

## Hypocholesterolemic and Antioxidant Activities of Dikanut (*Irvingia gabonensis*) Supplemented Diet in Abino Rats

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**Abstract:** Hypocholesterolemic and Antioxidant activities of Dikanut (*Irvingia gabonensis*) supplemented diet in Abino rats was investigated. Albino rats (males) were fed diets supplemented with 10, 20 and 30%, respectively dikanut for 14 days. Results showed significant ( $p < 0.05$ ) reductions in cholesterol levels compared to control;  $1.43 \pm 0.08$  to  $0.88 \pm 0.10$  mg/mL in the test animals. Lipid peroxidation levels also decreased significantly ( $p < 0.05$ ) from  $6.56 \pm 0.23$  mg/mL for control to  $4.16 \pm 0.48$  mg/mL for the test animals, while antioxidant enzymes (SOD and Catalase) levels increased significantly ( $p < 0.05$ ) compared to control,  $21.30 \pm 3.28$  to  $28.72 \pm 3.68$  and  $2.26 \pm 0.05$  to  $2.95 \pm 0.11$  U/L, respectively. Glutathione peroxidase level also showed significant ( $p < 0.05$ ) reductions from  $3.82 \pm 0.41$  U/L for control to  $3.06 \pm 0.62$  U/L in the test animals. The elicitation of these effects by the dikanut supplemented diet is a reflection of its hypocholesterolemic and antioxidative properties and could be of nutritional and clinical importance to persons at risk of cardiovascular disease.

**Keywords:** Cardiovascular disorders, cholesterol, free radicals, lipid, oxidative stress

### INTRODUCTION

Cholesterol is the major steroid found in animal tissues in the form of lipoproteins either in free forms inside the cells or combined form as esters in the blood circulation. Free cholesterol is usually converted to bile acids, while the cholesterol esters are the major storage and transport forms of cholesterol. Cholesterol is amphipathic and serves as the parent molecule from which other steroids such as sex hormones are synthesized (Murray *et al.*, 1990). Cholesterol is an important constituent of cell membrane and bile acids, which are essential for the utilization of fat-soluble vitamins (Boles *et al.*, 1994). The transport of cholesterol to and from the liver is mediated by Low Density and High Density Lipoproteins (LDL and HDL). HDL is called the 'good cholesterol since it can exchange it with other lipid carriers and transports it to the liver for disposal in bile (Vance, 1990).

Lipid peroxidation is a free radical mediated enzymatic or non-enzymatic oxidation of lipids especially polyunsaturated lipids to generate reactive peroxides and aldehydes that are associated with the development of several diseases (Esterbauer *et al.*, 1992; Steinberg, 2006). The aldehydes and peroxides are more dangerous than Reactive Oxygen Species (ROS) since they are long-lived and can easily spread to distant sites through blood circulation to cause further peroxidation of cells (Esterbauer *et al.*, 1992). Lipid peroxidation involves the formation of conjugated

diene structure. Molecular oxygen is taken up to form a peroxy Radical ( $RO_2$ ) which stabilizes by forming lipid hydroperoxide ( $ROOH$ ) that propagates further peroxidation. The hydroperoxide breaks down to form a dialdehyde called Malondialdehyde (MDA), which causes cytotoxicity and injury damage to cell (Del *et al.*, 2005).

The deleterious effects of oxidants and lipid peroxidation can be countered by an organized antioxidant defense mechanism in humans. This mechanism involves several enzymatic and non-enzymatic processes that protect cells against oxidative damage. The major enzymatic anti-oxidant systems include Superoxide Dismutase (SOD), glutathione peroxidase and catalase. The non-enzymatic antioxidants include glutathione and dietary constituents such as vitamins, flavonoids and carotenoids, which protect cells from the damaging effect of free radicals and lipid peroxidation (Farombi *et al.*, 2003).

Dikanut (*Irvingia gabonensis*) belongs to the family Simarubaceae (Eka, 1980). It is an oil bearing seed which is of local importance in West Africa and Congo. The fruit is usually collected from wild, dried and the kernel is split to release the nut. The kernel is usually processed into press cake and added to soup in mashed form to thicken it, confer flavor, aroma and draw ability. It contains mainly triglyceride the free fatty acid composition obtained from chromatography analysis shows it consists essentially of 59% linolenic fatty acid which is a polyunsaturated acid. It has a total

unsaturation of 95.28% which is similar to those of castor and safflower oils and higher than those of jatropha and soya bean oils (Bello *et al.*, 2011).

