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The effects of garlic (*Allium sativum*) on taste responses and relative organ weight in rats.

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Abstract
This study evaluated the effects of Garlic (*Allium sativum*) on taste responses and relative organ weights in albino rats. Graded dietary supplementations of garlic viz: 0, 5, 10, 15, 20, and 25 % administered to seventy-five (75) disease-free stock of weanling albino rats of the Wistar strain (all males) in five groups of fifteen (15) rats each for 30 days was designed for this study in a complete randomized manner. A sixth group of fifteen rats served as the control (zero supplementation). Garlic supplementation showed a modulatory effect on taste responses in rats. At 5 and 10 % supplementation, there was strong preference for salt. On the other hand, at 15 and 20 % supplementation, sucrose and glutamic acid were moderately preferred while at 25 % supplementation a strong preference for quinine sulphate occurred. There was no significant ($p<0.05$) effect on the relative carcass weights of the testes and kidneys. However, the relative weights of the liver and spleen were significantly affected particularly at higher supplementation levels. At 20 % supplementation, the liver weight was decreased from a control value of 8.4 ± 0.2 to 5.9 ± 1.5 grams (g) while the spleen was increased from a control value of 9.8 ±0.6 to 15.1±1.2 g. Likewise at 25 % supplementations the liver weight was further decreased from 8.4±0.2 to 4.9± 1.7 g while the spleen was increased from 9.8 ± 0.6 to15.4± 1.8 g. This study reveals that garlic when consumed in excess exerts modulatory effects on taste response which could irregularly influence appetite and food selection. Also high dietary levels in mammalian diet may predispose them to liver and spleen damage. It is therefore recommended that garlic intake should be regulated to avoid some possible adverse effects associated with high levels of intake.

Keywords: Garlic, organo-somatic index, rats, taste.

Introduction

Allium sativum commonly known as garlic is a specie in the onion family *Alliaceae* (Eric, 2010). It`s close relatives include the onion, shallot, leek and chive. Garlic has been used throughout history for both culinary and medicinal purposes. It has a characteristic pungent spicy flavor that mellows and sweetens considerably with cooking (Gernot, 2005). Garlic has attracted particular attention of modern medicine because of its widespread health use around the world, and the cherished belief that it helps in maintaining good health wading off illnesses and providing more vigor. To date, many favorable experimental and clinical effects of garlic preparations, including garlic extract, have been reported (Durak *et al*., 2002). These biological responses have been largely attributed to i) reduction of risk factors for cardiovascular diseases and cancer, ii) stimulation of immune function, iii) enhanced detoxification of foreign compound, iv) hepatoprotection, v) antimicrobial effect and vi) antioxidant effect.

In *in-vitro* studies garlic has been found to have antibacterial, antiviral and antifungal activities. Garlic is also claimed to help prevent heart diseases (including atheriosclerosis, high cholesterol, and high blood pressure) and cancer (Durak *et al*, 2002). Animal studies and some early investigational studies in humans, have suggested possible cardiovascular benefits of garlic by reducing accumulation of cholesterol on the vascular walls of animals (Sonova & Sova, 2004). It has been shown that supplementation with garlic extract inhibits vascular calcification in human patients with high blood cholesterol (Durak *et al.*, 2004). Although these studies showed protective vascular changes in garlic fed subjects, clinical trial in human subjects showed that the consumption of garlic in any form did not reduce blood cholesterol levels in patients with moderately high baseline cholesterol levels (Charlson & McFerren 2007; Gardner *et al*., 2007). Also, there are no reported effects of raw garlic or garlic supplements on low density lipoprotein (LDL), high density lipoprotein (HDL) or triacylglycerols (Charlson & McFerren, 2007).

Likewise, information on the hematological effects of *Allium sativum* have been well documented such as reduced platelet aggregation (Borreli *et al.,* 2007; Chan *et al*., 2007; Rahman, 2007) and hyperlipidemia (Steiner & Lin, 1998; Kojuri *et al.,* 2007), sugar levels in blood (Chang & Johnson, 1980). However, there is paucity of

information on the effect of garlic on taste modulation and organ weights as there is a direct relationship between taste and food consumption which in turn affects body weight, organ development and growth particularly at the early stages of life. Thus the aim of this study was to assess the effect of dietary supplementation of raw garlic on taste response and relative organ weights in growing albino rats.

