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BIOCHEMICAL STUDIES ON THE EFFECT OF DIET FORMULATED WITH ALLIUM SATIVUM (GARLIC) ON THE GASTROINTESTIONAL SECTIONS OF WISTAR ALBINO RATS.

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ABSTRACT

Biochemical studies on the stomach, large, and small intestines sections of the gastro intestinal tract (GIT) was done using a total of forty eight wistar albino rats. The rats were divided into four groups to represent control, group I (received 2% garlic), group II (received 4% garlic), and group III (received 6% garlic), respectively. Feeding was done for two months while data was collected every two weeks after sacrificing two rats selected from each group under light chloroform anaesthesia. The levels of cholesterol, glucose, and triacylglycerol significantly ($p \leq 0.05$) reduced in a dose dependent fashion throughout the period of study. Similarly, the activities of lipase, and amylase, generally decreased also in a dose dependent fashion in all the GIT sections throughout the study. However the activities of acid phosphates significantly increased in all the GIT sections also in a dose dependent manner. The implication of the result is that garlic enriched diet could have inhibitory effect on important digestive enzymes. However, it could facilitate nutrient transport by enhancing the membrane permeability of the GIT sections.

KEY WORDS: Garlic; GIT sections; Digestive enzymes; Hypoglycemia, Hypolipidemia.

INTRODUCTION

Allium sativum (garlic) is a member of the family *Amaryllidaceae*, which also include Leeks, Onions, and Shallots. It is a perennial with an under ground bulb composed of pungent bulblets known as cloves (Newatt *et al*, 1996). The major active ingredient in garlic is allicin which does not occur naturally. However garlic cloves contain the amino acid allin (S-allycysteine sulphoxide) and when crushed or damaged, allinase (occur naturally in garlic), catalyzes the transformation of allin to allicin (dially thiosulphinate) (Sanjay and Subir, 2002). When allicin is degraded, it produces various diallyl sulphides, the most common being diallyl disulphide.

Garlic reduces high LDL- cholesterol, and dilates peripheral blood vessels which in turn lowers blood pressure thereby reduces the incidence of circulatory problems. Blood pressure increases in response to the synthesis of angiotensin I-converting enzyme (ACE), and garlic contains γ-glutamyl cysteine, a natural ACE inhibitor (Jack,1995). Studies have shown that garlic may indeed help prevent cancer by (i) Stimulating the immune system more efficiently to fight tumour cells; (ii) Directly inhibiting tumour cell metabolism, and (iii) Preventing the initiation and propagating cancer cells. (Benjamin 1991; El-Mofty, 1994). Glutathione–s-transferase, an enzyme involved in the detoxification of carcinogens and other zenobiotics is almost higher among animals eating high garlic diets (El-Mofty, 1994).

Apart from the medicinal properties, garlic is utilized extensively as flavour enhancer in many dishes like soups, sauces, seasoned meats, sausages, and other home dishes. It has been pointed out that spices particularly those with pungent compounds are known to stimulate digestion, precisely through the stimulation of digestive enzymes like pancreatic lipase, amylase, and protease (Platel and Srinivasan, 2004). This increases the rate of nutrient absorption, decreases food transit time, and the rate of deposition of toxic substances that could have caused infection within the GIT. In this study, we intend to determine whether garlic has such stimulating effect on the digestive enzymes. Also, the effect on such biochemical parameters like glucose, and lipids will be investigated with a view to understand the possible mechanism of the medicinal properties attributed to garlic.

MATERIALS AND METHODS

Allium sativum, (garlic) was purchased from the Okigwe central market, Imo state, Nigeria, and was duly certified by a taxonomist.

Animal and Treatment:
Forty eight rats, two months old, mean weight 166.80 ± 0.40g were bought from the department of Zoology University of Nigeria Nsukka. They were divided into twelve rats of four groups and housed in metallic cages and allowed to acclimatize on normal rat feed (Top Feed Nigeria Limited) for one week before introducing the experimental diet. The four group represent the control, and three other different concentration of the garlic as shown below.

Feed Formulation:

Groups	Normal rat feed (g)	Garlic component (g)	Total composition(g)
Control	100.00	0.00	100.00
GP1	98.00	2.00	100.00
GP11	96.00	4.00	100.00
GP111	94.00	6.00	

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Preparation of GIT Sections of Rats:
(Homogenates) Three sections of the GIT, stomach, small and large intestines were studied. Twelve hours after the last ingestion every two weeks, two rats were selected from each group sacrificed under light chloroform anesthesia. Using sterilized dissecting sets, the GIT parts were isolated, and 1.0g of each weighed, and homogenized in 20.0ml of distilled water with the aid of mortar and pestle until a paste was formed. It was then filtered using white muslin cloth before centrifuging at 5000xg for 10min. The supernatant was then used for all the analysis.

