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COMPARATIVE EFFECTS OF THE LEAVES OF *GONGRONEMA LATIFOLIUM* AND *TELFAIRIA OCCIDENTALIS* INCORPORATED DIETS ON THE LIPID PROFILES OF RATS.

C.E. UGWU, J. E. OLAJIDE, E. O. ALUMANAH AND L. U. S. EZEANYIKA

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ABSTRACT

The hypolipidaemic effects of the leaves of *Gongronema latifolium* (GL) *and Telfairia occidentalis*(TO) diet preparations on the lipid profile of rats were compared. The rats were fed for 28 days on diet specially formulated to contain 5%, 15% and 30% by weight of the leaves of each plant respectively while the control groups were fed standard rat diet. The serum total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C) and low- density lipoprotein cholesterol (LDL-C) were determined on blood samples collected on the 28th day. The results show that at equal concentrations that the TO diet preparation induced a significantly lower total cholesterol levels than the GL diet preparation. At the 5% treatments, GL produced a significantly lower serum TGs while at the 15% and 30% treatments there were no significant differences between the effects of the two vegetable diets. For LDL-C, the results show that GL induced a significantly lower LDL-C level relative to the TO diet preparation. The results show also indicate that TO induced a significantly higher serum HDL-C level when compared to GL at the 5% and 15% concentrations. The general results from the study suggest that both GL and TO diet preparations may have equal effects on the lipid profile of wistar rats.

KEY WORDS: *Gongronema latifolium, Telfairia occidentalis*, lipid profile.

INTRODUCTION

There is evidence that hypercholesterolemia is a risk factor for cardiovascular diseases (CVD) such as atherosclerosis and myocardial infarction, which are common cause of mortality and morbidity (Wald and Law, 1995; Krieger,1998; Adaramoye *et al.,* 2008). Genetic factors and diet individually play a major role in regulating cholesterol and triacylglycerols concentrations in the plasma (Krieger, 1998). Epidemiological and metabolic studies have shown that serum cholesterol levels are strongly influenced by the amount and type of dietary fat, as well as by daily cholesterol intake (Schaefer *et al.*, 1995).

Most extrahepatic cells (with the exception of those synthesizing steroid hormones) are unable to metabolise cholesterol, which would accumulate if supply exceeded demand (Barter, 2005). High density lipoprotein-cholesterols (HDL-C) are the principal means by which excess cholesterol is removed from extrahepatic cells. HDL-C particles dispose of their load of cholesterol either by returning it directly to the liver via the scavenger receptor SR-B1 for excretion in bile or for recycling (Trigatti *et al.*, 2003) or by indirectly transferring it into the very low-density lipoproteins(VLDL) /LDL fraction in a process mediated by cholesterol ester transfer protein (CETP) (Barter, 2005).

Low-density lipoprotein cholesterol (LDL-C) has several characteristics that are linked to atherogenesis: long residence time in plasma, and enhanced oxidizability, atherial proteoglycan binding, and permeability through the endothelial barrier (Krauss, 1994; Sacks and Campos, 2003). These could lead to the hypothesis that small dense LDL is a potent atherogenic lipoprotein which can be used to improve risk prediction, and evaluate response to lipid therapy (Lamarche *etal*, 1999; Austin, 2000; Berneis and Krauss, 2002). Furthermore, LDL-C is often part of a group of high-risk characteristics including high triacylglycerols, low HDL, diabetes, insulin resistance, obesity, and the metabolic syndrome (McNamara *et al.*, 1987; Austin *et al.*,1990 and Reaven *et al.*,1993). This has led logically to the concept that it contributes to the high rate of chronic heart disease (CHD) in these groups (Sacks and Campos, 2003).

Plants have been the companions of man and formed the basis of useful drugs since they are less toxic than synthetic drugs. Screening of medicinal plants present an avenue for the discovery of new drugs. *Gongronema latifolium* (Asclepiadaceae) is a tropical rainforest plant primarily used as spice and vegetable in most Nigerian diets(Ugochukwu and Babady,2002; Ugochukwu *et al.*,2003).

