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Antifungal Activity in Human Urine and Serum After Ingestion of Garlic (*Allium sativum*)

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A fresh extract of garlic (*Allium sativum*) was administered orally to human volunteers. At intervals, serum and urine were collected and assayed for antifungal activity. The maximum tolerable dose was determined to be 25 ml of garlic extract. Larger amounts caused severe burning sensations in the esophagus and the stomach and vomiting. After oral ingestion of 25 ml of the extract, anticandidal and anticryptococcal activities were detected in undiluted serum 0.5 and 1 h after ingestion. No detectable antifungal activity was found in the excreted urine at any time after oral ingestion. Oral garlic is of limited value in the therapy of human fungal infections.

Garlic extracts have been shown to inhibit the growth of a variety of bacteria (2, 6) and fungi (1–3, 5). Fractionation and analysis of aqueous garlic extracts have shown that the active ingredient is allicin (1), a low-molecular-weight organosulfhydryl compound whose biological activity is rapidly abolished by exposure to thiols (such as L-cysteine), heat, or alkali.

The success of garlic extracts in the treatment of human cryptococcosis has been reported previously (4). Patients were given intravenous or intramuscular aqueous extracts of garlic in addition to garlic given orally. The success rate of this therapeutic regimen was reported to be 69%. However, the achievable antifungal activity in the urine or serum was not determined for those patients on oral therapy alone or for those on a combined regimen of oral and parenteral therapy. In this study the antifungal activity of aqueous garlic extract for the commonly encountered pathogenic fungi was measured in artificial medium, human serum, and human urine. To demonstrate the maximum achievable antifungal activity in these two body fluids after oral therapy, a maximum tolerable dose of garlic extract was administered to human volunteers and the antifungal activity in urine and serum was determined.

MATERIALS AND METHODS

Preparation of garlic extract. An extract of a single batch of garlic cloves was prepared from commercially available fresh garlic by using a modification of the procedure of Fromtling and Bulmer (3). Garlic cloves were hulled, and 100 g of garlic was homogenized in 10 ml of distilled water in a Waring blender at the high-speed setting with 2-min bursts for a total of 10 min. The vessel was chilled in an ice-water bath between bursts. This mixture was centrifuged at 2,000 × *g* for 20 min. The supernatant was filtered through Whatman no. 1 filter paper (Whatman Corp., Bedford, Mass.) and then filter sterilized by passing through a 0.2-μm Nalgene filter (Nalgene Labware Div., Nalge/Sybron Corp., Rochester, N.Y.). The filtrate was assayed for antifungal activity and stored at −25°C until used.

In addition to the fresh garlic extract, a commercial preparation of garlic extract was tested for antifungal activity. Each capsule (Windmill Natural Vitamin Co., Morton Grove, Ill.) containing 1,500 mg of garlic extract had its contents aspirated with a needle and syringe, and the antifungal activity of the aspirate was assayed as described above.

Testing of fungal isolates. All fungal strains tested were clinical isolates. All yeasts were maintained on glucose-Sabouraud agar (Difco Laboratories, Detroit, Mich.) and grown in glucose-yeast nitrogen base (YNB) broth (Difco) in the log phase before use. The mycelial fungi were grown on glucose-Sabouraud agar slopes (9 ml), and the spores were prepared by the addition of 5 ml of sterile 0.05% Tween 80 (Sigma Chemical Co., St. Louis, Mo.), gentle agitation, and the aspiration of the suspension of spores. These spores were washed three times with equal volumes of 0.05% Tween 80 before use.

Preparation of inocula. All yeast cell and spore densities were determined in a hemacytometer (American Opticals Corp., Buffalo, N.Y.). The inoculum was adjusted to 5×10^4 cells per ml in the test media (human urine, human serum, or glucose-YNB broth).

Susceptibility testing procedure. All susceptibility tests were performed in sterile microtiter trays (Flow Laboratories Inc., McLean, Va.). Serial twofold dilutions were made of the test fluid in glucose-YNB broth, urine, or serum. An equal volume of the inoculum was added. The trays were gently agitated and incubated at 30°C for 48 h. Growth was read visually

TABLE 1. Growth inhibition of yeasts by garlic extract in YNB broth, human serum, and human urine

Species (no. of isolates)	Inhibition[a]		
	YNB	Human urine	Human serum
Candida albicans (10)	512	512	64
Candida glabrata (2)	64	64	4
Candida guilliermondii (2)	1,024	1,024	128
Candida krusei (2)	128	128	16
Candida parapsilosis (2)	512	512	64
Candida stellatoidea (2)	512	512	128
Candida tropicalis (2)	128	128	16
Cryptococcus neoformans (5)	128	128	16
Cryptococcus albidus (2)	512	512	64

[a] Results are reported as the reciprocal of the geometric mean titer.

