African Journal of Biotechnology Vol. 10(35), pp. 6804-6808, 13 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2598
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effect of garlic (*Allium sativum*) on *Salmonella typhi* infection, gastrointestinal flora and hematological parameters of albino rats

Adebolu, T. T., Adeoye, O. O. and Oyetayo, V. O.*

Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.

Accepted 2 June, 2011

The effect of consumption of garlic (*Allium sativum*) in treating *Salmonella typhi* infection and on the gastrointestinal flora and hematological parameters of rats was investigated. Crude garlic extract inhibited the growth of *S. typhi* on agar plate with a zone of inhibition averaging 23.8 mm in diameter using the agar diffusion assay. This inhibition was superior to that of all the conventional antibiotics used except streptomycin which gave a growth inhibitory value of 24.0 mm. In the *in vivo* assay, although, pretreatment with the extract did not protect rats orogastrically dosed with the infective dose of *S. typhi* from the infection, the consumption of crude garlic extract however, caused a significant ($p \leq 0.05$) reduction in the number of *S. typhi* in the feaces of the infected rats from 2.0×10^8 to 9.0×10^1 cfu/ml and also reduced the duration of infection from 5 to 3 days. On the effect of consumption of garlic extract on the type and load of gastrointestinal flora and hematological parameters of rats, garlic extract caused a significant ($p \leq 0.05$) reduction in the type of bacteria present in the gastrointestinal tract of rats fed 1 ml daily for 7 weeks from six microbial types to one microbial species, reduction in microbial load of the flora from 1.64×10^{12} to 1.3×10^7 cfu/ml, reduction in packed cell volume (PCV), total white blood cells (WBC) and lymphocytes counts but caused an increase in neutrophils and monocytes counts of the rats. The observed increases in the neutrophils and monocytes counts of the blood suggested that garlic may possess immune-stimulatory effect in addition to its antibacterial effect.

Key words: *Salmonella typhi, Allium sativum,* antibacterial activity, rats.

INTRODUCTION

Salmonella typhi is the etiological agent of typhoid fever in man. This rod shaped, Gram negative bacterium is transmitted by milk, water, or solid food contaminated by feaces of typhoid victims or of carriers, that is, healthy persons who harbor typhoid bacilli without presenting symptoms (Jay, 1978). Symptoms of infection include nausea, vomiting, abdominal cramps, diarrhea, fever and headache. In chronic cases, arthritis symptoms may follow 3 to 4 weeks after the onset of acute symptoms (Cheesbrough, 1994; Prescott et al., 2002). The infection is normally treated using antibiotics such as chloromycetin, ampicillin, amoxycillin, ciprofloxacin (Morgan, 2007). Some *Salmonella* bacteria however, have become resistant to antibiotics; therefore there is the need to search for alternative therapeutic measures.

Garlic (*Allium sativum*) is a perennial plant in the family Alliaceae, a member of the same group of plants as the onions (Damir and Davor, 2004). Garlic is well known for its medicinal, antibacterial and pesticidal properties. It has also been shown to possess antiparasitic and anti-tumour properties. In addition, garlic extract has been reported to show an *in vitro* growth inhibition effect against a large number of yeasts including *Candida* species and fungi such as *Coccidiodes immitis* (Tung-Nsi and Chung-Mag, 1989). Syrup of garlic is an invaluable medicine for treating asthma, hoarseness, coughs, difficulty of breathing and most other disorder of the lungs (Amagase et al., 2001). Since garlic has been reported by many researchers, that it has antibacterial property and that it is being used to treat many ailments

*Corresponding author. E-mail: ovofuta@yahoo.com.

(McMahon and Vargas, 1993; Microsoft Encarta, 2008; Mikail, 2010), it became worthwhile to investigate its potential as an alternative for treating *S. typhi* infection. This study therefore was designed to assess antibacterial activity of garlic against *S. typhi* and also to examine the effect of garlic on the hematological and gastrointestinal flora of rats.

MATERIALS AND METHODS

Test bacteria

Stock cultures of *S. typhi* used in this study were collected from Phelab Medical Microbiology Laboratory, Akure, Ondo State, Nigeria. They were confirmed in the laboratory through Gram's staining, culturing on appropriate selective media and subjection to various biochemical tests.

