

Full Length Research Paper

Administration of *Hydrocotyl bonariensis* aqueous leaves extract regulates cardiovascular disease risk factors in galactose model of experimental cataractogenesis

Ajani, Emmanuel O.^{1*}, Salau, Bamidele A.², Adebayo, Olusegun L.², Adegbesan, Bukola O.³ and Odufuwa, Kuburat T.³

¹Department of Biochemistry, Faculty of Science and Science Education, Bowen University, Iwo, Osun State, Nigeria.

²Chemical Sciences Department, Redeemers University km46/48 Lagos Ibadan Express Road, Redemption City, Mowe, Ogun State, Nigeria.

³Department of Biochemistry, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

Accepted 26 March, 2012

Reports had indicated that *Hydrocotyl bonariensis* contains phytochemicals that can be exploited for the development of anticataractogenic agent. In the present study we investigated the effects of administration of the plant's leaves extract on some cardiovascular disease (CVD) risk factors in galactose model of experimental cataract. Twenty five (25) weanling rats randomly assigned to five groups were used in the study. Aqueous extract of *H. bonariensis* at 500 and 1000 mgKg⁻¹ were administered to two different groups of the rats placed on galactose diet. Plasma lipid profile, fibrinogen and platelet count were then compared after 4 weeks treatment period between these groups and with the baseline, normal and test control groups. Result of the study indicates that galactose diet elicit significant increase in the plasma cholesterol, triglyceride and artherogenic risk index (AI) whereas simultaneous administration of the extract significantly ($p<0.05$) lowers the parameters. Fibrinogen and platelet count were also significantly ($p<0.05$) reduced in rats placed on galactose diet when administered with the extract. The result also suggests that the cardio protective effect of the extract was more pronounced at lower dose administration of the extract. The study indicates that pretreatment with *H. bonariensis* will reduce predisposition to CVD associated with cataract.

Key words: Cardiovascular disease, cataract, lipid, phytomedicine, galactose.

INTRODUCTION

Cardiovascular disease (CVD) is the major cause of morbidity and mortality in adults hence, much attention is being focused on control of cardiovascular risk in patients on therapeutics (Lee et al., 2004; Lee et al., 2003; Ahn et al., 1998; Coreps et al., 1990). A physician may order a lipid profile as part of an annual exam or if there is

specific concern about CVD, especially coronary artery disease. A lipid profile measures total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides. Many studies have shown that increased lipid levels such as triacylglycerols, total cholesterol and LDL-C or HDL-C are associated with an increased risk of CVDs (Lee et al., 2001; MacRury et al., 1993).

Cataract is leading cause of opacity worldwide (Gabriel et al., 2009). Till date, the only known cure for cataract is surgery (Mohammed et al., 2009), hence, the ongoing

*Corresponding author. E-mail: immanbisi@yahoo.com. Tel: +2348055533192.

Table 1. Effect of treatment on plasma cholesterol profile and atherogenic risk.

Group/Treatment	Plasma cholesterol profile (mg/dl)				Atherogenic index (AI)
	Total cholesterol	Triglyceride	HDL-C	LDL-C	
A (Baseline)	82.18 ± 3.01 ^a	68.91 ± 4.23 ^a	48.14 ± 2.31	51.13 ± 7.23 ^{abc}	0.71
B (Control diet + normal control)	81.49 ± 5.61 ^a	68.40 ± 4.34 ^a	46.65 ± 4.82	48.52 ± 2.31 ^{ac}	0.75
C (Dietary galactose + normal saline)	130.62 ± 7.89 ^b	85.21 ± 3.42 ^b	42.15 ± 1.24	62.34 ± 4.20 ^b	2.10
D (Dietary galactose + 500 mgKg ⁻¹ extract)	98.82 ± 8.32 ^a	52.53 ± 6.45 ^c	50.65 ± 4.15	46.59 ± 2.70 ^c	0.95
E (Dietary galactose + 1000 mgKg ⁻¹ extract)	114.45 ± 10.52 ^c	63.54 ± 4.10 ^a	53.41 ± 4.33	56.72 ± 1.12 ^{ad}	1.14

Note: Results presented are mean ± SEM (n=5). Values in the same column with different superscripts are significantly different from each other (p<0.05).

investigation to identify plants with anticataract properties. *Hydrocotyl bonariensis* Comm. Ex Lam., araliacea (largeleaf pennywort) is an herbaceous plant with prostate, creeping or floating stems, roots forming at nodes. Its common name in Nigeria, West Africa, is Karo. In our previous investigation, *H. bonariensis* leaves extract was reported to possess significant ability to prevent lens opacification in galactose induced cataract (Ajani et al., 2009). For effective exploration of this extract as phytomedicine and/or in the development of it as a base for anticataractogenic agent, it is important to investigate the possible CVD risk associated with its administration.

