Application of Garlic in Management of Atherosclerosis: Tolerance and Impact of Some Local Therapies on Common Parameters of Lipid Analysis

Uhiara Ngozi Sunday[1, *], Ohanwe Cyprian Nnanwa[2], Clement Ezeaku Anikezie[2], Adesanya Oluwatosin Dorothy[3], Victoria Ada Abodenyi[2], Eduzor Esther[1], Samuel Emmanuel[1]

[1]Department of Food Science and Technology, School of Science and Technology, Federal Polytechnic Bauchi, Bauchi, Nigeria

[2]Department of Agricultural Engineering Tech, School of Engineering, Federal Polytechnic Bauchi, Bauchi, Nigeria

[3]Department of Nutrition and Dietetics, School of Science and Technology, Federal Polytechnic Bauchi, Bauchi, Nigeria

Email address:

ngoziuhiara@gmail.com (Uhiara Ngozi Sunday)

[*]Corresponding author

Abstract: Four groups of rats (three in each cage) were fed with conventional feed, supplemented with known percentages of crushed garlic paste – thoroughly mixed (5%, 10%, 20% and 30%). A fifth group – control was fed with garlic free feed. In the second phase of the work, three groups of three rats each, were kept in separate cages and fed with conventional rat feeds supplemented with 5% fresh garlic which was incooperated into the feed as (i) garlic water extract – sample G (ii) garlic paste/honey mix – sample H (iii) crushed garlic paste – sample I. The rats in both phases were fed for twenty one (21) days, during which period the weights, feed intake and weight of droppings were recorded at three days' intervals. The blood samples of the experimental rats were also harvested at the end of 21 days and lipid analysis carried out on them. Result of the preliminary experiments showed that group A rats (fed with 5% garlic supplemented feed) had a significantly lower level of total cholesterol 6.5 Mmol/ml compared to 7.3Mmol/ml of the control (group E). In the second phase, the 5% garlic incooperated as crushed garlic, recorded low density lipoprotein (LDL) of 0.5 Mmol/ml compared to 1.2 and 1.1 Mmol/ml shown respectively by the control an d group C (garlic water extract) groups respectively. Ingesting or applying garlic in different therapeutic forms was shown or demonstrated to be capable of producing different results with regards to studied lipid parameters.

Keywords: Garlic, Traditional Therapies, Combinations Impact, Lipid Parameters, Atherosclerosis

1. Introduction

Garlic (allium sativum) belongs to the plant Liliaceae family; genus Allium containing about 500 species [2]. It is a bulbous plant and grows up to 1.2m. Allicin (ally – 2 propenethiosulfinate) is the principal bioactive compound present in aqueous extract of garlic or raw garlic homogenate [2, 12]. It is responsible for the flavour, odour and significantly high antioxidant properties of garlic. Antioxidants fight free radicals; compounds that can cause disease and aging which explains the seemingly positive effects of garlic in treatment of diabetes, blood pressure, cholesterolemia and cancer [4, 5].

When garlic is chopped or crushed, allinase enzyme is activated and produces allicin from the allin present in intact bulb [15]. Garlic extract contains at least 33 sulfur compounds, several enzymes, 17 amino acids and minerals such as selenium. Its numerous biological activities have been attributed to the rich content of different volatile organo-sulfur compounds (OSC) and phyto-chemicals that work synergistically with hundreds of other organic compounds [17]. Investigations by Aouadi et al [1, 2] noted that garlic supplement increased high density lipoproteins and decreased

low density lipoproteins in normal hypercholesterolemic rats.

Hypercholesterolemia is a disease condition usually associated with cholesterol levels equal to or higher than 5Mmol/Ml. Low density lipoproteins are the major causes of atherosclerotic cardiovascular diseases. Evidence of this abounds from genetic epidemiologic and clinical studies. It is also a consensus statement from the European Atherosclerosis society consensus committee [17].

More research works have been conducted into newer processes for extracting garlic components; one recorded some significantly positive results upon testing a material extracted from garlic fermented with a mold – monascuspilosus on 15 hypercholesterolemic patients. The material significantly reduced total cholesterol and low density lipoproteins [15]. Other traditional therapies which have been employed by civilizations at different places include garlic water extracts, crushed garlic paste, crushed garlic paste mixed with honey etc. in management of different ailments including high blood pressure, asthma, digestive disorders etc. [3, 6, 9]. Science is yet to find comprehensive solution to the problem of atherosclerosis – an umbrella name for group of ailments associated with obstruction of blood flow in human systems [17]. In third world countries like Nigeria that is lacking in medical equipments and knowhow for management of atherosclerosis, these increasing number of positive outcomes in garlic therapies efficacy have necessitated need for more definite studies on impact of individual local therapy on certain parameters of atherosclerosis and lipid profiling; which was largely the aim of this research.

