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Biochemical Studies of the Effects of the Aqueous Extract of Nigerian Garlic on Lipid Profile and Atherogenic Risk Predictor Indices.

[1]Ojiako O.A. and [2]Nwanjo, H.U.

[1]Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
[2]Department of Medical Laboratory Science, College of Medicine and Health Sciences, Imo State University, Owerri, Nigeria.

Abstract: Following claims and counterclaims about the efficacy of aqueous extracts of garlic in different parts of the world, the effects of Nigerian garlic extract on some atherogenic risk predictor indices were investigated using thirty adult and apparently healthy Wistar albino rats. The animals were divided into five different groups of six animals each. Four different concentrations of aqueous garlic extract were administered by oral compulsion for four weeks to four groups while the fifth group served as control. The plasma lipid profile of the experimental animals was assayed and the resulting values statistically analysed. The values of Total Cholesterol (TC), LDL-Cholesterol (LDL-C) and Triglycerides (TG) significantly ($p < 0.05$) decreased as the garlic concentration increased. No significant differences existed in the levels of HDL-Cholesterol (HDL-C). Calculation of the atherogenic risk predictor indices HDL-C/TC and LDL-C/HDL-C showed that the positive predictor, HDL-C/TC increased with increased garlic extract concentrations while the negative predictor, LDL-C/HDL-C decreased as garlic concentration increased, indicating beneficial and desirable effects. Nigerian garlic extract therefore, has antiatherogenic properties and can be useful in the management/prevention of coronary events.

Key words: Nigerian garlic extract, lipid-lowering effects, albino rats.

INTRODUCTION

The use of plant extracts in managing various disorders is currently commonplace (Philip and Cephas, 1997). Many plant materials are also in current use as supplements (Block *et al.,* 2007). Sometimes the aim is to lower the levels of some markers of disease states in order to improve health conditions. An example may be found in the use of substances that lower the cholesterol level in the system. Many studies indicate that lowering serum cholesterol may prevent, control and even reverse atherosclerosis and coronary heart disease[3]. Low triacylglycerol and low-density lipoprotein cholesterol (LDL-C) levels or high high-density lipoprotein-cholesterol (HDL-C) levels are desirable health outcomes known to have resulted from the use of some plant materials. Amongst such plants reported to exhibit beneficial effects in atherosclerosis and ischaemic heart disease in experimental animals and in clinical and experimental studies in human beings garlic *(Allium sativum)* and onion *(Allium cepa),* both of the *Amaryllis* family *(Amaryllidaceae),* have been outstanding (Arora *et al.,* 1981; Jain, 1997). Evidence from earlier studies indicates that garlic and onion can bring about plasma lipid normalization, enhance fibrinolytic activity, inhibit platelet aggregation and thrombozane formation as well as have protective actions against stroke, coronary thrombosis and diabetic ketosis (Bordia and Bansal, 1973). These reasons and more explain why garlic is now a popular component of dietary supplements (Block *et al.,* 2007; Radimer *et al.,* 1999).

There however, have been varying reports of the chemical composition of garlic (Javier *et al.,* 2004; Muoio *et al.,* 2004) resulting in contradicting evidences of efficacy and inefficacy of its extracts. Some possible factors for the varying biological activity and inconsistent chemical composition of garlic may include soil differences (Bloem *et al.,* 2004), horticultural practices (Castellanos *et al.,* 2004), processing and storage methods (Montano *et al.,* 2004) among several other factors. Also, most reference studies on the antilipidaemic properties of garlic have used experimental animals upon which hyperlipidaemic conditions have been induced either by nutrition (Chi *et al.,* 1982) or by drugs (Jain, 1997). This study however, is aimed at investigating the possible antilipidaemic efficacy of garlic cloves from the open market in Nigeria with particular regard to

Corresponding Author: Dr Okey Alphonsus Ojiako (B.Sc., M.Sc., Ph.D). Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
Email: okeyojiako@yahoo.com. Tel.: +2348032637607

their effects on plasma lipoproteins and some atherogenic risk predictor indices in rats upon which the hyperlipidaemic state has not been induced. Since garlic is now taken as a supplement not necessarily by persons challenged by lipid-associated health conditions (Block *et al.,* 2007), the study was not aimed only at the lipid-lowering efficacy of Nigerian garlic in disease states but also on its possible beneficial use as a supplement to prevent or at least mitigate the effects of certain disease conditions that may involve lipid peroxidation in otherwise healthy individuals who are functional foods enthusiasts.