Experimental animals used

Albino rats, eight to ten weeks old were used for this assay. These rats were got from the Animal Production and Health Department, Federal University of Technology, Akure, Ondo State, Nigeria.

Garlic bulbs used

Fresh garlic (*A. sativum*) bulbs were purchased from Oba's market, Akure, Ondo State, Nigeria.

Source of antibiotics used

The antibiotics used in this study were purchased from a National Agency for Food Drug Administration and Control (NAFDAC) approved pharmaceutical shop in Akure, Ondo State, Nigeria.

Preparation of crude garlic extract

Garlic (bulblets) cloves were cleaned and surface sterilized using hypochloride solution and were again washed in sterile distilled water to remove any trait of the hypochloride solution. The cloves were then deskinned and crushed using sterile mortar and pestle. The filtrate was obtained by filtering the crushed garlic using sterile cheese cloth and the extract was collected into sterile universal bottle and then covered and labelled appropriately.

Determination of antibacterial activity of crude garlic extract on the test organism

This was carried out using agar diffusion method. One millilitre of *S. typhi* containing 10^7 cfu/ml was dispensed into a sterile Petri dish with the aid of a sterile syringe and needle, 10 ml of already prepared nutrient agar cooled to about 45°C was aseptically poured into the plate, swirled for even distribution of the bacterial cells, and left to gel. Two wells were made on the plate using a sterile cork borer, garlic extract was introduced into one of the wells, while sterile distilled water was put into the other well, this served as negative control. The plates were incubated at 37°C for 24 h after which the plates were observed for zones of inhibition. The diameter of the zones of inhibition was measured using a transparent ruler.

Determination of the minimum inhibition concentration (MIC) of crude garlic extract on *S. typhi*

To determine the MIC, crude garlic extract was diluted serially (0.1, 0.01, 0.001) using sterile distilled water (volume by volume) into a set of sterile tubes. Each tube was inoculated with 1 ml of *S. typhi* containing 10^7 cfu/ml and the tubes were plated using pour plate technique to enumerate the viable count after incubation at 37°C for 24 h.

Assessment of the growth inhibitory activity of some antibiotics on the test organism

Instead of boring two wells and introducing garlic extract, conventional antibiotic discs were placed on the surface of plates of *S. typhi* that were prepared as aforestated. The antibiotics served as positive control. The plates were incubated at 37°C for 24 h after which the plates were observed for zones of inhibition. The diameter of the zones of inhibition was measured using a transparent ruler.

Challenging apparently healthy rats with *S. typhi* infective dose and treatment with crude garlic extract

Ten rats were orogastrically challenged with the infective dose of *S. typhi* which was calculated to be 2.0×10^8 cfu/ml for the rats used in this study and after infection had set in, were fed with crude garlic extract, 1 ml daily. Their stool was daily observed and analyzed to determine whether garlic had reduced or eliminated *S. typhi* from their stool and the animals were also examined for any sign of recovery. Apparently, healthy rats dosed with the organism but not treated served as control.

Assessment of the administration of crude garlic extract to protect apparently healthy rats from *S. typhi* infection, effect on their GIT flora and hematological parameters

Ten rats were fed with crude garlic extract in addition to their normal feed (Grower's mash) for seven weeks (1 ml daily) using feeding loop. Their stool was collected weekly to check the microbial load and types using standard microbiological techniques. At the end of seven weeks, four of the rats were randomly picked and their blood was collected for hematological assay using the method of Cheesbrough (1994). The remaining rats were challenged with the infective dose of *S. typhi* and observed for signs of infection. Their stools were daily analyzed to determine whether garlic had reduced or eliminated *S. typhi*. Apparently, healthy rats not fed with garlic were used as control.

Isolation, identification and enumeration of the microbial flora in the GIT of apparently healthy rats

Faeces of rats that were caged separately were aseptically taken and 1 g each was introduced into separate McCartney bottles and serially diluted. One milliliter of each suspension was plated separately using pour plate technique on nutrient agar for bacterial growth and potato dextrose agar for fungal growth and the plates were incubated at 37°C for 24 h and at room temperature (30 $\pm$ 2°C) for 72 h respectively after which the resulting number of colonies were counted. For isolation, spreading method was used instead of pour plate technique and the resulting colonies were subcultured to obtain pure cultures before they were identified using standard microbiological techniques.