Reports by various authors showed that it contains essential oils, saponins, and pregnans among others(Schneider *et al.*, 1993; Morebise and Fafunso,1998; Morebise *et al.*,2002). The leaves of *Gongronema latifolium* have protective role against diabetes, hypertension, stomach upsets and pains, and

C. E. Ugwu, Department of Biochemistry, Kogi State University, Anyigba, Nigeria.
J. E. Olajide, Department of Biochemistry, Kogi State University, Anyigba, Nigeria.
E. O. Alumanah, Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
L. U. S. Ezeanyika, Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

typhoid fever (Etim *et al.*,2008). *Telfairia occidentalis* (Cucurbitacea) leaves and young shoots are frequently eaten as a potherb (Okigbo, 1977; Okoli and Mgbeogu, 1983). The root and leaves have been shown to contain highly toxic alkaloids and saponins (Alada, 2000). In Nigeria, the herbal preparation of the plant has been employed in the treatment of sudden attack of convulsion, malaria, and anaemia (Gbile, 1986). Based on the pharmacological functions of the leaves of *Gongronema latifolium* and *Telafiria occidentalis* the present study was designed to compare the effects of the diet preparations of the leaves of the two plants on the lipid profiles of rats.

MATERIALS AND METHODS

Animals

Twenty five male albino rats (Wistar strain) weighing between 93-120g were used as experimental animals. The rats were kept in cages for two weeks to acclimatize, and were allowed free access to food and water *ad libitum*. The protocol is in line with the guidelines of the National Institute of Health (NIH) (NIH Publication 85-23, 1985) for laboratory animal care and use.

The experimental animals were randomly divided into four groups of five animals each. Group 1 rats were fed on standard rat diet (Vital feeds, Nigeria), as control, while groups 2,3,4 were fed on diets containing 5%, 15% and 30% by weight of *Gongronema latifolium* or *Telfairia occidentalis* leaves respectively for 28 days.

Feed formulation

The leaves of *Gongronema latifolium* and *Telfairia occidentalis* were purchased from a local market in Anyigba, Kogi state, Nigeria. The plants samples were collected on September, 2007. The botanical identification and authentication was confirmed at the Department of Biological Sciences, Kogi State University, Anyigba. The leaves were dried at room temperature for 2 weeks to a constant weight and then powdered. The standard rat diet was similarly milled. The feed for each leaf type was mixed to contain 5%, 15% and 30% by weight of the leaves for groups 2, 3, and 4 respectively.

Sample collections.

The animals were starved of food over night prior to treatment. After treatment blood samples were collected from the ocular median-cantus vein of the rats with the aid of capillary tubes and transferred to test tubes and allowed to clot and subsequently centrifuged to obtain the serum component used for lipid analysis.

Lipid analysis.

The lipid profiles were determined using kits manufactured by TECO Diagnostics, Lakeview Ave, Anaheim, CA, USA. Serum total cholesterol (TC) was determined by the method of Aliain *et al.*,(1974) while triacylglycerols was determined by the method of Burstein *et al.,*(1980). The lipoproteins, VLDL and HDL were precipitated using phosphotungstic acid and magnesium chloride. After centrifugation, the supernatant contained the high-density lipoprotein cholesterol (HDL-C) fraction which was assayed for cholesterol by the method of Grove (1979). The low-density lipoprotein cholesterol (LDL-C) was calculated using the method of Friedewald *et al.*, (1972).

STATISTICAL ANALYSIS

Data collected were subjected to analysis of variance (ANOVA) using the paired T- test. The mean ± SD of each parameter was taken for each group. Test probability value of $P< 0.05$ was considered significant. The analysis was carried out on SPSS for windows version 10.

RESULTS

Table1: Effects of *Gongronema latifolium* and *Telfairia occidentalis* diet preparations on the serum total cholesterol and triacylglycerols (mg/dl).