Determination of Glucose, Cholesterol and Triacylglycerol (mg/ml) from GIT Homogenates

Glucose was determined according to the method of Trindex (1969), Cholesterol by the method of Roeschlau *et al* (1974), and triglycerol by the method of Fossati and Prencipe, (1982),using spectrophotometer (model SP 300. Optima).

Enzyme Assay (i.u/L) from the GIT sections

Lipase, and acid phosphatase activities were assayed using spectrophotometer (model SP 300. Optima) according to the method of Francis (1996), and Tietz *et al* (1995) respectively while amylase activity was also determined spectrophotometrically by the method of Smith and Roe, (1949).

Statistical Analysis:
The degree of difference between the different values and the control was analyzed using two way ANOVA, where $P < 0.05$ was regarded as statistically significant.

RESULT

The result shows that glucose, cholesterol and Triacylglycerol levels in all the GIT sections significantly decreased ($P< 0.05$) in a dose dependent manner compared to the control (tables 3.1, 3.2, 3.3). The hypoglycemic and hypocholesterolemic effects are important since reduction in values of glucose and cholesterol are utilized in the management of certain ailments, like diabetes, and cardiovascular diseases(Davis, 2000). Similarly, the activities of lipase and amylase decreased significantly in all the GIT sections (tables 3.5 and 3.6,). This implies that garlic does not stimulate digestive enzymes. However, the activity of acid phosphatase increased only in the stomach section, while it decreased in dose dependent manner in the small, and the large intestine sections (tables 3.4). Reduction in activities of acid phosphatase in the small and large intestine may be due to unfavorable P^H condition rather than labilization of membrane.

TABLE

Table 3.1 GLUCOSE CONTENT(mg/ml) OF THE GIT SECTIONS.

GROUP	TIME (Weeks)			
Stomach :	2nd	4th	6th	8th
Control	70.80±3.90	70.10±3.90	69.00±4.70	72.30±7.40
GP I	70.10±3.60	69.20±3.40*	62.20±1.20*	60.10±1.30*
GP II	60.20±1.40*	60.00±1.20*	59.10±0.40*	53.20±2.10*
GP III	50.30±6.30*	50.10±6.10*	48.80±5.50*	44.10±6.60*
Small Intestine				
Control	70.01±0.20	69.84±0.30	70.94±0.20	71.02±0.30
GP I	62.00±2.90	57.14±0.50*	55.41±0.40*	50.08±3.06*
GP II	54.30±2.80*	50.42±0.90*	48.36±0.10*	41.12±3.70*
GP III	40.00±1.40*	38.21±0.50*	36.43±0.40*	34.14±1.50*
Large Intestine				
Control	60.43±1.30	61.40±0.10	63.40±0.85	62.50±1.15
GP I	58.15±2.04*	56.20±0.29*	55.25±1.21*	53.20±0.10*
GP II	50.60±1.18*	49.62±0.23*	47.00±1.06*	46.14±1.47*
GP III	41.20±1.50*	39.80±0.66*	36.10±0.10*	33.50±0.52*

All values are mean ±SD 3 determinations
* = Significantly different from the control values.

Table 3.2 CHOLESTEROL CONTENT (mg/ml) OF THE GIT SECTION.

GROUP	TIME (Weeks)			
Stomach :	2nd	4th	6th	8th
Control	140.30±7.90	150.30±12.70	148.00±13.00	145.50±15.90
GP I	133.20±4.40*	130.50±2.80*	128.30±3.10*	120.30±3.30*
GP II	120.00±2.20*	118.30±6.60*	113.50±4.30*	99.00±7.40*
GP III	104.20±10.10*	100.50±12.20*	98.50±11.80*	90.20±11.80*
Small Intestine :				
Control	192.80±0.50	190.60±0.60	191.40±0.20	192.62±0.40
GP I	174.13±4.20*	167.00±0.70*	161.10±2.30*	160.41±2.60
GP III	153.14±3.60*	148.10±1.13*	142.23±1.80*	139.63±3.00
GPIII	90.13±4.20*	86.20±2.20*	78.40±1.60*	72.10±4.80
Large Intestine :				
Control	170.20±0.38	173.40±1.36	168.93±0.27	172.31±0.63
GP I	152.30±0.15	146.16±0.98	138.32±1.00	130.00±0.58
GPII	126.40±0.06	118.24±0.83	107.42±0.43	98.12±0.64
GP III	85.15±0.01	83.14±0.18	80.40±0.47	76.30±0.08

All values are mean ±SD 3 determinations.
* = Significantly different from the control values.