Table 1. Diameter of zones of inhibition of the growth of *Salmonella typhi* using crude garlic extract or standard commercial antibiotic discs.

Treatment	Average diameter of zone of inhibition (mm)
Garlic extract	23.8
Streptomycin	24.0
Gentamycin	22.0
Chloramphenicol	20.0
Ofloxacin	19.5
Erythromycin	16.0
Penicillin	0.0
Ampicillin	0.0
Cloxacillin	0.0
Sterile distilled water (Control)	0.0

Statistical analysis

All experiments were carried out with replicates. Mean were compared using Duncan's multiple range tests (SPSS 11.5 version). Differences were considered significant at $p<0.05$.

RESULTS

Growth inhibitory activity of crude garlic extract on *S. typhi*

Crude garlic extract inhibited the growth of *S. typhi* on agar plate with diameter of zone of inhibition averaging 23.8 mm (Table 1). The minimum inhibitory concentration of the crude garlic extract on the organism was observed to be 0.01%.

Growth inhibitory activity of standard commercial antibiotics on *S. typhi*

Streptomycin gave the highest growth inhibition with diameter of zone of inhibition of 24.0 mm on the growth of *S. typhi* on agar plate followed by gentamycin (22.0 mm), antibiotics like ampicillin and cloxacillin did not inhibit the growth of the organism at all (Table 1).

Effect of administration of crude garlic extract on rat infected with *S. typhi* infective dose

Administration of crude garlic extract to rats infected with *S. typhi* caused the rats to start recovering by the fifth day, unlike the control rats that did not recover throughout the duration of the experiment (Table 2).

Effect of feeding apparently healthy rats with crude garlic extract in protecting them against *S. typhi* infection

Feeding of apparently healthy rats with garlic for 7 weeks did not protect them from becoming infected with the infective dose of *S. typhi* used. The rats fed with garlic started showing symptoms of infection compared with the control rats that were not given garlic after 24 h of infection. The rats became weak, their fur became scattered, fell off and their stool not formed. Administration of crude garlic extract to treat the infected rats however, caused the rats to start recovering by the third day unlike the control rats that were weak throughout the duration of the experiment (Table 2).

Effect of treating infected rats with crude garlic extract on the load of *S. typhi* recovered in their faeces

For rats fed with crude garlic extract for 7 weeks before infecting with the infective dose of *S. typhi*, there was a reduction in the load of *S. typhi* in the faeces from 2.0×10^8 to 1.0×10^1 cfu/ml by the 5th day of daily feeding them with 1 ml crude garlic extract. By the 6th day of feeding with the extract, the organism was no longer found in their faeces. There was also a reduction in the load of *S. typhi* in the faeces of infected rats not fed with crude garlic extract prior infection with the organism but treated with the extract when infection had set in, the load reduced from 2.0×10^8 to 9.0×10^1 cfu/ml by the 6th day of daily feeding them with 1 ml crude garlic extract and the organism was no longer seen by the 7th day of feeding with the extract (Table 3).

Types and number of bacteria isolated from the intestine of apparently healthy rats after feeding with garlic

A total of six different types of bacteria were isolated from the faeces of apparently healthy rats before feeding with garlic. These were *Aerobacter aeromonas, Lactobacillus jenseni, Staphylococcus epidermidis, Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Escherichia freundii.* The microbial population was 1.64×10^{12} cfu/ml. One week after feeding with garlic, the type of organisms present reduced to two; *B. subtilis* and *E. coli* with load of 4.0×10^9 cfu/ml. Two weeks after feeding with garlic, *E. coli, B. subtilis,* and *E. freundii* were isolated but with slight reduction in population to 1.2×10^8 cfu/ml. By the 5th week however, a new organism emerged, *Zygosaccharomyces bailli*, a yeast which persisted to the 6th week. By the 6th week, the total microbial load reduced to 6.0×10^7 cfu/ml and by the 7th week, the microbial load reduced to 1.3×10^7 cfu/ml with *E. coli* being the only organism isolated (Table 4).