TABLE 2. Growth inhibition of mycelial fungi by garlic extract in YNB broth, human serum, and human urine

Species (no. of isolates)	Inhibition[a]		
	YNB	Human urine	Human serum
Aspergillus fumigatus (5)	128	128	16
Aspergillus niger (3)	512	64	8
Aspergillus flavus (3)	256	64	8
Mucor pusillus (2)	1,024	1,024	16
Rhizopus arrhizus (2)	64	64	16
Rhizopus rhizopodiformis (2)	128	64	16

[a] Results are reported as the reciprocal of the geometric mean titer.

with a Titertek reading mirror (Flow Laboratories). The antifungal activity in the test fluid was recorded as the highest dilution inhibiting visible growth of the fungi.

Human volunteers. Five normal human volunteers were fasted overnight and then given 10 to 25 ml of the garlic extract orally, followed by 100 ml of water. Blood samples and urine specimens were obtained at 0, 0.5, 1, 2, and 3 h after oral ingestion of the extract. The urine samples and sera were assayed immediately for antifungal activity and stored at −25°C.

RESULTS

All the yeast species tested were susceptible to garlic extract when diluted in YNB broth, even at high dilutions (Table 1). The least susceptible isolates were strains of *Candida glabrata*, which were inhibited only with a 1:64 or lower dilution of the extract. When the tests were performed in urine, the susceptibilities of these yeasts were similar to those obtained in YNB broth. However, when human serum was used as the diluent, a marked drop in susceptibility to garlic extract of all the yeasts was noted. Initially, the microtiter plates were incubated at 30°C without CO_2. However, very little antifungal activity was noted in the presence of human serum. Since the active ingredient of the extract was believed to be allicin, which has been reported to be unstable at a high pH, the drop in antifungal activity noted in serum may in part be due to the high pH of serum in the absence of CO_2. Such a phenomenon has previously been observed by Wolff et al. (7). All subsequent test plates were incubated in an atmosphere of 5% CO_2, and the antifungal activity of the extract in serum was noted to be slightly higher (Tables 1, 2, and 3).

The mycelial fungi varied in their susceptibility to garlic extract in YNB broth (Table 2). *Mucor pusillus* was most susceptible, being inhibited at a dilution of 1:1,024, whereas strains of *Rhizopus arrhizus* were inhibited only at a 1:64 or lower dilution. In urine, most isolates tested were equally susceptible as compared with YNB broth. In serum, a marked decrease in antifungal activity was noted as seen previously with the yeasts.

A commercial preparation of garlic extract was similarly assayed. No antifungal activity was detected for the yeasts or the mycelial fungi.

All volunteers who ingested the fresh garlic extract complained of burning sensations in the mouth, esophagus, and stomach which lasted for less than 15 min. In the volunteer who took a 25-ml dose of the extract, additional symptoms of nausea, diaphoresis, and light-headedness lasting for 30 min were noted. The antifungal activities of the serum and the double-voided urine are shown in Table 3. All urine and serum samples collected from the volunteers before the ingestion of garlic failed to inhibit the growth of any of the fungal strains tested. Detectable anticandidal and anticryptococcal activity was noted only in serum collected 30 and 60 min after garlic extract ingestion.

DISCUSSION

Garlic (*Allium sativum*) is an intriguing herb with alleged powers ranging from warding off vampires to the more recently reported power of curing fungal infections (4). Fungal infections have become an important aspect of modern infectious disease practice. The prominence of fungi as pathogens may be due to the longer survival of immunocompromised patients, the recent development and usage of broader-spectrum antibiotics, or the wider use of immunosuppressive and cancer chemotherapeutic agents.