MATERIALS AND METHODS

Plant authentication and extraction

Mature fresh leaves of *H. bonariensis* were collected from a local garden and were authenticated at the herbarium of the Botany Department, University of Lagos, Nigeria. The herbarium voucher number is 13478. The leaves were then oven dried at 40°C for 24 h. They were blended using a local kitchen blender, and 240 g of the blended leaves were soaked in 500 ml of water at room temperature for 48 h. The extract was then sieved into a clean container and further concentrated using a rotary evaporator at 40°C. The concentrated product was then lyophilized. The yield of the extract was 5.8%.

Experimental design and dietary regimen

Twenty five (25) male Wistar albino rats (21 days old) having an average body weight of 30 g, bred in the animal house, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Remo Campus, Ikenne, Nigeria, were used in the study. The rats were randomized into five groups of five rats each and treated as follows;

Group A: Rats in this group were sacrificed before the commencement of treatment (baseline); Group B: Rats were fed with a normal stock diet based on the AIN-93 (Reeves, 1997) formula; Group C: Rats were fed with 30% galactose in the above diet;

Group D: Rats in this group received the Group C diet and were administered with 500 mgkg⁻¹ extract;

Group E: Rats in this group received the Group C diet and was administered with 1000 mgkg⁻¹ extract.

Animals were housed in individual cage in a temperature and humidity controlled room, having a 12 h light and dark cycle. All the animals had access to their respective feed and clean drinking water *ad libitum*. The treatment was carried out for 4 weeks after which food was withdrawn from the animals overnight. Blood was then withdrawn by cardiac puncture after diethyl ether anaesthesia. The care of the animals was in accordance with the U.S. Public Health Service Guidelines (NRC, 1999).

Biochemical analysis

Triglyceride levels were determined using the Triglyceride GPO-PAP kit (Roche, SA) (Stein and Myers, 1995). Cholesterol CHOD-PAP kit (Roche, SA) was used for total cholesterol determination (NIH, 1990). Determination of HDL-C was done using the HDL-C precipitant (Roche, SA) on the same pooled samples (Lopes-Virella et al., 1977). LDL-C was calculated by difference using Friedwald formula. Atherogenic risk index (AI) was calculated using the formula of Abott et al. (1988). Fibrinogen was estimated based on clotting by thrombin physicochemical transformation. Platelet count was done microscopically.

Statistical analysis

Data analyses were performed using SPSS software (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL). All data are expressed as mean ± SEM. Analysis of variance was used to test for differences between the groups. Duncan's multiple range test was used to determine the significance of differences among the mean values at the level of P ≤ 0.05 (Sokal and Rohlf, 1969).

RESULTS

Dietary galactose was observed in the study to significantly increase the plasma total cholesterol and triglyceride level from the normal control values of 81.49 ± 5.61 and 68.40 ± 4.34 to 130.62 ± 7.89 and 85.21 ± 3.42 mgdl⁻¹, respectively (Table 1). No significant alteration was however observed in the HDL-C and LDL-C values. Simultaneous administration of the extract at the two tested doses lowered the total cholesterol and

Table 2. Effect of treatment on plasma fibrinogen and platelet count.

Group/ Treatment	Plasma fibrinogen ($\times 10^9/L$)	Platelet count ($\times 10^9/L$)
A (Baseline)	3.44 ± 0.50^a	4.51 ± 0.03^a
B (Control diet + normal control 1)	3.48 ± 0.20^a	4.49 ± 0.03^a
C (Dietary galactose + normal saline)	6.56 ± 0.50^b	8.27 ± 0.11^b
D (Dietary galactose + 500 mg/Kg extract)	3.34 ± 0.62^a	4.81 ± 0.22^a
E (Dietary galactose + 1000 mg/Kg extract)	3.91 ± 0.01^a	6.59 ± 0.30^c

Note: Results presented are mean $\pm$ SEM (n=5). Values in the same column with different superscripts are significantly different from each other ($p < 0.05$).

triglyceride level. The observed triglyceride level at 500 mgKg⁻¹ dose extract administration was lowered than the pre-treatment value. No significant alteration was observed in the HDL-C level with the extract administration at the two different dosages. An increased AI was observed with dietary galactose whereas simultaneous administration of the extract lowers the AI to the pretreatment value.