Dikanut is very viscous and gives a mucillagenous 'draw' to soups when used as a thickening agent. The dikanut kernels contain protein 10%, fats 62-73%, low iodine value and high saponification. Judith *et al.* (2005, 2009), reported dikanut seeds decreased significantly total cholesterol, LDL cholesterol, triglycerides and increased HDL cholesterol while body weight decreased in a study of obese subjects. Iwu (1986) also reported that dikanut has medicinal properties and lowers blood sugar levels often associated with diabetes mellitus when incorporated in the diet.

Since dikanut seeds are used as soup thickener in most homes in the South East of Nigeria, the aim of the present study was to investigate its effect on serum cholesterol, lipid peroxidation and some antioxidant enzymes, since the blood concentration of these biomarkers are associated with the development of several diseases (Esterbauer *et al.*, 1992; Steinberg, 2006).

## MATERIALS AND METHODS

**Dikanut seeds:** The dikanut seeds were bought as sold in the Okigwe main market. They were identified in the Department of Plant Science and Biotechnology, Abia State University, Uturu.

**Preparation of dikanut supplemented diet:** Clean healthy dikanut seeds were cut into small pieces, air dried and milled into fine powder and used for preparation of the diet (Table 1).

**Animals:** Forty healthy male albino rats weighing between 140-150 g and aged 6 weeks were used in this study. Approval for animal studies was obtained from the Animal Ethics Committee of College of Health Sciences, Abia State University, Uturu. The animals were randomly placed into 4 groups of 10 animals each.

**Group I :** Animals were placed on the diet with no dikanut supplement and served as control.

**Group II :** Animals were placed on dikanut supplemented diet (10%).

**Group III:** Animals were placed on dikanut supplemented diet (20%).

**Group IV :** Animals were placed on dikanut supplemented diet (30%).

The animals were starved overnight before they were placed on the different diets and had access to feed and water ad libitum for 14 days.

Table 1: Composition of diets (%)

Diet composition	Control diet (control) g	Dikanut supplemented diet (g)		
		10%	20%	30%
Corn	69.0	59.0	49.0	39.0
Fish meal	5.0	5.0	5.0	5.0
Groundnut	10.0	10.0	10.0	10.0
Bone meal	5.0	5.0	5.0	5.0
Palm oil	5.0	5.0	5.0	5.0
Dikanut	-	10.0	20.0	30.0
Vitamin premix	1.0	1.0	1.0	1.0
Corn starch	5.0	5.0	5.0	5.0
Total	100.0	100.0	100.0	100.0

**Blood sample collection:** At the 15<sup>th</sup> day the animals were sacrificed and blood collected by cardiac puncture.

**Chemicals:** All chemicals used were of analytical grade.

**Analysis:** Serum total cholesterol level was determined by the quantitative method described by Richmond (1973), using diagnostic kits supplied by Biosystems Ltd., USA

Lipid peroxidation was determined as described by Wallin *et al.* (1993).

While the antioxidant enzymes: SOD, Catalase and Glutathione peroxidase were assayed as described by Weydert and Cullen (2010) also using Biosystem kits.

**Statistical analysis:** Values were represented as Mean±S.D. Data obtained were subjected to one way Analysis of Variance (ANOVA) and group means were compared using Duncan's new multiple range tests. Differences were considered to be significant at (p<0.05).

## RESULTS AND DISCUSSION

The serum total cholesterol levels in animals fed the dikanut supplemented diets were reduced significantly (p<0.05) when compared to the control (Table 2), 1.43±0.18 mg/100 mL for control to 0.88±0.16 mg/100 mL for animals fed on 30% dikanut supplemented diet. Elevated serum cholesterol and LDL-cholesterol constitute risk factors in the development of cardiovascular diseases (Austin, 1991). The reduction in cholesterol level could be due to several factors. The fiber and gum content of dikanut are known to play a role in reducing the absorption of cholesterol from the small intestines (Jenkins *et al.*, 1979). The presence of the fiber and gum in the dikanut supplemented diet may be responsible for the hypocholesterolemia (Hassel, 1998). Several dietary fiber sources lower blood cholesterol levels, specifically that fraction transported by low density lipoproteins (Marlett, 2001). Two of these fibers, beta glucan in oats and psyllium husk have been sufficiently

Table 2: Cholesterol concentration mg/100 mL

	Group I (control)	Group 2	Group 3	Group 4
Cholesterol	1.43±0.18	1.32±0.10	1.08±0.25*	0.88±0.16*