Because of past reports of the antifungal activity of garlic in vitro (1, 3–5), it is imperative to determine whether maximally tolerable oral doses of this herb can produce therapeutic antifungal effects. It can be speculated from previ-

TABLE 3. Anticandidal activity in human serum and human urine after garlic ingestions

Sample	Oral dose (ml)	Activity[a] at following time (min):				
		0	30	60	120	180
Serum						
Volunteer 1	25	ND[b]	2	2	ND	ND
Volunteer 1	10	ND	1	1	ND	ND
Volunteers 2–5	10	ND	1	1	ND	ND
Urine (all volunteers)	10–25	ND	ND	ND	ND	ND

[a] Results are reported as the reciprocal of the geometric mean titer.

[b] ND, No detectable activity.

ous reports that garlic can be used to suppress oropharyngeal or vaginal colonization by candida, to cure candida cystitis in diabetics or patients with chronic indwelling Foley catheters, to cure dermatophytic infections, and even to ameliorate systemic infections caused by fungi such as cryptococci, aspergilli, or zygomycetes.

The antifungal activity of garlic extracts has been confirmed in this study. All yeasts tested, with the exception of *C. glabrata*, were inhibited by a 1:100 dilution of the extract. Similarly, all of the mycelial fungi tested, with the exception of *R. arrhizus*, were inhibited by a 1:100 dilution of the extract. In general, the activity of the extract remained intact in urine but was markedly depressed in the presence of serum. It is unclear from the available data whether the loss of activity in serum is related to high serum protein binding of the active compound allicin, or whether serum irreversibly inactivated allicin.

Oral doses of the garlic extract caused significant discomfort. The commercially prepared garlic capsule, when taken in a one-capsule dose, caused little discomfort. However, these capsules contained no antifungal activity—a fact consistent with a previous observation that allicin is unstable at room temperature (1). There is no reason to believe that fresh garlic in the amounts of 10 to 25 ml would be better tolerated orally when administered in the form of capsules. This would require 5 to 10 capsules, and the burning sensation would still be expected to occur in the stomach.

After ingestion of a maximally tolerable dose of fresh garlic extract, detectable anticandidal and anticryptococcal activity was found only in serum at 30 and 60 min after ingestion. No activity was detected in the urine at any time. Because urine did not appear to inhibit the antifungal activity of garlic extract, one might conclude that allicin was not excreted in the urine as an active compound in any significant proportion. The mode of inactivation or elimination of the antifungal activity in the serum is not known. The subsequent decrease in serum antifungal activity after 60 min may also have been due to the distribution of the absorbed allicin in the body. The administration of multiple doses of garlic extract should clarify this point, but such a suggestion was untenable to the volunteers.

It is conceivable that the mucosa-irritating substance of the garlic extract can be separated from the ingredient with antifungal activity (allicin). Oral garlic therapy might then be an attractive therapy for fungal infections. Unfortunately, diallyl disulfide, a precursor of allicin in a one-step synthesis, has the odor of garlic and is similarly irritating to the oral mucosa. Thus, garlic extract or allicin has limited potential as an oral therapy for fungal infections. Further investigation in an animal model is needed to determine whether parenteral therapy can be tolerated or yield better antifungal activity in the serum.

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LITERATURE CITED

1. **Barone, F. E., and M. R. Tansey.** 1977. Isolation, purification, identification, synthesis, and kinetics of activity of the anticandidal component of *Allium sativum* and a hypothesis for its mode of action. Mycologia **69**:793–825.
2. **Dankert, J., T. F. J. Tromp, H. De Vries, and H. J. Klasen.** 1979. Antimicrobial activity of crude juices of *Allium ascalonicum, Allium ceps*, and *Allium sativum*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1: Orig. Reihe A **245**:229–239.
3. **Fromtling, R. A., and G. S. Bulmer.** 1978. *In vitro* effect of aqueous extract of garlic (*Allium sativum*) on the growth and viability of *Cryptococcus neoformans*. Mycologia **70**:397–405.
4. **Hunan Medical College.** 1980. Garlic in cryptococcal meningitis. Chin. Med. J. **93**:123–126.
5. **Moore, G. S., and R. D. Atkin.** 1977. The fungicidal and fungistatic effects of an aqueous garlic extract on medically important yeast-like fungi. Mycologia **69**:341–348.
6. **Sharma, V. D., M. S. Sethi, A. Kumar, and J. R. Rarotra.** 1977. Antibacterial property of *Allium sativum: in vivo* and *in vitro* studies. Indian J. Exp. Biol. **15**:466–468.
7. **Wolff, M., H. Chmel, and R. H. K. Eng.** 1982. Aminoglycoside bioassay: observations on accuracy. J. Infect. Dis. **145**:585.