. This is of importance in the therapeutic applications of β – sitosterol glycoside in chronic diseases in which the scientific world has not found a particular and less expensive therapy for.

REFERENCES

Agada, P.O. and Braide, V.B. (2009). Effect of Dietary Garcinia Kola Seed on selected serum electrolytes and Trace Metals inmale Albino rats. *Nig J Physiol Sci* **24**: *53-57.*

Awad, A.B., Smith, A.J. and Fink, C.S. (2001). Plant sterols regulate rat vascular smooth muscle cell growth and prostacyclin release in culture. *Prostaglandins Leukot Essent Fatty Acids* **64**:*323-330.*

Bartels, H., Bohmer, M. and Heierli, C. (1972). Serum creatinine determination without protein precipitation. *Clinica Chemica Acta* **37**: *193-197.*

Catapano, A.L. (1997). Calcium antagonists and atherosclerosis: Experimental evidence. European Heart Journal

18 *{Supplement A), A80-A86.*

Henry, P.D. (1990). Calcium channel blockers and atherosclerosis. *J Cardiovasc Pharmacol* **16**: *S12-S15.*

Katan, M.B., Grundy, S.M., Jones, P., Law, M., Miettinen, T. and Paoletti, R. (2003). Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clin Proc* **78**: *965- 978.*

Kovesdy, C.P. and Kalantar-Zadeh, K. (2008). Bone and mineral disorders in pre-dialysis Chronic Kidney Disease (CKD). *Int Urol Nephrol* **40**: *427–440.*

Marther, H.M., Levin, G.E. and Nisbet, J.A. (1982). Hypomagnesaemia and ischemic heart disease in diabetes. *Diabetes Care* **5**: *452- 453.*

Meites, S. and Faulkner, W.R. (1962). Manual of Practical Micro and General Procedures in Clinical Chemistry, Charles C Thomas, Springfield, IL, pp 5-9.

Moe, S.M., Drueke, T. and Lameire, N. (2007). Chronic kidney disease-mineral-bone disorder: a new paradigm. *Adv Chronic Kidney Dis* **14**: *3–12.*

National Kidney Foundation I (2003). Kidney Disease-Dialysis Outcome Quality Initiative. K/DOQI Clinical Practice Guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis **42**: S1–S202.*
Nature Life SA. (2005). Cardiovascular Journal of South Africa 16 (3), May/June 2005.

NCEP (2002). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report: Circulation, vol 106, *pp. 3143-3421.*

Olaiya, C.O and Omolekan, T.O. (2013). Antihypercholesterolemic activity of ethanolic extract of *Buccholzia coriacea* in rats. *Afr. Health Sci*. **13**(4): *1084 – 1090. PMCID: PMC4056488*

Olaiya, C.O., Choudhary, M.I., Ogunyemi, O.M. and Nwauzoma, A.B. (2013). Nutraceuticals from Bitter Leaf (*Vernonia amygdalina* Del.) protects against cadium chloride induced hypertension in albino rats. *Nature and Science* **11** (6); *136 – 145*.

Olaiya, C.O., Esan, A.M. and Alabi, T.D. (2014). Ameliorative effects of β-sitosterol on some biochemical indices of hypertension in wistar albino rats. *African Journal of Medicine and Medical Sciences* (In Press).

Omolekan, T.O. and Olaiya, C.O. (2013). Anti–atherogenic activity of ethanol extract of *Buccholzia coriaceae* seeds on hypercholesterolemic rats. *International J. Biochem Research Rev*. **3** (3): *190 – 199.*

Ouchi, Y. and Orimo, H. (1990). The role of calcium antagonists in the treatment of atherosclerosis and hypertension. *J Cardiovasc Pharmacol* **16**: *S1-S4.*

Puri, V.N. (1999). Cadmium induced hypertension. *Clin Exp Hypertens*. **21**(1-2) : *79-84.*

Sabanayagam, C., Teo, B.W, Tai, E.S, Jafar, T.H, and Wong, T.Y. (2013). Ethnic differences in the association between blood pressure components and chronic kidney disease in middle aged and older Asian adults. BMC Nephrology **14**: 86: *1 – 11.*

Shanmuganayagam, D., Warner, T.F., Krueger, C.G., Reed, J.D. and Folts, J. (2007). Concord grape juice attenuates platelet aggregation, serum cholesterol and development of atheroma in hypercholesterolemic rabbits. *Atherosclerosis* **190**:*135–142.*

Spilburg, C.A., Goldberg, A.C., McGill, J.B., Stenson, W.F., Racette, S.B., Bateman, J., McPherson, T.B. and Ostlund, R.E. (2003). Fat free foods supplemented with soy stanollecithin powder reduce cholesterol absorption and LDL cholesterol. J Am Diet Assoc 103: *577–581*.