Table 3.3 TRIGLYCEROL CONTENT (mg/ml) OF GIT SECTIONS.

GROUP	TIME (Weeks)			
Stomach :	2nd	4th	6th	8th
Control	131.20±6.60	138.40±10.60	135.30±11.50	136.20±8.70
GP I	128.30±5.10	121.20±4.10	116.10±1.90	113.00±2.90
GPII	120.50±1.20	118.10±0.50	108.70±1.80	101.20±8.80
GP III	92.10±13.00	90.70±13.20	89.30±11.50	83.40±17.70
Small Intestine :				
Control	152.60±0.95	151.46±0.40	148.20±1.25	150.60±0.05
GP I	137.20±4.05	131.20±1.05	128.60±0.25	119.40±4.80
GP II	103.40±1.90	100.30±0.30	98.70±0.50	96.30±1.70
GP III	84.70±3.15	80.20±0.90	75.10±1.70	73.42±2.50
Large Intestine :				
Control	145.20±1.70	148.00±1.04	142.83±2.58	146.20±0.52
GP I	121.40±1.27	118.23±1.30	116.50±2.00	114.40±1.80
GP II	106.33±1.00	102.62±0.81	98.41±0.42	94.30±1.86
GP III	78.11±0.37	75.10±0.33	70.20±0.77	68.24±0.52

All values are mean ±SD 3 determinations
All values are significantly different from the control values.

Table 3.4 ACID PHOSPHATASE ACTIVITY (I.U/L) FROM THE GIT SECTIONS

GROUP	TIME (WEEKS)			
Stomach :	2nd	4th	6th	8th
Control	7.00±12.10	7.50±2.30	6.90±2.90	7.80±3.00
GP I	9.20±0.90*	10.40±0.80*	12.10±0.30*	13.40±0.20*
GP II	12.40±0.60*	13.30±0.64*	14.00±0.60*	15.50±8.40*
GP III	16.10±2.50*	16.89±2.40*	17.90±0.90*	18.50±1.10*
Small Intestine :				
Control	23.50±0.80	22.80±0.50	21.00±0.50	20.20±0.80
GP I	18.40±0.50*	17.60±0.10*	17.10±0.15*	16.60±0.40*
GP II	14.30±0.30*	11.00±0.15*	13.60±0.05*	12.70±0.50*
GP III	10.00±0.25*	10.00±0.15*	10.10±0.10*	9.80±0.25*
Large Intestine :				
Control	22.41±0.03	21.00±0.15	20.50±0.15	22.15±0.08
GP I	20.00±0.17	16.50±0.07	18.30±0.14	17.42±0.19*
GP II	17.40±0.19*	16.83±0.01*	15.10±0.29*	14.33±0.41*
GP III	14.31±0.34*	13.42±0.19*	12.18±0.58*	11.14±0.45*

All values are mean ±SD 3 determinations
* = Significantly different from the control values

Table 3.5: AMYLASE ACTIVITY (I.U/L) FROM THE GIT SECTIONS.

GROUP	TIME (Weeks)			
Stomach :	2nd	4th	6th	8th
Control	1.51±0.11	1.50±0.20	1.61±0.23	1.52±2.20
GP I	1.42±0.06	1.21±0.02*	1.10±0.03*	1.05±0.05*
GP II	1.20±0.03*	1.11±0.04*	1.10±0.07*	0.93±0.06*
GP III	0.90±0.20*	0.91±0.13*	0.80±0.04*	0.72±0.20*
Small Intestine :				
Control	1.70±0.09	1.51±0.05	1.48±0.02	1.37±0.08
GP I	1.53±0.06	1.42±0.01	1.39±0.05	1.32±0.04
GP II	1.46±0.05	1.38±0.01	1.34±0.01	1.26±0.05
GP III	1.34±0.07	1.30±0.05	1.24±0.02	0.95±0.12
Large Intestine :				
Control	1.60±0.01	1.68±0.01	1.71±0.05	0.58±0.01
GP I	1.85±0.04	1.40±0.07	1.37±0.10*	1.30±0.05*
GP II	1.36±0.02*	1.30±0.06*	1.28±0.06*	1.15±0.04*
GP III	1.28±0.03*	1.24±0.05*	1.20±0.08*	1.00±0.04*

All values are mean ±SD 3 determinations
* = Significantly different from the control values.