\*: Values are mean±S.D. of triplicate determinations (n = 10)

Table 3: Lipid peroxidation (TBARS) mg/mL

	Group I (control)	Group 2	Group 3	Group 4
Lipid peroxidation	6.56±0.23	5.58±0.17	4.65±0.39*	4.16±0.48*

\*: Values are mean±S.D. of triplicate determinations (n = 10)

Table 4: Enzyme activities (U/L)

Enzyme	Group I (control)	Group 2	Group 3	Group 4
SOD	21.30±3.28	23.46±2.45	25.34±6.85*	28.72±3.68*
Glutathione peroxidase	3.82±0.41	3.51±0.62	3.22±1.23*	3.06±0.62*
Catalase	2.26±0.05	2.69±0.03	2.87±0.12	2.95±0.11*

\*: Values are mean±S.D. of triplicate determinations (n = 10); Values with \* are statistically significant (p<0.05)

studied for FDA to authorize a health claim that foods meeting specific compositional requirements and containing 0.75 or 1.7 g of soluble fiber per serving respectively, can reduce the risk of heart diseases (FDA, 2001). The level of Cholesterol especially LDL-cholesterol in the body has been associated with certain diseases such as cardiovascular disorders. Reductions in circulating cholesterol levels can have profound positive impacts on cardiovascular disease, particularly on atherosclerosis, as well as other metabolic disruptions of the vasculature. Cholesterol is derived from diet. However, it is not an essential nutrient since the body has the machinery to synthesize adequate amount of cholesterol. The dietary intake of cholesterol lowers the rate of its synthesis in the liver (Scriver and Beaudet, 1995). Cardiovascular disorders are consequences of the deposition of cholesteryl esters during their transport in the blood vessels leading to hardening and narrowing of the vessels, a condition known as atherosclerosis. There is an established relationship between blood concentration of cholesterol and atherosclerosis. Concentrations above 250 mg/100 mL normally increase the risk of atherosclerosis (Brown and Goldstein, 1984). However, this increased risk is associated with LDL-cholesterol and appears to be reduced by high levels of HDL-cholesterol. Also cholesterol is deposited in the gall bladder, which is important in the development of gallstones.

Lipid peroxidation was significantly (p<0.05) reduced in all the test animals fed dikanut supplemented diet (Table 3), 6.56±0.23 mg/mL for control to 4.16±0.48 mg/mL for animals fed on 30% dikanut supplemented diet. This is in similar to the findings of Eidange *et al.* (2010) who reported the lowering of serum lipid profile and improvement of antioxidant activity by melon oil. The reaction with ROS protects lipids from peroxidation and by extension prevents damage to cells that result into disease condition (Cai *et al.*, 1997; Olatunde *et al.*, 2004). Extensive lipid peroxidation leads to disorganization of membrane by peroxidation of unsaturated fatty acids which also alters the ratio of polyunsaturated to other fatty acids. This would lead to a decrease in the membrane fluidity and

the death of the cell (Devaki *et al.*, 2004). From the result of our investigation, dikanut seed has the potential to prevent this cell death due to lipid peroxidation by inhibiting the lipid peroxidation process. The radical chain of lipid peroxidation is a continuous process which can alter essential functions of cells that may lead to death (Cullis *et al.*, 1996). Lipid peroxidation is an underlying cause of degenerative diseases including cancers (Ochani and Mello, 2009). Conversely, lipid peroxidation is beneficial and has been used by nature to destroy microorganisms, produce molecules that signal new cell growth and in plants produce fungicides and bacteriocides (Trible *et al.*, 1987).

In this study, SOD was increased significantly (p<0.05) in the test animals compared to the control, 21.30±3.28 U/L for control to 28.72±3.68 U/L for animals fed 30% dikanut supplemented diet (Table 4). The increase in SOD activity is suggestive of the ability of Dikanut to boost the production of the antioxidant SOD within the system of the experimental animals, which showed that the seeds of Dikanut may possess antilipoperoxidative effect. This is a likely proof of its antioxidative properties which has high inhibition of lipid peroxidation. SOD is a spread enzyme that protects cells against the deleterious effects of ROS especially the superoxide radical (Moore and Roberts, 1998). SOD is crucial to limiting ROS formation and controlling lipid peroxidation by dismutation of superoxide radical.