MATERIALS AND METHODS

Collection and Preparation of Garlic Samples:

Garlic bulbs were obtained from the open market in Owerri, Imo State, Nigeria and confirmed by taxonomists at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. Fleshy cloves were selected and dried overnight on a laboratory tray in a carbolite moisture extraction oven at 65°C before grinding with a kitchen blender as in an earlier work (Ojiako and Nwanjo, 2006).

A 100g portion of the garlic powder was soaked overnight with occasional shaking in 500ml of distilled water before filtering. The resulting filtrate was heated in a rotary evaporator to produce a solid residue from which small appropriate weights were measured and dissolved in 2.5ml of physiological saline to obtain the desired concentrations of 10mg, 7.5mg, 5.0mg and 2.5mg per kilogramme body weight of experimental animals respectively. The control group received 2.5ml of physiological saline per kilogramme body weight.

Preparation of Experimental Animals:

A total of 30 Wistar albino rats were obtained from the animal house of a research institute, in Abia State, Nigeria and transported in an air-free cage to our laboratory. The rats were randomly grouped into five groups of six animals per group and then housed separately in partitioned polypropylene cages labeled A - E and allowed free access to water and feed diet (product of Pfizer Nigeria Ltd) *ad libitum* for 14 days under controlled environmental conditions of temperature of 20°C and relative humidity and a 12-hour light and dark cycle to allow the rats acclimatize with the laboratory conditions. Treatment was administered daily by oral compulsion at doses of 2.5, 5.0, 7.5 and 10.0 mg of garlic extract per kg body weight of experimental animals respectively to groups A - D while animals in group E received an equivalent of 2.5ml of normal saline per kg body weight.

Biochemical Assays:

At the end of experiment, the rats were fasted for 24 hours and then as permitted by the University Ethics Committee, the animals were euthanized by anaesthesia using the tuhalant, halothane and then sacrificed. Using a sterile syringe and needle, 6ml of blood was collected from the heart into EDTA bottles and immediately centrifuged at 3000 rpm for 10 min to remove formed elements. The resulting plasma was then used for the estimation of the different lipid fractions (Nwanjo and Ojiako, 2006).

Serum triacylglycerol (TG) was measured using the extraction method of Mendez *et al* ((Nwanjo and Ojiako, 2006), total cholesterol (TC) was measured using the method of Lopez -Vitrella *et al.,* (1977). HDL-Cholesterol (HDL-C) was also measured according to the method of Lopez Vitrella *et al* (1977). after extraction. LDL-cholesterol (LDL-C) was then calculated using the formula of Friedwald *et al.,* (1972). The atherogenic risk predictor indices were calculated using the formulae of Dobiasova and Frohlich (Dobiasova and Frohlich, 2001).

Statistical Analysis of Results:

All values were expressed as mean ± S.D. and then subjected to statistical analyses (Statistical Analysis System Program Institute, Inc. Cary, N. C) using Duncan Multiple Range Test to detect significant differences (at $P<0.05$).

RESULTS AND DISCUSSION

The plasma lipid-lowering effect of garlic extract on Wistar albino rats is presented in Table 1. The mean plasma total cholesterol, LDL-cholesterol and triacylglycerol levels significantly decreased as the concentration of the garlic extract administered increased until, but not beyond, 7.5mg per kg bodyweight. There was no significant difference in the HDL-cholesterol levels of the different groups. Table 11 shows that the mean ratio of the positive indicator HDL-C/TC increased with increasing concentration of garlic extract while the negative indicator LDL-C/HDLC decreased as concentration of garlic extract administered increased.

Table 1: Lipid profile of the different groups of albino rats treated with different concentrations of garlic extract.

Para- meters	Control 2.5ml saline	GRP A 2.5mg/dl	GRP B 5.0mg/dl	GRP C 7.5mg/dl	GRP D 10.0mg/dl
TC (mg/dl)	115.O±O.36	95.4O±O.36*	84.1±O.54**	76.7±0.46***	75.8±O.50***
HDL-C (mg/dl)	31.86±O.66	30.5±O.70	31.2±O.53	32.38±O.34	30.2±O.65
LDL-C (mg/dl)	60.96±O.22	45.O±0.31 *	34.7±O.43**	28.7±O.31***	29.2±O.18***
TG (mg/dl)	11O.9±O.69	99.5±O.43*	91.O±O.36**	83.1±O.66***	82.O±O.40***

Values are means of the six animals per group ± standard deviation
HDH-C = HDL-cholesterol,
LDL-C = LDL-cholesterol,
TC = Total cholesterol,
TG = Triacylglycerol.
* Significantly different from control group (P<O.05)
** Significantly different from control and group A (P<O.05).
*** Significantly different from control, group A and B (P<O.05

Table 2: Atherogenic risk predictor indices of the different groups of albino rats treated with different concentrations of aqueous garlic extract.

