

# The Effect of Garlic Powder on Human Urinary Cytokine Excretion

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**Purpose:** To evaluate the effects of orally administered dehydrated garlic powder on cytokine excretion in the urinary tract.

**Materials and Methods:** A total of 60 healthy volunteers, randomized into 3 groups, were given a single oral dose of 1 g or 3 g of dehydrated garlic powder or placebo. Urine samples were obtained 6.0 and 24.0 h after garlic intake and assayed for interleukin-8 (IL-8), interleukin-12 (IL-12), tumor necrosis factor-alpha (TNF- $\alpha$ ), diallyl disulfide (DADS) and diallyl sulfide (DAS).

**Results:** Significant increases in IL-12 levels over baseline were noted in urine samples obtained after oral intake of 1 g and 3 g of garlic powder ( $P < .001$ ). In the 1 g and 3 g garlic powder treatment groups, time-dependent variations in IL-12 levels over the study period were significantly different from the placebo group ( $P < .001$ ). In both garlic treatment groups, urinary levels of IL-8 and TNF- $\alpha$  were not significantly different from baseline and placebo levels ( $P > .017$ ). DADS and DAS were not detected in the urine samples at any time after garlic powder intake.

**Conclusion:** Oral intake of doses of garlic traditionally used for daily supplementation increases urinary levels of IL-12, which is a potent stimulator of T helper cell 1 (Th-1) immune responses. This observation encourages further studies investigating the immunostimulatory role of garlic in the urinary tract.

**Keywords:** cytokines; secretion; garlic; humans; administration; oral; interleukin.

## INTRODUCTION

Since ancient times, garlic has been used as a phyto-pharmaceutical agent and a dietary supplement. Ancient Egyptian documents dating to 1550 after death (AD) describe oral and topical use of garlic as a remedy for tumors.<sup>(1)</sup> Since the 1950s, the scientific basis of garlic's medicinal effects has been partially elucidated by studies demonstrating that thiosulfate extracts from garlic inhibited tumor growth in sarcomas.<sup>(2)</sup> In a rat model of intravesical transitional cell carcinoma, intravesical aqueous garlic extract instillation enhanced local lymphocyte and macrophage responses and inhibited macroscopic tumor growth.<sup>(3)</sup> In addition, a reduction of tumor volume was reported in a rat model when garlic extract was administered orally.<sup>(4)</sup>

Attempts to elucidate the mechanisms responsible for the antitumor effects of garlic revealed that the component diallyl disulfide (DADS) induces apoptosis through caspase-3 activity. DADS and diallyl sulfide (DAS) also inhibited N-acetyl-transferase activity in the T-24 human bladder cancer cell line.<sup>(5,6)</sup>

To our knowledge, the cytokine response in the human urinary tract after oral intake of garlic has not been documented. We assessed garlic's immune potency by monitoring urinary cytokine excretion following administration of oral garlic supplements to healthy subjects.

## MATERIALS AND METHODS

After receiving approval from the institutional ethics committee, a total of 60 healthy volunteers, comprised of 34 male participants and 26 female participants between 20 and 40 years of age were enrolled in this study (Table 1).

### *Study Participants*

All volunteers included in this study were determined to be healthy by a physician, who performed the following laboratory tests: complete blood count, C - reactive protein, erythrocyte sedimentation rate, urine analysis, fasting blood glucose and plasma cholesterol levels, kidney function tests (creatinine, blood urea nitrogen) and liver function tests. Participants were also required to have a normal body mass index. All participants underwent abdominal and pelvic ultrasonographic evaluation and electrocardiographic examination before the study. Because inflammatory cytokine levels can

be affected by a variety of factors, the following list of exclusion criteria was used: the existence of urinary, respiratory, cardiovascular, gastrointestinal and hepatic disorders, the use of medications, including antimicrobial, antiviral and anti-inflammatory medications, in the 2 weeks prior to the study, and pregnancy or menstruation at the time of the study.

Written informed consent was obtained from eligible participants. Participants were asked to avoid ingestion of vegetables in the allium family and processed derivatives of these vegetables for 15 days prior to the study. The participants were randomized into 3 groups: one group received placebo, and two groups received dried garlic powder in a dose of either 1 g or 3 g. Investigators and outcome assessors were blinded to treatment group assignments.

### *Preparation of Garlic Powder*

Denuded fresh garlic was frozen at -70°C in a lyophilizer and dried under a vacuum. The resulting dried garlic was blended in a blender until it was ground into a fine powder. From approximately 8.75 g of denuded raw garlic, 1 g of dried powder was obtained. On a precise scale, powder aliquots weighing 1 g and 3 g were allocated to individual packets. On the study day, the content of each packet was thawed and placed in caches, which were similar to those of the placebo (flour).

### *Sampling and Laboratory Analysis*

Placebo, 1 g and 3 g of garlic powder were administered orally to participants in the appropriate treatment group after 12 h of overnight fasting. Urine samples were obtained from each participant before and 6 h and 24 h after powder administration. The collected urine was immediately centrifuged at 3000 rpm for 5 minutes, and the supernatant was divided into 3 tubes and stored at 80°C until further analysis. Urine cytokine assays were performed in duplicate by enzyme linked Immunosorbent assay (ELISA) using commercially available kits for IL-8, IL-12 and TNF- $\alpha$  (Quantikine, R&D Systems Europe, Ltd. Abingdon OX14 3NB, UK) according to manufacturer instructions.