Table 2 shows the result of treatment on total plasma fibrinogen content and platelet count. The Table indicates that dietary galactose induced a significant increase ($p < 0.05$) in plasma fibrinogen level from the normal control value of $3.48 \pm 0.20 \times 10^9/L$ to $6.56 \pm 0.50 \times 10^9/L$. The observed value was also higher than the baseline value of $3.44 \pm 0.50 \times 10^9/L$. Administration of the extract to rats placed on dietary galactose prevented the increase in fibrinogen level. The observed fibrinogen level of $3.34 \pm 0.62 \times 10^9/L$ in rats treated with 500 mgkg⁻¹ dose and $3.91 \pm 0.01 \times 10^9/L$ in those treated with 1000 mgkg⁻¹ dose of the extract did not differ between each other. Whereas administration of *H. bonariensis* extract at 500 mgKg⁻¹ was observed to prevent increased in platelet count in the rats placed on dietary galactose, the observed platelet count at 1000 mgKg⁻¹ dose was however higher than the pre treatment level.

DISCUSSION

A CVD risk factor that has been implicated in some forms of cataract is fibrinogen (Goodrich et al., 1999). High fibrinogen level can hinder blood flow by encouraging atheroma formation and platelet aggregation as well as by increasing plasma viscosity. Goodrich et al. (1999) noted that increasing plasma fibrinogen may in some way lead to impaired nutrient supply to the lens. Findings from this study revealed that increased dietary galactose induce increased plasma fibrinogen level; we thus opined that this may be an important factor in the lens becoming cataractous in individual placed on high dietary galactose. Our result also suggests that increased dietary galactose may predispose to CVD. This is consistent with previous report of an increased association between cataract development and CVD (Girao et al., 1999). The present

study also indicates that administration of *H. bonariensis* leaf extract prevented increase in fibrinogen content associated with high dietary galactose. This thus suggests that the extract may help prevent CVD risk that may be associated with lens opacity. Platelet has been noted to contain significant amounts of various plasma coagulation factors. Increased platelet production has thus been reported in thrombocytopenia attributable to accelerated platelet destruction (Goodrich et al., 1999). The present study indicates that dietary galactose increased platelet production and that this can be prevented with simultaneous administration of *H. bonariensis* extract.

Literature has shown that there is a positive correlation between the risk of developing ischemic heart disease and raising plasma cholesterol and LDL-C concentrations and a negative one with raising plasma HDL-C. High levels of total cholesterol and LDL-C are major risk factors for coronary diseases, whereas increased HDL-C is associated with a decrease in coronary disease risk (Wilson, 1990). Elevated triacylglycerols have also been reported to increase the incidence of coronary heart disease (Bainim et al., 1992). Pitsin et al. (1986) and Iams and Wexler (1977) reported increased serum triacylglycerol levels in diabetic patients and rats. The significant increase in total cholesterol, triglyceride and LDL-C with increased dietary galactose observed in this study suggests that dietary galactose may predispose to increased CVD risk. Our study also suggests that administration of *H. bonariensis* leaf extract in individual placed on increased dietary galactose may reduce the incidence of CVDs. This may be a significant contribution to the reduction of lens opacity in such individual (Ajani et al., 2009; Noorouz-Zadeh, 1997).

Because increased plasma cholesterol, particularly that portion associated with LDL, is an established risk factor for coronary heart disease (Brown, 1994; Stamler et al., 1993); it is widely recommended (Lee et al., 2001) that LDL-C be determined in individuals with increased total cholesterol. Findings from this study indicate that whereas increased dietary galactose increased LDL-C concentration, administration of *H. bonariensis* leaf extract prevented the increase.

In a previous study (Ajani et al., 2011), we reported on

the phytochemical components of aqueous extract of *H. bonariensis* leaves. Results of the present study also suggest that *H. bonariensis* contain phytochemicals that may not only reduce lens opacity, but also prevent CVDs diseases that may be associated with cataract development.

Conclusion

Results obtained in this study suggest that lens opacity occasioned by increased dietary galactose may be associated with an increased CVD risk and that aqueous extract of *H. bonariensis* is efficacious in reducing this risk.