Table 2. Effect of feeding rats infected with *S. typhi* with crude garlic extract.

Day	Appearance of rat and their stool		
	Rat infected and treated with garlic	**Infected rat**	**Control rat**
1	W, SF, LA	W, SF, LA	W, SF, LA
2	W, SF, LA, US,FO	W, SF, LA, FO, US	W, SF, LA, FO, US
3	W, SF,LA, US,FO	A, LA.SF,SFS	VW, SF, LA, FO, US
4	W, SF,LA, US,FO	VA, FS	W, SF, LA, FO, US
5	A, SPF, SF	VA, FS	W, SF, SPF
6	A, SPF	VA, FS	W, SF, SPF
7	A, SPF	VA, FS	W, SF, SPF
8	A, SPF	VA, FS	W, SF, SPF

A= Active; SF= Scattered fur; FO= Fur falling off; LA= Loss of appetite; W= Weak; VW= Very weak; SPF= Stool partially formed; US= unformed stool; FS= Formed stool.

Table 3. Effect of pretreatment of apparently healthy rats with crude garlic extract on the recovery of *S. typhi* in the feaces of rats infected with the organism and treated with the extract.

Day	***S. typhi* count (cfu/ml)**	
	Rat pretreated with garlic	**Control rat**
1	4.0×10^5	1.5×10^7
2	5.0×10^3	8.0×10^6
3	8.0×10^2	1.0×10^6
4	6.0×10^2	1.9×10^4
5	1.0×10^1	5.0×10^2
6	0.0	9.0×10^1
7	0.0	0.0
8	0.0	0.0

Table 4. The effect of garlic extract on the types and load of microbial flora in the faeces of apparently healthy rats.

Week	Organism isolated	Total plate count (cfu/ml)
0	*E. coli, A. aeromonas, L. jenseni, S. epidermidis, B. subtilis, S. aureus* and *E. freundii*	1.64×10^{12}
1	*E.coli* and *B. subtilis*	4.0×10^9
2	*E. coli, B. subtilis,*and *E. freundii*	2.8×10^9
3	*E. coli, .S. aureus* and *E. freundii*	1.4×10^8
4	*E. coli* and *S. aureus*	1.2×10^8
5	*E. coli* and *Z. bailli*	9.4×10^7
6	*E. coli* and *Z. bailli*	6.0×10^7
7	*E. coli*	1.3×10^7

Effect of feeding apparently healthy rats with crude garlic extract on their hematological parameters

Table 5 shows the effect of feeding rats with crude garlic extract on their hematological parameters. The average PCV of rats fed with garlic reduced from 42.3 to 26.4% when compared with that of the control rats. However, the neutrophils and monocytes of rats fed with garlic on the other hand had higher values than the values in the control rats.

DISCUSSION

The *in vitro* and *in vivo* assays employed in this study showed that garlic (*A. sativum*) has antibacterial activities against *S. typhi.* Garlic contains several components such as alliin, alline, ajorene, diallyl sulfides (DAS), diallyl disulfides (DADS), diallyl trisulfides (DAT), S-allylcysteine (SACS), organosulfur compounds and allylsulfur compounds which make it to be a powerful disease fighting agent (Fleschauer and Arab, 2001; Banerjee and

Table 5. Effect of feeding apparently healthy rats with crude garlic extract on their hematological parameters.

Hematological parameter	Rat fed with garlic (%)	Control (%)
PCV	26.4 ± 1.2	42.3 ± 1.3
WBC (Total)*	60.3 ± 9.7	92.8 ± 7.9
Lymphocytes	47.5 ± 2.1	53.2 ± 2.6
Neutrophils	51.5 ± 2.4	45.5 ± 2.3
Monocytes	3.6 ± 3.3	1.3 ± 0.2

* = Expressed in mm^3

Maulik, 2002). In this study, administration of raw garlic extract to albino rats for a period of seven weeks affected the type and load of microbial flora present in their GIT (Table 4). This showed that the consumption of raw garlic may cause upset in the balance of microbial flora in the gut of consumers which might result in deleterious effect such as indigestion, malabsorption and diarrhoea. Before feeding apparently healthy rats with garlic extract, six different types of bacteria were isolated from their feaces with an average population of 1.64×10^{12} cfu/ml but by the end of the 7^{th} week of feeding with the extract, only *E. coli* was left in the GIT with a population of 1.3×10^{7} cfu/ml (Table 3).