	Total cholesterol(mg/dl)		**Triacylglycerols(mg/dl)**	
	Gongronema latifolium	***Telfairia occidentalis***	***Gongronema latifolium***	***Telfairia occidentalis***
Control	133.80±5.95	133.80±5.95	150.73±6.8	150.73±6.8
5%	120.30±7.04^{*a}	115.55±4.89^{*b}	141.00±4.53^{*a}	144.75±9.62^{*b}
15%	116.06±2.07^{*a}	112.50 ±6.29^{*b}	132.12±4.44^{*a}	133.93±3.13^{*a}
30%	110.94±5.09^{*a}	105.10±9.32^{*b}	125.53±3.46^{*a}	127.00±7.72^{*a}

Results are mean ±SD. For the same parameter, values with different alphabetical superscripts in a row are significant with respect to each other ($P<0.05$). Asterisked values (*) in a column are significant with respect to the control ($P<0.05$).

Table 2: Effects of *Gongronema latifolium* and *Telfairia occidentalis* Diet Preparations on the Serum LDL- C and HDL-C (mg/dl).

	LDL-C(mg/dl)		HDL-C(mg/dl)	
	Gongronema latifolium	***Telfairia occidentalis***	***Gongronema latifolium***	***Telfairia occidentalis***
Control	127.33±5.64	127.33±5.64	22.13±2.49	22.13±2.49
5%	123.35±8.59^{*a}	120.00±2.68^{*b}	25.85±4.46^{*a}	29.40±5.78^{*b}
15%	119.20±5.03^{*b}	115.30±3.68^{*b}	31.78±2.76^{*a}	35.85±3.48^{*b}
30%	114.36±1.76^{*a}	110.65±5.34^{*b}	36.85±2.77^{*a}	37.50±8.78^{*a}

Results are mean ±SD. Values with different alphabetical superscripts in a row are significant with respect to each other (P<0.05). Asterisked values (*) in a column are significant with respect to the control (P<0.05).

The effect of *Gongronema latifolium* and *Telfairia occidentalis* diet preparations on the serum total cholesterol and triacylglycerol concentrations are shown in Table 1. The results show that the two diet preparations decreased the serum total cholesterol and triacylglycerols levels in a concentration related manner. The decrease was significant compared to the control (P<0.05) in both *Gongronema latifolium* and *Telfairia occidentalis* diets preparations. At equal concentrations the results show that the *Telfairia occidentalis* diet induced a significantly lower serum total cholesterol levels(P<0.05).

The result of the effect of *Gongronema latifolium* and *Telfairia occcidentalis* diets on the serum triacylglycerols is shown in Table 1. The results showed decreased serum triacylglycerols values in concentration related fashion. The decrease in the serum value was significantly lower than the control (P<0.05) in both cases. At the 5% treatments *Gongronema latifolium* produced a significantly lower (P<0.05) serum triacylglycerols than *Telfairia occidentalis* while at the 15%, 30% treatments there were no significant differences between the effects of the two vegetables (P>0.05).

Table 2 depicts the effects of the two vegetable diets on the serum LDL-C and HDL-C concentrations. There was a significant decrease in the serum LDL-C concentrations at the various levels of treatments relative to the control (P<0.05). The results show that *Gongronema latifolium* induced a significant decrease in the LDL-C level relative to the effect of *Telfairia occidentalis* at the 30% concentration while at the 15% concentration there was no significant decrease between the two plant diets. The results also showed a significant (P<0.05) increase in the serum HDL-C values following the effects of the *Gongronema latifolium* and *Telfairia occidentalis* diet preparations (Table 2). The results also indicate that *Telfairia occidentalis* induced a significantly higher serum HDL-C when compared to *Gongronema latifolium* at the 5% and 15% concentrations while at the 30% concentration there was no significant difference (P>0.05) between the effects of the two plants.