2. Materials and Methods

In the preliminary stage of the work, fifteen (15) winster/albino rats were kept in five (5) groups with three rats in each. Four groups wereeach fed with conventional rat feed supplemented with known percentages of crushed garlic pastes thoroughly mixed (5%, 10%, 20% and 30%). The fifth group was fed with garlic free feed and labeled E, control. Note that 18 rats were purchased for the experiments. Three (3) selected at random had their blood harvested (almost on arrival) for preliminary test on the blood on certain blood lipid parameters.

The second phase of the experiments was designed to probe into the impact of mode of administration/ingestion (as different preparations or therapies). Twelve (12), two months old rats were kept in four groups of three rats each. One group the control was fed with garlic free feed and labeled F. Into the other three (3), 5% garlic was incooperated as garlic water extract (sample G), garlic/honey mix (sample H), and crushed garlic (sample I). The rats in both stages were fed for 21 days. During the period of feeding, the rats were weighed at three (3) days interval; the droppings as well as the feed intake were checked at the same intervals.

3. Sample Preparation

Garlic water: five (5g) grammes of peeled fresh garlic lobes were pounded/crushed using clean mortar and piston. This was scooped into a shallow cup. Then 50ml of distilled water used for washing/rinsing the mortar was poured into the shallow cup; shaken for three (3) minutes and stood for 5 minutes before 5ml was pippeted out as garlic water extract. Five (5) ml garlic water extract was thoroughly mixed with100g rat feed to give sample G.

Garlic/honey mix: five (5)g of peeled fresh lobes were crushed in a mortar, scooped into a shallow cup and thoroughly mixed with 20ml of honey; allowed to stand for 30 minutes before mixing into 100g of conventional rat feed to become sample H.

Crushed garlic: five (5)g of peeled fresh lobes were crushed in a mortar, scooped into shallow cup containing 100g of rat feed, thoroughly mixed to give sample I.

Lipid analysis: Lipid analysis of the blood samples of the experimental rats (which included total cholesterol, triglycerides, LDL – low density lipoproteins and HDL – high density lipoproteins) were carried out according to the procedures outlined by Schmidt and Schimidt [13] and Tolman [16].

4. Results and Discussions

From the first set or preliminary experiments (see Table 2), group A rats (fed with 5% garlic feed), showed a significant reduction ($P \leq 0.05$) in total cholesterol; 6.5 compared to 7.3 of the control (group E rats).

The triglycerides of the A group rats also reduced significantly (1.2, compared to 1.8 for the control). The low density lipoprotein (LDL) came down to 4.5 from 4.9 (see table 1). Citing LDL as example, the level came down from 4.9 for control to (3.3) a value within the (NR) normal range (2.39 – 3.55Mmol/ml). At higher concentrations of garlic in the feed, there were more drastic reductions in blood lipid parameters. For example, at 10% and 30%, the LDL for groups B and D rats came down to 3.8 and 3.3 respectively from 4.9 recorded by the E or control group (Table 1). However, from same table 2, it can be postulated or deduced that feeds above 5% garlic supplementation were less tolerable. The repulsive impact of the numerous organosulfides on the taste of the feedis expected to increase with increasing concentration of garlic supplementation [7, 8. 10]. The average feed consumption for group A (5% garlic rats) was 23.11%; B (10% garlic) and C (15% garlic) respectively reduced to 19.3 and 17.40 (Table 1).

In the second stage of the work, designed to focus on 5% garlic feeds in varied forms (in line with traditional therapies), the results show the highest impact was recorded by sample B (5% crushed garlic) which reduced low density lipoprotein (LDL) from 1.2 (recorded by the control) to 0.5Mmol/ml; this is also significantly lower than 0.8 and 0.9 reported by sample C (garlic honey mix) and sample D (garlic water) respectively (see Table 3).

Ingesting or applying garlic in different therapeutic forms has always been considered as being capable of producing different results or observations [9, 11, 14]. The result on

Table 3, tends to lend weight to the preceding postulation; using levels of triglycerides as example, 5% crushed garlic (sample B) reported 1.9Mmol/ml while same 5% garlic administered as water extract (sample C) had 1.1Mmol/ml, significantly lower ($p \leq 0.05$). The same observation goes for LDL; between samples B (crushed garlic) and D (garlic water extract): both scored 0.5 and 0.9Mmol/ml, respectively and significantly different.