REFERENCES

- Abott RD, Wilson PW, Karvel WB, Carsel WP (1988). HDL- cholesterol, total cholesterol screening and myocardial infarction. *Arteriosclerosis*, 8: 207-211.
- Ahn HS, Park SY, Kim HR (1998). Effects of vitamin E supplementation on renal glycosylation products in diabetic KK mice. *Korean J. Nutr.* 31(6): 1024 -1030.
- Ajani EO, Salako AA, Salau BA, Shallie PD, Akinleye WA, Adeoye AO, Adebawo OO (2009). Chemopreventive and remediation effect of *Hydrocotyl bonariensis* comm.. ex. Lam (apiacea) leave extract in galactose induced cataract. *J. Ethnopharma.*, 123: 134-142.
- Ajani EO, Salau BA, Adegbesan BO, Ogunsola MO, Adenuga GA (2011). Inhibitory effect of aqueous and organic extracts of *Hydrocotyl bonariensis* (Largeleaf Pennyworth) on Rat Lens Aldose Reductase Activity. *Arch. App. Sci.*, 2: 12-21.
- Bainim D, Miller NE, Botton CH, Yarnell JWG, Suretman PM, Baker IA (1992). Plasma triglyceride and high density lipoprotein cholesterol as predictors of ischaemic heart disease in British men. *Br. Heart J.*, 68: 60-66.
- Brown WV (1994). Lipoprotein disorders in diabetes mellitus. *Med. Clin. North Am.*, 78: 143- 161.
- Coreps ET, Llambias EBC, Grinstein M (1990). Studies on regulatory mechanisms of heme biosynthesis in hepatocytes from normal and experimental-diabetic rats. Role of insulin. *Biochem. Cell Biol.*, 68: 914-921.
- Gabriel AC, Silvana AS, Rodrigo F, Flávia PL, Antônio CLR, Carlos RP (2009). Cataract prevalence in Central-West region of São Paulo State, Brazil *Arq Brazilian Ophthalmology*. 72: 13-16.
- Girao H, Mota M C, Ramaho C, Pereira P (1999). Cholesterol oxides in human cataractous lens. *Exper. Eye Res.*, 8: 645-652.
- Goodrich ME, Cumming RG, Mitchel P, Koutts J, Burnett L (1999). Plasma fibrinogen and other cardiovascular disease risk factors and cataract. *Ophthal. Epidem.*, 6: 279-290.
- Iams SG, Wexler BC (1977). Alloxan diabetes in spontaneously hypertensive rats: gravimetric, metabolic and histopathological alterations. *Br. J. Exp. Pathol.*, 58: 177- 199.
- Lee JS, Kawakubo K, Kobayashi Y, Mori K, Kashira H, Tamura M (2001). Effect of ten year body weight variability on cardiovascular risk factors in Japanese middle- aged men and women. *Inter. J. Obes.*, 25: 1063-1067.
- Lee SH, Park HJ, Chun HK, Rhie SG, Lee YS (2003). Supplementary effect of the rice germ oil on lipid metabolism in diabetic KK mice. *Kor. J. Comm. Liv. Sci.*, 14(3): 59 - 65.
- Lee SH, Park HJ, Cho SY, Han GJ, Chun HK, Hwang HG (2004). Supplementary effect of the high dietary fiber rice on lipid metabolism in diabetic KK mice. *Korean J. Nutr.*, 37(2): 81 -87.
- Lopes-Virella MF, Stone P, Ellis S, Colwell JA (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin. Chem.*, 23(5): 882-884.
- MacRury SM, Gordon D, Wilson R, Bradley H, Gemmell CG, Paterson JR (1993). Comparison of different methods of assessing free radical activity in type 2 diabetes and peripheral vascular disease. *Diabet. Med.*, 10: 331-335.
- National Research Council. (1999). Guide for the care and use of laboratory animals. 2nd ed. Washington DC, Nat. Acad. Pres., pp. 54-63.
- NIH (1990). Recommendations for improving cholesterol measurement: A report from the laboratory standardization panel of the National Cholesterol Educ. Program., 90-2964.
- Noorouz-Zadeh J, Rahimi A, Tajaddini-Sarnadi J, Tritschler H, Rosen P, Halliwell B (1997). Relationship between plasma measures of oxidative stress and glycemic control in NIDDM. *Diabetologia*. 40: 647- 653.
- Pitsin D, Polonov K, Penev P, Chernev K (1986). Lipid fraction content of puncture biopsy material from the liver of patients with diabetes mellitus and obesity. *Vutr. Boles.*, 25: 73-77.
- Reeves PG (1997). Components of the AIN-93 diet as an improvement in the AIN-76A Diet. *J. Nut.*, 127: 838S-841S.
- Sokal RR, Rohlf FJ (1969). The principles and practice of statistics in biological research. San Francisco (Calif)7 Freeman and Co; pp. 469-484.
- Stamler J, Vaccaro O, Neaton JD, Wentworth D (1993). Diabetes, other risk factors, and 12 year cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabet. Care*, 15: 434 -444.
- Stein EA, Myers GL (1995). National Cholesterol Education Program Recommendations for Triglyceride Measurement: Executive Summary. *Clin. Chem.*, 41: 1421-1426.
- Wilson PWF (1990). High-density lipoprotein, low-density lipoprotein, and coronary heart disease. *Am. J. Cardiol.*, 66: 7A-10A.