Glutathione peroxidase was found to be significantly (p<0.05) reduced (Table 4) in the test animals from 3.82±0.41 U/L for control to 3.06±0.62 U/L for animals fed on 30% dikanut supplemented diet. Peroxidase is involved in the reduction of lipid and hydrogen peroxides to eliminate oxidative stress. Its reduction in the test animals suggests that the dikanut may offer protection against lipid peroxidation. However, induction of peroxidase provides an important chemopreventive strategy against carcinogens (Chae *et al.*, 1998), as well as diseases caused by oxidative damage.

Catalase concentration was also significantly ( $p < 0.05$ ) increased (Table 4) from  $2.26 \pm 0.05$  U/L for control to  $2.95 \pm 0.11$  U/L for test animals fed on 30% dikanut supplemented diet. Catalase works closely with superoxide dismutase to prevent free radical damage to the body SOD converts the dangerous superoxide radical to hydrogen peroxide, which catalase converts to harmless water and oxygen. Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde (Del *et al.*, 2005). This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as Advanced Lipoxidation End products (ALE), in analogy to Advanced Glycation Endproducts (AGE). The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism (Farmer and Davoine, 2007).

An imbalance in the oxidant versus the anti-oxidant levels is called oxidative stress and is implicated in the development of diseases such as cancer, diabetes, ageing etc (Nordmann, 1993). Conversely the free radicals find usefulness in the functions of the immune system, hepatic Cyp<sub>450</sub> and smooth muscles (Bast *et al.*, 1991).

## CONCLUSION

This investigation showed that the dikanut supplemented diet caused significant reductions in total cholesterol and lipid peroxidation levels and significant increase in the activity of superoxide dismutase and catalase and also a decrease in glutathione peroxidase activity in the experimental animals. These results suggest the use of dikanut as a hypocholesterolemic and antioxidant agent in foods.

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## REFERENCES

- Austin, M.A., 1991. Plasma triglycerides and coronary disease. *Arterioscler. Thromb.*, 11: 2-14.
- Bast, A., G.R. Haenen and C.J. Doelman, 1991. Oxidants and antioxidants state of the art. *Am. J. Med.*, 91: 2S-13S.
- Bello, E.I., A.O. Fade-Aluko, S.A. Anjorin and T.S. Mogaji, 2011. Characterization and evaluation of African bush mango Nut (Dika nut) (*Irvingia gabonensis*) oil biodiesel as alternative fuel for diesel engines. *J. Petrol. Technol. Altern. Fuels*, 2(9): 176-180.
- Boles, S.J., D. Bradley, R.A. Force and B.R. Brown, 1994. The new dietary fats in health and diseases. *J. Am. Diabetic. Assoc.*, 97: 280-286.
- Brown, M.S. and S.L. Goldstein, 1984. How LDL receptor influences cholesterol and atherosclerosis. *Sci. Am.*, 251: 58-66.
- Cai, Q., R.O. Rahan and R. Zang, 1997. Dietary flavonoids, quercetin, luteolin and genistein, reduce DNA damage and lipid peroxidation and quench free radicals. *Cancer Lett.*, 119: 99-108.
- Chae, Y.H., J. Rosa, L.K. Williams, D. Desai, S.G. Amin, E. Fiala and K. El-Bayoumy, 1998. Induction of oxidative DNA damage by 7, 12-dimethylaminobenzo [a] anthracene and 2-amino-1-methyl-6-phenylimidazo[4,5,6] pyridine in rats. *Proc. Am. Assoc. Cancer Res.*, 39: 490.
- Cullis, P.R., D.B. Fennscke and M.J. Hope, 1996. Physical Properties and Functional Roles of Lipids in Membrane. In: Vance, D.E. and J.E. Vance (Eds.), *Biochemistry of Lipids, Lipoproteins and Membranes*, Elsevier, Amsterdam, pp: 1-33.
- Del, R.D., A.J. Stewart and N. Pellegrini, 2005. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr. Metab. Cardiovasc. Dis.*, 15(4): 316-328.
- Devaki, T., H.R.B. Raghavendran and A. Sathivel, 2004. Hepathoprotective nature of seaweed alcoholic extract on acetaminophen-induced hepatic oxidative stress. *J. Hlth. Sci.*, 50: 42-46.
- Eidange, G.O., G.C. Ojieh, B.O. Idonije and O.M. Oluba, 2010. Palm oil and egusi melon oil lower serum and liver profile and improve antioxidant activity in rats fed a high fat diet. *J. Food Technol.*, 8: 154-158.
- Eka, S.A., 1980. Proximate composition of bush mango seeds and some properties of dikanut fat. *Niger. J. Nutr. Sci.*, 11: 3-35.
- Esterbauer, H., J. Gebicki, H. Puhl and G. Jurgens, 1992. The role of lipid peroxidation and antioxidants in oxidation modification of LDL. *Free Radic. Biol. Med.*, 13: 341-390.
- Farmer, E.E. and C. Davoine, 2007. Reactive electrophile species. *Curr. Opin. Plant Biol.*, 10(4): 380-386.
- Farombi, E.O., J.O. Nwankwo and G.O. Emerole, 2003. Evaluation of the antioxidant and partial characterization of extracts from browned yam flour. *Food Res. Int.*, 6: 33.
- FDA (Food and Drug Administration US), 2001. Health claims: Soluble fiber from certain foods and risk of heart diseases. Code of Federal Regulations, 21: 81-110.
- Hassel, C.A., 1998. Animal models: New cholesterol raising and lowering nutrients. *Curr. Opin. Lipidol.*, 9(1): 7-10.
- Iwu, M.M., 1986. Empirical Investigation of Dietary Plants used in Igbo Ethnomedicine. In: Nina Etkin (Ed.), *Plants in Indigenous Medicine and Diet: Biobehavioral Approaches*. Redgrave Publishing Co., New York, pp: 131-150.