GROUPS	GARLIC CONCENTRATION (mg/dl)	HDL-C/TC	LDL-C/HDL-C
Control	Nil (2.5ml saline)	0.27±0.006	1.91±0.070
GROUP A	2.5	0.31±0.007*	1.47±0.050*
GROUP B	5.0	0.37±0.006**	1.11±0.025**
GROUP C	7.5	0.40±0.007***	0.91±0.01 **
GROUP D	10.0	0.39±0.130**	0.96±0.006**

Values are means of the six animals per group ± standard deviation
HDH-C = HDL-cholesterol,
LDL-C = LDL-cholesterol,
TC = Total cholesterol,
TG = Triacylglycerol.
* Significantly different from control group (P<0.05)
** Significantly different from control and group A (P<0.05).
*** Significantly different from control, group A and B (P<0.05).

Discussion:

Various studies (Durak *et al.,* 2004; Thomson and Ali, 2003) indicate that serum levels of cholesterol are strongly related to coronary atherosclerosis and increased risk of coronary heart disease. Clinical studies in humans have shown that lowering levels of serum cholesterol with diet or drugs decreases the incidence of coronary heart disease. Whereas some studies point to the cholesterol lowering effect of garlic (Durak *et al.,* 2004; Thomson and Ali, 2003), others have questioned the validity of this effecf (Lerner and Hulley, 1994; Simons *et al.,* 1995). Even a recent study (Islam and Choi, 2008) concluded that the aqueous extract of garlic had no effect on the lipid profile of experimental animals. Results from the present investigation show however that in Wistar albino rats, after one month of consumption of the aqueous extract of Nigerian garlic, the plasma total cholesterol (TC), LDL-cholesterol and triacylglycerol (LDL-C) levels significantly (P<0.05) decreased as the concentration of the extract increased. There was no significant difference (P<0.05) in the levels of HDL-cholesterol. In contrast to the usually stated inverse correlation between levels of triacylglycerol and HDL-cholesterol, the significant reduction of triacylglycerol levels observed in our study, was not associated with a significant increase in HDL-cholesterol.

Considered in their entirety, these results unequivocally show that the aqueous extract of Nigerian garlic has beneficial health effects. An increase in any of the parameters (except HDLCholesterol) and a decrease in HDL-Cholesterol is a significant and independent marker of possible coronary event (Wierzbicki and Mikhailidis, 2002). The atherogenic risk predictor indices HDL-C/TC and LDL-C/HDL-C also corroborate our finding of a salutary effect of the aqueous garlic extract on lipid profile as HDL-C/TC increased with increased garlic extract concentration and LDL-C/HDL-C decreased as garlic concentration increased, indicating that the extract is antiatherogenic and desirable and can reduce the development of coronary atherosclerosis (Dobiasova and Frohlich, 2001). Some workers (Plourde, 2002), have recommended the measurement of TC/HDL-C. Even this will not have affected the outcome of our work since the value is the reciprocal of the HDL-C/TC that we measured and the interpretation of results is exactly the reverse.

There are several possible explanations for the results we obtained. It has been previously shown that administration of garlic to humans (Thomson and Ali, 2003), to rats and to cell culture (Orekhov *et al.,* 1995) is effective in decreasing cholesterol levels. The mechanism for the cholesterol lowering effect of garlic has also been attributed to the inhibition of the specific activity of the enzyme HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis. This enzyme has been variously reported to be significantly lowered in rat liver microsomes after garlic consumption (Merat and Fallahzadeh, 1996; Omkumat *et al.,* 1991).

The mechanism for the triacylglycerol-lowering effect of garlic is not well understood. However, Yeh and Yeh (1994), demonstrated that the rate of C (Nwanjo and Ojiako, 2006)-acetate incorporation into fatty acids was reduced in hepatocyte cell culture treated with garlic extract. Thus, the triacylglycerol-lowering effect of garlic may be due to the inhibition of fatty acid synthesis. These mechanisms are however open to further studies.

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