On this basis (after further investigation perhaps), two different therapies of same quantities of garlic could be prescribed for use at the same time by one patient and this is what is culturally obtainable in several quarters [3].

From Table 1, titled feed consumption, weight gain and weight of droppings, there is no significant difference ($p \leq 0.05$) down the columns (IA, IB, IC, ID) meaning intra (within) the groups, there is no significant difference in feed consumption. However across the row, there were significant differences meaning that the rats consumed crushed garlic, garlic water extract and garlic/honey mix supplemented feeds, in quantities that differ significantly. In other words, with regards to the attribute 'feed consumption' (I), while there were no significant differences in the seven occasions

(3- days' interval) the ones that had crushed garlic in their feed were evaluated. The same for those with garlic honey mix and garlic water extract. Between groups, there were significant differences. The pattern on feed consumption was replicated by weight gain (II) and weight of droppings (III). Despite the seeming generalization, there were no significant differences ($p \leq 0.05$) between the rats fed with Feed sample B (crushed garlic supplemented feed) and those feed on D (garlic water extract supplemented feed) in terms of weight gain and rat droppings. The significant difference observed in terms of quantity of feed consumed could be interpreted to mean among other things that the impact of the two preparations on the taste (strong factor in tolerance) of the feed samples was significantly different.

While tolerance (based on pleasant taste) might appear very logical and easily convincing for the significantly higher consumption ($p \leq 0.05$) of the garlic- honey mix supplemented feed, enhancing or improvements on rat's metabolic processes could be a strong possibility. The honey as well as the crushed fresh garlic all contain among other things, enzymes that could facilitate digestion absorption and cellular metabolism of feed [1].

Table1. Feed Consumption, weight gain and weight of droppings of experimental rats.

3 Days Interval	Consumption of food comparison			
	iA	iB	iC	iD
1	$99.0 \pm .001^a$	$95 \pm .01^b$	$98.0 \pm .002^a$	60 ± 1.1^c
2	99.9 ± 012^a	$98 \pm .11^b$	99.8 ± 012^a	66 ± 09^c
3	$98.0 \pm .001^a$	$95 \pm .13^b$	98.9 ± 001^a	60 ± 1.5^c
4	98.5 ± 09^a	$95 \pm .10^b$	$99.9 \pm .113^a$	80 ± 1.9^c
5	$98.0 \pm .021^a$	$95 \pm .90^b$	$99.8 \pm .111^a$	75 ± 1.4^c
6	$90.0 \pm .01^a$	95 ± 1.1^b	99.8 ± 101^a	80 ± 1.0^c
7	$99.0 \pm .11^a$	$90 \pm .97^b$	99.9 ± 014^a	70 ± 0.09^c
Intra group Mean	99.8	94	99.8	70
Inter group Mean		90.93		

3 Days Interval	Weight gain (g) comparison			
	iiA	iiB	iiC	iiD
1	157.3 ± 1.1^d	103.1 ± 6.9^e	$133 \pm .01^f$	102 ± 001^e
2	157 ± 1.0^d	102 ± 0.11^e	$132 \pm .19^f$	102.1 ± 012^e
3	159 ± 0.9^d	103.9 ± 0.9^e	$134 \pm .67^f$	101 ± 041^e
4	$158 \pm 1.^{9d}$	101.9 ± 009^e	130 ± 1.0^f	103 ± 001^e
5	160 ± 1.8^d	103 ± 1.1^e	$132 \pm .01^f$	103 ± 063^e
6	165 ± 1.2^d	102 ± 1.12^e	$132.2 \pm .02^f$	$101 \pm .15^e$
7	163 ± 1.95^d	102.7 ± 0.79^e	$131 \pm .03^f$	$102 \pm .19^e$
Intra group Mean	159.9	102.7	132.02	102
Inter group Mean		124.16		

3 Days Interval	Dropping (g) Comparison			
	iiiA	iiiB	iiiC	iiiD
1	20 ± 1.9^g	17 ± 1.1^g	29 ± 1.01^h	18 ± 1.1^g
2	$17.1 \pm .09^g$	19 ± 1.6^g	30 ± 0.92^h	19 ± 0.97^g
3	15 ± 2.0^g	17.5 ± 1.91^g	30.9 ± 1.32^h	19.5 ± 1.3^g
4	22.1 ± 1.9^g	18 ± 1.8^g	32 ± 0.97^h	18.5 ± 1.67^g
5	20 ± 2.1^g	19 ± 1.0^g	30 ± 1.4^h	17.9 ± 0.98^g
6	21 ± 1.87^g	21 ± 1.9^f	31 ± 1.83^h	-22 ± 1.21^g
7	23 ± 1.56^g	16 ± 1.96^f	30.5 ± 1.0^h	24 ± 1.97^g
Intra group Mean	19.74	18.21	30.49	19.8
Inter group Mean		22.0		