Table 3.6: LIPASE ACTIVITY(i.u/L) FROM THE GIT SECTIONS.

GROUP		TIME (Weeks)		
Stomach :	2nd	4th	6th	8th
Control	ND	0.70±0.10	0.60±0.30	0.40±0.70
GP I	ND	0.60±0.07	0.50±0.06	0.35±0.40
GP II	ND	0.35±0.05	0.25±0.01	0.20±0.03
GP III	ND	0.20±0.13	0.15±0.10	0.10±0.80
Small Intestine :				
Control	ND	0.39±0.05	0.33±0.02	0.17±0.07
GP I	ND	0.28±0.04*	0.14±0.06*	0.14±0.04
GP II	ND	0.22±0.03*	0.17±0.01*	0.11±0.03*
GPIII	ND	0.17±0.03*	0.11±0.01*	0.06±0.02*
Large Intestine :				
Control	ND	2.00±0.03	2.30±0.05	2.00±0.05
GP I	ND	1.50±0.06	1.33±0.03*	1.00±0.10*
GP II	ND	1.00±0.07*	0.83±0.06*	0.67±0.03*
GP III	ND	0.67±0.03*	0.50±0.02*	0.33±0.06*

All values are mean ±SD 3 determinations
* Significantly different compared to the control values

DISCUSSION

It has been reported that some spice principles enhance the activity of intestinal enzymes leading to increase in the rate of digestion, decreased in food transit time, and improved nutrient absorption (Platel and Srinivasan, 2004). Reduction in activities of acid phosphatase and alkaline phosphatase within the GIT sections has been attributed to severe labilization of the plasma as well as the lysosomal membranes (Ngaha,1982). Based on these, we studied the biochemical effect of A. Sativum on the GIT sections of rats. The result showed hypoglycemic effect which is dose dependent (Table 3.1). This effect persisted throughout the period of the study. This is important because sometimes hypoglycemic effect of certain medicinal plants e.g. *Piper guinense*, reverts when the assay is prolonged (In pres). Garlic has been reported to dilate peripheral blood vessels (Jack, 1995), which probably enhances glucose transport and utilization. There is need to evaluate this effect on the serum glucose level since hypoglycemic value in this tissue can be of benefit medically in the treatment of diabetic conditions.

There was also significant reduction in the cholesterol and triacylglycerol levels in a dose dependent manner in the GIT sections possibly by (i) Inhibition of small intestine biosynthesis of cholesterol.

(ii) Stimulation of pancreatic bile secretion which plays a role in increased absorption of cholesterol and hence hypocholesterolemia (Murray et al, 1990). It is also ideal to investigate whether similar effect could be observed in the serum. Jack (1995), reported that garlic reduced low density lipoprotein and thus the risk of circulatory problem. Infact the first lesion of artherosclerosis is considered to be the fatty streak which begins to appear when LDL particles change and form deposits of cholesterol and other lipids on the arterial intima (Davis, 2000) In tables 3.4, the activities of acid phosphatase significantly decreased in a dose dependent fashion as compared to the control. According to Platel and Srinivasan (2004), ginger and other spice principles are known to enhance the activity of intestinal enzymes. Serum elevated levels of acid phosphatase is associated with bone diseases and prostatic cancer. Activities of amylase and lipase (table 3.5, 3.6) significantly reduced ($P < 0.05$) as compared to the control. According to Adesokan and Akanji (2003), labilization of membranes of the GIT sections due to administration of E-chloranta, an antimalaria extract can lead to reduction in the activities of such enzymes like alkaline phosphatase, acid phosphatase and amino transferase. However if there is labilization, there should be a corresponding increase in the serum activities of the enzymes (Adesokan and Akanji (2003). In this study, the significant reduction in the activities of the enzymes (lipase, amylase) could be attributed to inactivation or inhibition by some active ingredients of garlic. Infact the sulphur containing amino acid component of garlic (r-glutamyl cysteine) can inhibit a number of enzymes.

SUMMARY AND CONCLUSION

The hypoglycemic, and hypocholesterolemic effects of garlic diet within the GIT sections have been demonstrated in this study. The result of enzyme activities shows that garlic does not stimulate digestive enzymes, rather they were inhibited (amylase, lipase). There is also the possibility of GIT membrane labilization due to garlic intake. However to establish this the values of the parameters studied should be evaluated in the serum.

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