DISCUSSION

The results of this study clearly show that the administration of the diet preparations of *Gongronema latifolium* and *Telfairia occidentalis* produced hypolipidaemic effects on rats. There are many bioactive phytochemicals in the leaves of these vegetables and at present, it is not certain, which of them is/ are responsible for the observed effects. Reports have shown that flavonoids, tannins, and saponins may play some roles in hypolipidaemic effect (Ezekwe and Obidoa, 2001; Nwanjo, 2005; Yuldasheva *et al.*, 2005). In this study the hypolipidaemic effects of the two diet preparations were compared and it was observed that *Telfairia occidentalis* elicited a greater effect than *Gongronema latifolium.* Adaramoye *et al.*, (2007), has reported that *Telfairia occidentalis* has hypolipidaemic effect in rats fed on cholesterol rich diet while Ugochukwu *et al.,* (2003) reported that *Gongronema latifolium* leaf extract exhibited cholesterol lowering effect. The hypocholesterolaemic effects of *Telfairia occidentalis* and *Gongronema latifoliun* may be due to inhibition of the absorption of the cholesterol in the intestine or its production by the liver (Menendez *et al.*, 1997) or the stimulation of the biliary secretion of cholesterol and cholesterol excretion in the faeces(Ahmad-Raus *et al.*, 2001). Furthermore, flavonoids and saponins elicit hypolipidaemic activity owing to a lower rate of lipogenesis and higher rate of degradation (Price *et al.*, 1987; Koshy *et al.*, 2001).

Dietary cholesterol has been shown to reduce fatty acid oxidation, which, in turn, increases the levels of hepatic and plasma triacylglycerols (Fungwe *et al.*, 1993). The result shows that *Gongronema latifolium* diet preparation has a higher triacylglycerol lowering effect than *Telfairia occidentalis* at 5% level of incorporation. There is consistent evidence that raised levels of triacylglycerols are associated with coronary atherosclerosis (Cullen, 2003). Evidence for an association with other forms of atherosclerotic disease is much less certain. Triacylglycerols enter the walls of the coronary artery and adhere to proteoglycans, either directly or via the enzyme lipoprotein lipase, which acts as molecular tether (Cullen, 2003). One of the most important factors in the development of cardiovascular diseases (CVD) is low-density lipoprotein cholesterol (LDL-C) (Kwiterovich, 1997; Barter, 2005).

The entry of LDL-C into the artery walls begins a cycle that both commences artherosclerosis and leads

to its progression. The cholesterol required by the peripheral tissues, including vascular cells, is provided both by new synthesis in the cells and by a delivery from low-density lipoproteins (LDL). When the level of LDL-C is high, they accumulate in the artery walls where they are oxidized and taken up by foam cells in a process that leads to the development and progression of atherosclerosis (Philip, 2007). Low levels of high-density lipoprotein-cholesterol (HDL-C) is a crucial risk factor for CVD (Assmann and Gotto, 2004). Because low levels of HDL-C plays a direct role in the development of atherosclerosis, therapeutic intervention to raise HDL-C is encouraged (Philip,2007). The results of this study indicate that both the *Gongronema latifolium* and *Telfairia occidentalis* diet preparations induced a significantly higher serum HDL-C levels relative to the control. The treatment with *Telfairia occidentalis* however induced a significantly higher serum levels of HDL-C at lower concentrations relative to the effects of *Gongronema latifolium* diet.

HDL particles dispose of their load of cholesterol either by returning it directly to the liver scavenger receptor SR-B1 for excretion in the bile or for recycling (Trigatti *et al*., 2003) or by indirectly transferring it to the very low density lipoprotein / low-density lipoprotein fraction in a process mediated by cholesteryl ester transfer protein (CETP) (Barter *et al*., 2003). Therefore, HDLs oppose atherosclerosis directly, by removing cholesterol from foam cells, by inhibiting the oxidation of LDLs, and by limiting the inflammatory processes that underlie atherosclerosis.

On the basis of this result, *Telfairia occidentalis* and *Gongronema latifolium* may perform anti-atherogenic role through their elevation of the serum HDL-C levels. In conclusion, evidence from this study suggests that *Gongronema latifolium* and *Telfairia occidentalis* have lipid lowering effects which could be beneficial to people at risk of CVD. They may elicit their health benefits through the modulation of physiologic functions including the atherogenic lipid profile. The general results from the study suggest that both *Gongronema latifolium* and *Telfairia occidentalis* diet preparations may have equal effects on the lipid profile of wistar rats.

REFERENCES

Adaramoye, O. A., Achem, J., Akintayo, O. O. and Fafunso, M. A., 2007. Hypolipidemic effect of *Telfairia occidentalis* (Fluted pumpkin) in rats fed a cholesterol rich diet. J. Med. Food. 10(2): 330-336.

Adaramoye, O.A., Akintayo, O., Achem, J. and Fafunso, M.A. 2008. Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed high cholesterol diet. Vascular Health and Risk Management. 4(1):235-241.