Keys: i = Feed consumption in (g); ii = Weight gain (g); iii =Rat droppings (g) A = Zero Garlic Feed, B = 5% Crushed Garlic Feed, C = 5% Crushed Garlic + Honey feed, D = 5% garlic (water extract) supplemented feed.
Means ± standard deviations across row in each block with same superscripts are not significantly different ($p \leq 0.05$)

Table 2. *Lipid Profile/feed metabolism results.*

Samples	Total Cholesterol (Up to 5.17)* v 5.0	Triglyceride (0.7 – 1.7)* v1.3	HDL (1.04 – 1.7)* v1.8	LDL (2.39 – 3.55)* v3.3	Initial Rat Weight	Ave. rat gain	Ave feed intake	Weight of droppings Ave.
A	6.5 ± .12[a]	1.2 ± .21[a]	1.5 ± .011[a]	4.5 ± .39[a]	60.2 ± .1[a]	90.6 ± 1.0[a]	23.11 ± .1[a]	11.13 ± .12[a]
B	5.9 ± .22[a]	1.0 ± .09[a]	1.3 ± .02[b]	3.8 ± .19[b]	54.6 ± .41[b]	66.8 ± 1.2[b]	19.31 ± .9[b]	9.4 ± .93[b]
C	4.8 ± .9[b]	1.0 ± .12[a]	1.1 ± .11[a]	3.7 ± .97[b]	57.3 ± .31[b]	59.1 ± 1.09[c]	17.40 ± .92[b]	9.10 ± .94[b]
D	4.3 ± .18[b]	1.2 ± .1[a]	1.3 ± .21[a]	3.3 ± .78[b]	53.4 ± .33[b]	56.3 ± .99[c]	18.6 ± 1.1[b]	10.0 ± .96[b]
E (Control)	7.3 ± .97[b]	1.8 ± .01[b]	1.4 ± .31[a]	4.9 ± .86[a]	56 ± .6[b]	146.5 ± .97[d]	29.49 ± .2[c]	12.3 ± .1[c]

Means ± Standard deviations, down the same column with same superscripts are not significantly different.
A = 5% Crushed Garlic
* = Normal Range
B = 10% Crushed Garlic
V = Value before experimental feeding
C = 20% Crushed Garlic
HDL = High Density lipoprotein
D = 30% Crushed Garlic
LDL = Low Density lipoprotein
E = Control (Zero Garlic)

Table 3. *Lipid Profile of rats feed 5% Garlic supplement in various (forms) therapies.*

Samples	Total cholesterol NR = up to 5.17 (3.6)	Triglyceride NR= 0.7- 1.7 (1.8)	HDL NR= 1.04 – 1.55 (1.6)	LDL NR= 2.31 – 3.55 (1.2) VBEF
A	2.2 ± .9[a]	1.8 ± .11[a]	1.0 ± .1[a]	0.4 ± .01[a]
B	2.4 ± .8[a]	1.9 ± .12[a]	1.1 ± .12[a]	0.5 ± .09[a]
C	2.3 ± .81[a]	1.1 ± .21[b]	1.0 ± .12[a]	0.8 ± .19[b]
D	2.5 ± .96[a]	1.4 ± .34[b]	1.0 ± .61[a]	0.9 ± .08[b]

Means ± Standard deviations, down the same column with same superscripts are not significantly different.
HDL = High density lipoprotein
LDL = Low density lipoprotein
A = 100% rat feed
B = 5% garlic supplement
C = 5% garlic/honeymix
D = 5% garlic water contact
NR = Normal Range
VBEF = Value before experimental feeding

5. Conclusion

This research has collaborated the postulations of traditional believe as well as several other research efforts: garlic has great potentials in the fight against atherosclerosis. The mode of administration (crushed or water extract, garlic – honey mix etc) as well as quantity ingested affects the efficacy. A major observation, is that, the 5% crushed garlic lobe properly mixed into the feed and administered as quickly as possible proved more effective or made significant impact to reduction of total cholesterol, with necessary consideration to feed tolerance.

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