Table 1: Effects of Lisinopril, LBSSG and HBSSG on serum creatinine, BUN levels and eGFR of normal and hypertensive rats

Groups	Creatinine (µmol/l)	BUN (mmol/l)	eGFR (ml/min)
Control	1.57 ± 0.25	40.5 ± 12.02	31.48
Hypertensive	1.68 ± 0.05 *	46.67 ± 3.86 *	29.42
Hypertensive + lisinopril	1.35 ± 0.13 **	42.50 ± 4.95 **	36.61 **
Hypertensive + LBSSG	1.60 ± 0.20 **	36.00 ± 6.00 **	30.89 **
Hypertensive + HBSSG	1.47 ± 0.35 **, ***	27.00 ± 5.66 **, ***	33.63 **

Values expressed as means ± SEM, $n = 5$
* = statistical significant when compared with control (p = .05).
** = statistical significant when compared with untreated hypertensive group (p = .05).
*** = statistical significant when compared with hypertensive + LBSSG group (p = .05).

Table 2: Effects of Lisinopril, LBSSG and HBSSG on serum electrolytes of normal and hypertensive rats

Groups	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)	Bicarbonate (mmol/l)
Control	145.00 ± 1.00	6.77 ± 0.25	113.00 ±1.73	18.67 ± 0.58
Hypertensive	146.00 ± 2.63 *	6.43 ± 0.57 *	112.75 ± 1.80	19.75 ± 1.50
Hypertensive + lisinopril	141.75 ± 1.71 **	5.70 ± 0.77 **	108.75±2.06 **	19. 50 ± 1.20
Hypertensive + LBSSG	145.60 ± 1.60 **	6.47 ± 0.06	110.33±1.53 **	20.00 ± 0.00
Hypertensive + HBSSG	143.33 ±4.93 **, ***	6.60 ± 0.78	112.67 ± 3.06	18.00 ± 1.00

Values expressed as means ± SEM, $n = 5$
* = statistical significant when compared with control (p = .001).
** = statistical significant when compared with untreated hypertensive group (p = .001).
*** = statistical significant when compared with hypertensive + LBSSG group (p = .001).

Table 3: Effects of Lisinopril, LBSSG and HBSSG on serum alkaline phosphatase activity and calcium level of normal and hypertensive rats

Groups	ALP (IU)	Calcium (mmol/l)
Control	135.30± 25.77	7.97 ± 0.31
Hypertensive	145.00±33.22 *	8.67 ± 0.38 *
Hypertensive + lisinopril	144.10 ± 16.28	8.85 ± 0.38
Hypertensive + LBSSG	138.40±46.67 **	8.53±0.31 **
Hypertensive + HBSSG	134.30±32.67 **	8.40±0.20 **

Values expressed as means ± SEM, $n = 5$
* = statistical significant when compared with control (p = .01).
** = statistical significant when compared with untreated hypertensive group (p=.01).
*** = statistical significant when compared with hypertensive + LBSSG group (p= .01).

Table 4: Effects of Lisinopril, LBSSG and HBSSG on lipid profile and atherosclerosis index of normal and hypertensive rats

Groups	Total Cholesterol	TAG	HDL – cholesterol	LDL – Cholesterol	Atherosclerosis index
Control	56.00 ± 6.36	79.33 ± 43.66	22.67 ± 4.04	14.65 ± 5.20	2.18
Hypertensive	77.5 ± 2.12 *	125.33±18.04 *	35.00 ± 2.65 *	17.43 ± 2.39 *	2.21 *
Hypertensive + lisinopril	59.00 ± 6.93 **	85.00 ± 7.02 **	28.50±4.65 **	13.50 ± 5.79 **	2.07 **
Hypertensive + LBSSG	67.5 ± 2.12 **	105.33±6.51 **	33.00±5.57 **	13.43 ± 3.84 **	2.04 **
Hypertensive + HBSSG	60.75 ± 1.53 **	84.5 ± 0.71 **	30.5 ± 4.99 **	13.35 ± 3.26 **	1.99 **

Values expressed as means ± SEM, $n = 5$
* = statistical significant when compared with control (p = .05).
** = statistical significant when compared with untreated hypertensive group (p = .05).
*** = statistical significant when compared with hypertensive + LBSSG group (p = .05).