- Jenkins, D.J.A., D. Reynolds, A.R. Leeds and J.H. Cummings, 1979. Hypocholesterolemic action of dietary fiber unrelated to fecal bulking effect. *Am. J. Clin. Nutr.*, 32: 2430-2435.
- Judith, L.N., E.O. Julius and R.M. Samuel, 2005. The effect of *Irvingia gabonensis* seeds on body weight and body lipids of obese subjects in Cameroon. *Lipid Health Dis.*, 4: 12.
- Judith, L.N., C.E. Blanche, B.N. Christine, M.F.M. Carl and E.O. Julius, 2009. IGOB131, a novel seed extract of the West African plant *Irvingia gabonensis*, significantly reduces body weight and improves metabolic parameters in overweight humans in a randomized double-blind placebo controlled investigation. *Lipids Health Dis.*, 8: 7.
- Marlett, J.A., 2001. Dietary Fiber and Cardiovascular Disease. In: Cho, S.S. and M.L. Dreher (Eds.), *Handbook of Dietary Fiber*. Marcel Dekker Inc., New York, pp: 17-30.
- Moore, K. and L.J. Roberts, 1998. Measurement of lipid peroxidation. *Free Radic. Res.*, 28(6): 659-671.
- Murray, I.K., P.A. Mayer, O.K. Graner and V. Rodwel, 1990. Cholesterol Biosynthesis, Degradation and Transport. In: *Harper's Biochemistry*. 2nd Edn., Prentice Hall International Inc., pp: 240-254.
- Nordmann, R., 1993. Free radicals, oxidative stress and antioxidant vitamins. *C R Seances Soc. Biol. Fil.*, 187: 277-285.
- Ochani, P.C. and P.D. Mello, 2009. Antioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa* Linn. Leaves and calyces extracts in rats. *Indian J. Exp. Biol.*, 47: 276-282.
- Olatunde, F.E., M. Hansen, P. Rain-Haren and L.O. Dragsted, 2004. Commonly consumed and naturally occurring dietary substances affect biomarkers of oxidative stress and DNA-damage in healthy rats. *Food Chem. Toxicol.*, 42: 1315-1322.
- Richmond, N., 1973. Determination of cholesterol in serum. *Clin. Chem.*, 19: 1350-1356.
- Scriver, C.R. and A.I. Beaudet, 1995. Fate of cholesterol: An overview. *Science*, 300: 1120.
- Steinberg, D., 2006. Thematic review series: The pathogenesis of atherosclerosis. An interpretive history of cholesterol controversy, part V: The discovery of the statins and the end of the controversy. *J. Lipid Res.*, 47: 1339-1351.
- Trible, D.L., T.Y. Aw and D.P. Jones, 1987. The pathophysiological significance of lipid peroxidation in oxidative cell injury. *Hepatology*, 7: 377-387.
- Vance, J.S., 1990. Metabolism of Cholesterol. In: *Biochemistry of Lipids and Lipoprotein and Membranes*. Amsterdam Elsevier Science Publishers, Amsterdam.
- Wallin, B., B. Rosengren, H.G. Shertzer and G. Camejo, 1993. Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtiter plate: Its use for evaluation of antioxidants. *Anal. Biochem.*, 208: 10-15.
- Weydert, C.J. and J.J. Cullen, 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cell and tissues. *Nat. Protoc.*, 5: 51-60.