Ahmed-Raus, R.R., Abdul-Latif, E.A., Mohammed, J.I., 2001. Lowering of lipid composition in aorta of Guinea Pigs by *Curcuma domestica*. BMC Complement Altern. Med. (1) 6.

Alada, A. R. A., 2000. The haematological effect of *Telfaira occidentalis* diet preparation. African Journal of Biomedical Research. 3(20): 185-186.

Aliain, C.C., Pon, L.S., Chan, C.S.G., Richmond, W and Wu, P.C., 1974. Enzymatic determination of total cholesterol. Clin. Chem.20: 470-475.

Assmann, G. and Gotto, A.M. Jr., 2004. HDL-cholesterol and protective factors in atherosclerosis. Circulation. 109: 1118-11114.

Austin, M.A., King, M.C., Vranizan, K.M. and Krauss, R.M., 1990. Atherogenic lipoprotein phenotype: a proposed genetic marker for coronary heart disease risk. Circulation. 82:495-506.

Austin, M.A., 2000. Triglyceride, small, dense low-density lipoprotein, and the atherogenic lipoprotein phenotype. Curr. Atheroscler. Rep. 2: 200-207.

Barter, P.J., Brewer, H.B. Jr, and Chapman, M.J. *et al*., 2003. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. *Arterioscler Thromb Vasc Biol* .23:160–167.

Barter, P., 2005. The role of HDL-cholesterol in preventing atherosclerotic disease. European Heart Journal Supplements.7 (Supplement F) F_{4-} F_8.

Berneis, K.K. and Krauss, R.M., 2002. Metabolic origins and clinical significance of LDL heterogeneity. Journal of Lipid Research. 43: 1363-1379.

Burstein, M., Schnolnic, H.R. and Marlin, R., 1980. Rapid method for the isolation of lipoprotein from human serum by precipitation with polyanions. Scan. J. Clin. Lab. Invest. 40: 583-595.

Cullen, P., 2003. Triacylglycerol-rich lipoproteins and atherosclerosis- where is the link? Bio. Soc. Trans. (31): part 5: 1080-1083.

Etim, O. E., Akpan, E.J. and Usoh, I.F., 2008. Hepatoxicity of carbon tetrachloride: protective effect of *Gongronema latifolium*. Pak. J. Pharm. Sci. Vol. 21(3): 268-274.

Ezekwe, C.I. and Obidoa, O., 2001. Biochemical effect of *Vernonia amygdalina* on rats liver microsomes. Nigerian Journal of Biochemistry and Molecular Biology. 16(3):1745-1798.

Friedwal, W.T., Levy, R.I. and Fredrickson, D.S., 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem.. 18: 499-502.

Fungwe, T, V., Cagen, L.M., Cook, G.A., Wilcox, H.G. and Heimberg, M., 1993. Dietary cholesterol

stimulate biosynthesis of triglyceride and reduces oxidation of fatty acids in rats. J. lipid Res. (34); 933-941.

Gbile, Z.O., 1986. Ethnobotany, taxonomy and conservation of medicinal plant. In: the state of medicinal plant research in Nigeria. Sofoworo,A.O.(ed.), Ife University press. Pg 19.

Grove, T.H., 1979. Effect of reagent pH on determinationof high density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. Clin. Chem.. 25: 560-564.

Koshy, A.S., Anila, L., and Vijayalakshmi, N.R., 2001. Flavonoids from *Garcinia camgogia* lowers lipid levels in hypercholesterolemic rats. Food Chemistry. 72: 289-294.

Krauss, R.M., 1994. Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk. Curr. Opin. Lipidol. 5: 339-349.

Krieger, M., 1998. The 'best' of cholesterols, the 'worst' of cholesterols: A tale of two receptors. Proc. Natl. Acad. Sci. USA. 95: 4077- 4080.

Kwiterovich, P.O. Jr., 1997. The effect of dietary fat, antioxidants, and proxidants on blood lipids, lipoproteins, and atherosclerosis. J. Am. Diet. Assoc. 97: 531-541.