Materials and Methods

Animals/Feeding

Sixty (60) weaned albino rats (both sexes) of the Wistar strains purchased from Sokoto Sokoto State, Nigeria were used for this study. They had an average body weight of 183.4±3.5g upon purchase. They were kept in metal cages for a twenty-one day (21) acclimatization period, during which they were fed with poultry growers (Vital feed® Jos-Nigeria) only. Water was provided *ad libitium*. Within the acclimatization period those that showed unthriftness were removed and replaced with other healthy ones that were kept for replacement. No medication was given except multivitamins (Vitalyte®) which was given three times in a week. They were later divided into six groups of ten rats each and each group was kept in a metal cage with a flat base made of metal. Grouping was according to body weight. Feed was offered daily *ad libitium* in a metal cup that was fastened to the cage to avoid spillage. The daily feed consumption of each group was ascertained by subtracting the weight of food left in the metal cup after a 24 hour feeding period from weight of the feed offered.

Plant collection and preparation

Dried cloves of garlic obtained commercially from local farmers were pulverized, using a local mortar, into fine granules of powder. After pulverization, the powder was further dried under the sun for proper desiccation. A total of 3 kilograms of dried raw garlic powder was obtained and stored in well cocked plastic container at room temperature until use.

Experimental design and experimentation

Five groups of ten rats each were designated groups A, B, C, D and E. The sixth group (F) of ten rats served as the control. The average daily ration of each group, as obtained during the acclimatization period, were supplemented daily with garlic powder at 5, 10, 15, 20 and 25 % supplementations for groups A, B, C, D and E respectively for 30 days as earlier described by Rahman, (2007).

Taste response evaluation

During the 30 day feeding period, starting on day 5 during which the animal must have adapted to the effect of garlic (Kamalu *et al.,* 2006), five taste test solutions prepared as described by Kamalu, *et al* (2006); namely, 0.5 % salt solution (T_1), 2 % sucrose solution (T_2), 0.09 % Quinine sulphate solution (bitter) (T_3), 5 % Sodium hydrogen trioxocarbonate solution (sour) (T_4) and 10 % Glutamic acid solution (umami) (T_5) were offered daily and simultaneously to all groups for 24 hrs using metal nipple drinkers. The daily consumption of each taste test solution per group was recorded after each 24 hr period (daily) of presentation to the rats as described by Aka (2010). Percentage preference (response) was directly related to percentage consumption which was given as follows:

$$\% \text{ Consumption} = \frac{\text{Quantity offered} - \text{Quantity not consumed}}{\text{Quantity offered}} \times 100$$

The percentage preference score was as given by Goatcher & Church (1970a).

Relative organ weight

Five rats in each group and control were sacrificed on day 31 by cervical dislocation and weighed. The liver, spleen, testes and lungs were removed from each rat carcass and weighed using Metler weighing balance (EB 3006 SEW, England). The mean weight of each organ relative to the mean carcass weight was obtained for each group. The relative weight of individual organ to the total carcass weight was calculated as follows:

$$\frac{\text{Weight of organ}}{\text{Total carcass weight}} \times 100$$

Statistical analysis

The mean differences in the daily consumption of each test taste solution as well as the weights of different visceral organs at different levels of garlic supplementation were analyzed by the analysis of variance (ANOVA). The means were compared for statistical variation by the least significant difference method of mean comparison. (SPSS version 1.0).

Results

The results of the study are presented in table 1 and 2. In table one, the preference for salt was significantly ($p<0.05$) high at 5 and 10 % garlic supplementation with 60 and 67 % consumption respectively compared to other levels of supplementations. Sucrose was moderately preferred at 15 and 20 % supplementation with percentage consumption of 65 and 64 % respectively. Contrary to salt and sucrose, the preference for quinine sulphate (bitter), sodium hydrogen trioxocarbonate (sour) and glutamic acid (umami) appeared to be dose dependent as preference tend to increase as garlic supplementation increase. This trend was however more evident with quinine sulphate which had 68 and 88 % consumption (moderate and strong preference respectively) at 20 and 25 % supplementation levels respectively.