In the protection/therapeutic experiment, rats fed with garlic for 7 weeks prior challenge with the infective dose of *S. typhi* recovered faster than those not fed with garlic prior infection. This may be because of the higher number of neutrophils and monocytes present in them compared to the rats not fed with garlic (Table 5). Neutrophils provide protection against a variety of intracellular organisms by both phagocytic and non phagocytic mechanisms while monocytes replenish resident macrophages and dendritic cells which migrate quickly to sites of infection in the tissues and ingest invading bacteria. The administration of garlic extract to apparently healthy rats caused an increase in the count of these two cell types which play significant role in innate immunity. This observation agrees with the report of Bjarnsholt et al. (2005) that innate immunity of mice increased when fed with garlic. The increase in the count of these two cell types in rats fed with the extract showed that garlic can also boost the innate immunity of rats. Feeding of rats with garlic however, caused a decrease in the packed cell volume of their blood. This showed that garlic is toxic to red blood cells. This supports the claims of Banerjee and Maulic (2002), that prolong feeding with high levels of raw garlic in rats may result to anemia, weight loss and failure of the rat to grow. They further said that raw garlic juice at a dose of 5 ml/kg resulted in death of rat due to stomach injury.

This study showed that garlic has antibacterial activity against *S. typhi* as verified by *in vitro* and *in vivo* experiments. These results indicated that it is possible to use garlic for the treatment of *S. typhi* infection. However, since consumption of raw garlic for a long period can lead to reduction and elimination of some gastrointestinal flora leading to disruption of the intestinal microflora balance, its consumption should only be restricted to treat specific ailments and should not be consumed on a regular basis. Moreover, since it can also reduce PCV in consumers, the use of hematinics should be encouraged when raw garlic is to be consumed for a long duration.

REFERENCES

Amagase H, Petesch BL, Matsuura H (2001). Intake of garlic and its bioactive components. J. Nutr., 131: 9555-9265.

Banerjee SK, Maulik SK (2002). Effect of garlic on cardiovascular disorders: a review. Nutr. J. 1: p. 4.

Bjarnsholt T, Ostrup Jensen P, Rasmussen TB, Christophersen L, Calum H, Hougen HP, Rygaard J, Mosen C, Eberl L, Halby N, Gisvkovs M (2005). Garlic blocks quorum sensing and promotes rapid clearing of pulmonary *Pseudomonas aeruginosa* infections. Microbiology, 151: 3878-3880.

Cheesbrough M (1994). Medical Laboratory Manual for Tropical Countries. MicrobiologyUniversity Press, Cambridge, 2 : 257 - 261.Damir Zucic, Davor Juretic (2004). Precise annotation of transmembrane visualization program. Crit. Chem. Acta. 77(1-2): 397-401.

Fleischauer AJ, Arab L (2001). Garlic and Cancer: a critical review of epidemiologic literature. Nutrition, 131: 10325-10405.

Jay MJ (1978). Modern Food Microbiology 2nd Edition. D. Van Nostrand Company, New York, pp. 117-133.

McMahon FG, Vargas R (1993) Can garlic lower blood pressure? A pilot study. Pharmacotherapy, 13(4): 406-407.

Microsoft Encarta (2008) Microsoft Corporation. All rights reserved © 1993-2007.

Mikail HG (2010). Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of *Allium sativum* L. bulbs in experimental rabbits. J. Med. Plants Res. 4(4): 322-326.

Morgan HR (2007). Typhoid Fever. Microsoft (R) Student 2008 (DVD), Redmond, WA: Microsoft Corporation, 2007.

Tung-Nis Yu, Chung- May Wu (1989). Stability of allicin in garlic juice. J. Food Sci. 54(4): p. 977.

Prescott LM, Harley JP, Klein DA (2002). Microbiology. Fifth Edition. Mc Graw Hill Publishers, USA. (N.Y). pp. 926-933.