Larmarche, B., Lemieux, I. and Despres, J.P., 1999. The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, patho-physiology, and therapeutic aspects. Diabetes Metab. 25: 199-211.

McNamaara, J.R., Campos, H., Ordovas, J.M., Peterson, J., Wilson, P.W.F. and Schaefer, E.J., 1987. Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution. Results of the Framingham offspring study. Atherosclerosis. 7: 483-490.

Menendez, R., Arruzazabala, L. and Mas, R., 1997. Cholesterol-lowering effect of poliscosanol on rabbit with hypercholesterolemia induced by a wheat-starch casein diet. Br. J. Nutr. 77: 923-33.

Morebise, O. and Fafunso, M.A., 1998. Antimicrobial and phytotoxic activities of saponins extracts from two Nigeria edible medicinal plants. Biokemistri. 8:(2):69-77.

Morebise, O., Fafunso, M.A., Makinde, J.M., Olajide, O.A. and Awe, E.O., 2002. Antiinflamatory property of the leaves of *Gongronema latifolium*. Phytother. Res. 16(51): s75-s77.

Nwanjo, H. U., 2005. Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. Nigerian Journal of Physiological Sciences. 20: (1-2): 39-42.

Okigbo, B.N., 1977. Neglected plants of horticultural and nutritional importance in traditional farming systems of tropical Africa. Acta. Hortic. 55: 131.

Okoli, B.E. and Mgbeogu, C.M., 1983. Fluted pumkin (*Telfaira occidentalis*). West Africa Veg. crop. Eco. Botany. 37(2):145.

Philip, B.M.D., 2007. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular event. New England Journal of Medicine. 357: 1301-1310.

Price, K.K., Johnson, L.I. and Feriwick, H., 1987. The chemical and biological significance of saponins in foods and feeding stuffs. CRC Crit. Rev. in Food Sci. and Nutr. 26: 127-135.

Reaven, G.M., Chen, Y.D., Jeppesen, J., Maheux, P. and Krauss, R.M., 1993. Insulin resistance and hyperinsulinemia in individuals with small dense low density lipoprotein particles. Journal of Clinical Investigation. 92: 141-146.

Sacks, F.M. and Campos, H., 2003. Low-density lipoprotein, size and cardiovascular disease: A reappraisal. Journal of Clinical Endocrinology and Metabolism. 88: 104525- 104532.

Schaefer, E.J., Lichtenstein, A.H., Lamon-Fava, S., McNamara, J.R., and Ordovas., 1995. Lipoproteins, nutition, aging and atherosclerosis. American Journal of Clinical. Nutrition. 61: (Supplement 3): 726S-740S.

Schneider, C., Rotscheidt, K. and Breitmaimeri, E., 1993. Four new pregnane glycosides from *Gongronema latifolium* (Asclpidaeceae). Annalen Der Chemie. 10: 1057-1062.

Trease, G.E. and Evans, W.C., 1983. Textbook of Pharmacognosy. 12th edition. Balliese Tindall and Company publishers, London. Pp 343-368.

Trigatti, B.L., Krieger, M. and Rigotti, A., 2003. Influence of the HDL receptor SR-BI on lipoprotein metabolism and atherosclerosis. *Arterioscler Thromb Vasc Biol* 23:1732–1738.

Ugochukwu, N.H. and Babady, N.E., 2002. Antioxidant effects of *Gongronema latifolium* in hepatocytes of rat models of non-insulin dependent diabetes mellitus. Fitoterapia. 73(7-8):612-618.

Ugochukwu, W.H., Babady, N.E., Coburne, M. and Gasset, S.R., 2003. The effect of *Gongronema latifolium* leaf extract on serum lipid profile and oxidative stress of hepatocytes of diabetic rats. J. Biosci. 28(1): 1-5.

Wald, W.J. and Law, M.A., 1995. Serum cholesterol and ischaemic heart disease. Atherosclerosis. 118:1-5.

Yuldasheva, L.N., Carvalho, E.B., Catanho, M.T.J.A. and Krasilnikov, O.V., 2005. Cholesterol-

dependent hemolytic activity of *Passiflora quadrangularis* leaves. Brazilian Journal of Medical and Biological Research. 38(7): 1061-1070.