Table 2 shows the relative organ weights of rats following daily intake of garlic supplemented feeds for the 30 day feeding period. There was significant decrease in the relative weight of the liver particularly at 20 and 25 % supplementation levels with values at 5.9±1.5 and 4.9±1.7 respectively compared to the control which had a relative liver weight of 8.4±0.2. At other supplementation levels there were no significant ($p<0.05$) differences in the relative organ weights within treatment as well as when compared with the control. For the spleen, there was an increase in the relative organ weight at all supplementation levels. The increase was however significant ($p<0.05$) at 20 and 25 % levels of

supplementation. There were no treatment effects on both the testes and the kidney.

Table1: Taste Responses in rats fed graded supplemental levels of *Garlic* (*Allium sativum*)

Treatments	Absolute percentage preferences at various supplementation levels					
	0 %	5 %	10 %	15 %	20 %	25 %
Salt	36^{b}	60^{a}	67^{a}	39^{b}	40^{b}	38^{b}
Sucrose	46^{a}	43^{a}	47^{a}	65^{b}	64^{b}	50^{a}
Quinine sulphate	44^{a}	51^{a}	48^{a}	43^{a}	68^{b}	88^{c}
$NaHCO_3$	32^{a}	36^{a}	38^{a}	35^{a}	40^{a}	42^{a}
Glutamic acid	50^{a}	48^{a}	52^{a}	66^{b}	67^{b}	69^{b}
Water	58^{a}	55^{a}	46^{b}	44^{b}	66^{c}	68^{c}

a,b,c= means within row with different superscript are significantly different at $p<0.05$

Table 2: Relative organ weights (%) in rats fed graded supplemental levels of Garlic (*Allium sativum*)

Treament group	*Total BW*	*Liver*	*Spleen*	*Testes*	*Kidney*
A (5%)	198.3±4.5	$7.9±0.6^{a}$	$11.1±1.0^{a}$	9.2±0.0	11.1±1.3
B (10%)	201.2±6.2	$8.7±0.3^{a}$	$10.0±1.\ 8^{a}$	9.1±0.1	11.7±2.0
C (15%)	193.4±4.2	$9.3±0.7^{a}$	$12.1±0.4^{a}$	9.3±0.0	11.4±1.2
D (20%)	196.1±6.3	$5.9±1.5^{b}$	$15.1±1.2^{c}$	9.8±1.1	12.6±1.5
E (25%)	201.5±5.5	$4.9±1.7^{b}$	$15.4±1.8^{c}$	10.3±0.2	13.1±1.4
Control	197.8 ±7.1	$8.4±0.2^{a}$	$9.8±0.6^{a}$	9.3±0.1	12.2±1.2

a,b,c= means within column with different superscript are significantly different at p<0.05.

Discussion

This study has demonstrated that *Allium sativum* (Garlic) is a potential modulator of taste responses in mammals using rat as a model. Its ability to influence the weight of some vital visceral organs is however probably not in doubt. It was observed that different supplementation levels of garlic resulted in different forms of taste responses (Table 1). There was moderate preference for salt and indifference response to quinine sulphate and glutamic acid at 5 and 10 % supplementation levels with corresponding negative modulatory effect on sucrose and sodium hydrogen trioxocarbonate. This observation suggests that even at low consumption rate of garlic, rats (which by extension, mammals) are likely to have high threshold for salt compared to all other tastants. Increase in threshold for any tastant implies that high concentration of such substance is required to trigger off its sensation.

It was also observed that increasing garlic supplementation beyond 10 % had no significant effect on taste responses to salt. On the contrary, at 15 % garlic supplementation a progressive preference of 65 % for sucrose was recorded compared to the control preference value of 46 %. This indicates that increasing the intake of garlic up to 15 % of daily dietary intake or prolonged consumption of it would cause a strong stimulatory effect on sucrose intake with minimal effect on other investigated tastants. This probably could be due to stimulatory effect of garlic, at this consumption level, on the chorda tympani nerve that supply the proximal two third of the tongue that detect sweet taste. It could also be that the threshold of the tatse buds for sucrose at the proximal two third was decreased at this level of garlic supplementation.

We also observed that taste responses to Quinine sulphate and glutamic acid was enhanced at 20 and 25 % garlic supplementation, indicating that higher consumption of garlic could predispose mammals to higher threshold levels for or tolerance to Quinine sulphate (bitter) and glutamic acid (amino acid) solutions.

Changes in taste responses have been described to result from changes in the number of amiloride-sensitive sodium (Na) channels in taste receptors as well as the responsiveness of isolated taste receptors cell (Froloff, Faurion, & Macload, 1996). This probably accounts for the modulatory effects of raw garlic on taste responses as observed in this study. Again, taste responses have been shown to be influenced by hormonal effects, particularly reproductive hormones, on the peripheral nerves (Thompson, 2004). It is probable that the taste response observed at different supplementation levels were influenced by levels of reproductive hormones in serum. It would be worthwhile therefore to evaluate the relationship between garlic consumption and serum levels of reproductive hormones in male and female rats in other to lay better credence to their possible interactions. Different sensory nerves are involved in detecting the taste of different solutions in different animals (Smith & Frank, 1993). An animal's response to different taste solutions is also based on the diversity of the chemical structure of solutions and the interpretation of genetic, physiological and behavioral attributes (Froloff *et al*, 1996). These variables could have played some role along side with the primary effect of the raw garlic in taste modulation and responses observed in this study. This however provides avenues for broader investigations for a better understanding of the taste modulatory mechanism of raw garlic.

The observed effects of garlic supplementation on the relative organ weights showed that higher garlic supplementation decreased the relative weight of liver and increased that of the spleen. There was however no significant effect on those of the testes and kidney (see Table 2). In another study, garlic oil showed a clear improvement in kidney functions, perhaps due to the antioxidant properties of garlic in scavenging free radicals leading to increased of superoxide dismutase production and consequently reduced lipid peroxidation (Hanaa, Sherif, Rajiv, Augusta, Madhiva, & Allal, 2009, Veena, Arti, & Leena, 2010). Garlic extracts have been observed to possess hypotensive action, anti-platelet function and activation of the endogenous antioxidants, superoxide dismutase and catalase in laboratory rats (Banerjee, Pulok, & Mukherjee, 2003).

The observed decrease in the weight of the liver relative to the total carcass probably suggests that high garlic intake probably predisposed the rats to poor liver development. *Allium spp*. are known to contain N-propyl disulphide. Excess intake of *Allium spps*. has been associated with several syndromes including hemolytic anemia, blindness, pulmonary emphysema, digestive disturbances, pallor, hemoglobinuria, jaundice (in chronic conditions) (Radostits, Clive, Douglas, & Kenneth, 2000). Histological findings in allium poisoning have revealed moderate periacinar, hepatocyte necrosis in the liver, compatible with the effects of hypoxia. Thus at high dose (20 to 25 %) supplementation, liver poisoning probably occurred. The observed increase in spleenic weight at high intake of garlic (20 and 25 % supplementation) was suggestive of splenomegaly. This has been previously described in excessive intake of *Allium spps.* (Radostits *et al*., 2000).

Various preparations of garlic, mainly Aged Garlic Extract, have been shown to have promising antioxidant potential. However, the presence of more than one compound in garlic, with apparently opposite biological effects, has added to the complexity of the subject. Raw garlic homogenate has been reported to exert antioxidant potential but higher doses have been shown to be toxic to the heart, liver and kidney (Banerjee *et al*., 2003). This point probably explains why there were marked liver atrophy and splenomegaly at higher doses of the raw garlic used in this study.

In conclusion, this study has demonstrated that garlic modulates taste responses in mammals with differences in taste preferences at different supplemental levels. The taste effects on taste preferences at any tolerable level, in line with other effects, could be exploited by adding garlic at such levels that could favor the intake of a desired tastant or diets containing such tastants. Also, at higher supplemental levels (above 10 %) possible deleterious effects occurred on the liver and spleen indicative of poor liver development or atrophy and splenomegaly respectively. Though, various beneficial effects of garlic have been spelt out in literatures, the result of this experiment provides some evidences suggestive of its restriction to not more that 10 % in diet.

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