Detection of DADS and DAS in the collected urine samples was performed using a gas chromatograph (6890N, Agilent Technologies, Massy, France) coupled to a Mass Selective Detector (5973, Agilent Technologies, Massy, France). Manual sampling was performed by a fiber column (Supelco Solid Phase Microextraction, Supelco Park, Bellefonte, PA,

**Table 1.** Characteristic data of study groups.

Variables	Placebo	1 g Garlic	3 g Garlic
Total	20	20	20
Male	10	13	12
Female	10	7	8
Mean age, years (range)	30.2 (20-38)	31.7 (23-40)	32.3 (26-39)

16823 USA) mounted to the analyzer. The following conditions were used for gas chromatography and mass spectrometry: column, HP-5 MS 0.25 mm × 30 m × 0.25 µm; carrier gas, helium (2 mL/min); temperature of the injection port, 280°C; mode, scan; applied method, pulsed splitless solvent; and delay, 1 min. The heating program was as follows: initial temperature, 40°C; increase rate, 10°C/min, target temperature, 150°C; and duration, 3 min. Samples were thawed at room temperature before analysis. Extraction was performed by the solid phase micro extraction (SPME) method using 85 µm film thickness polyacrylate fiber. The inlet temperature was adjusted to 280°C, and the fiber was conditioned for one hour. Two mL aliquots were removed from each urine sample tube, transferred to vials and mixed with 1 g of NaCl. The vials were stoppered and stirred in a stirrer for 20 min at room temperature. During mixing, fiber was placed into the vial without contacting the urine. Garlic metabolites absorbed into the fiber were manually injected onto the gas chromatography/mass spectrometry apparatus. Calibration curves were drawn for DAS and DADS standards for concentrations ranging from 1.25- 20 parts-per billion (ppb). The retention times for DAS and DADS were 4.84 min (correlation coefficient,  $r^2 = 1.0$ ) and 9.62 min (correlation coefficient  $r^2 = 0.99$ ), respectively. The detection limit was 0.5 ppb. The values were expressed as a function of urine creatinine concentration (pg/mg creatinine).

#### Statistical Analysis

Deviations from the normal distribution were evaluated for IL-8, IL-12 and TNF- $\alpha$  concentrations using the Shapiro-Wilk W test for normality. Descriptive statistics were expressed as the median and percentiles (25th - 75th). Significant time-dependent variations in IL-8, IL-12 and TNF- $\alpha$  levels within groups were assessed using the Bonferroni-

corrected Wilcoxon signed-rank test. A  $P$  value  $< .0056$  was considered significant for comparisons within groups. Variations in the urinary levels of IL-8, IL-12 and TNF- $\alpha$  between the baseline and 6 h samples, between the baseline and 24 h samples and between the 6 h and 24 h samples were calculated. The significance of time-dependent variations in levels of IL-8, IL-12 and TNF- $\alpha$  between groups was evaluated using the Bonferroni-corrected Kruskal-Wallis test, with  $P$  values  $< .017$  considered statistically significant. For variations that were determined to be significant using the Kruskal-Wallis test, the groups with significant variation were identified using the nonparametric multiple comparison test. All statistical analyses were conducted using the statistical package for the social science (SPSS Inc, Chicago, Illinois, USA) for Windows version 11.5.

## RESULTS

### IL-8 Assays

The median baseline urinary IL-8 level for the healthy participants was 31.88 pg/mg creatinine, ranging from 11.52 pg/mg creatinine (25th percentile) to 62.72 pg/mg creatinine (75th percentile). The median baseline IL-8 levels of the placebo, 1 g and 3 g garlic powder groups were not significantly different ( $P > .017$ ). Compared to baseline levels, median IL-8 levels were not significantly different 6 h or 24 h after administration of placebo, 1 g or 3 g of garlic powder (each  $P > .0056$ ). IL-8 levels after 24 h were not significantly different from IL-8 levels after 6 h ( $P > .0056$ ). When the groups were compared sequentially, significant time-dependent median IL-8 variations were not observed between the placebo and 3 g garlic powder treatment groups or between the 1g and 3g garlic powder treatment groups ( $P > .017$ ) (Table 2).

### IL-12 Assays

**Table 2.** Comparison of time dependent variation of urinary interleukin-8 levels among the study groups.

Variables	Placebo	1 g Powder	3 g Powder	P*	P**	P***
Baseline, 6th h	7.77 (-49.68-27.65)	-12.01 (-39.73-19.23)	-13.22 (-32.74-8.33)	.320	.445	.816
Baseline, 24th h	1.74 (-11.49-34.68)	-31.38 (-46.68- -10.55)	-3.50 (23.82-20.78)	.002	.365	.019
6-24th h	7.61 (-14.00-48.08)	-10.52 (-54.57-0)	2.58 (-11.25-27.86)	.013	.921	.017

Values are as median (25th-75th percentile), (pg/mg urinary creatinine), n = 20.

\* 1 g powder vs. placebo, where  $P < .01$  is considered as significant due to Bonferroni correction.

\*\* 3 g powder vs. placebo.

\*\*\* 1 g vs. 3 g powder.

The median baseline urinary IL-12 level for all participants was 0.00 pg/mg creatinine (0.00 - 1.42 pg/mg creatinine). There were no significant differences in the median baseline levels of the three groups ( $P > .017$ ). In the placebo group, the median IL-12 levels after 6 h and 24 h were not different from baseline levels ( $P > .0056$  for each) (Table 3). In the group that was given 1 g garlic powder, the median IL-12 levels after 6 h and 24 h were significantly higher than the baseline level ( $P < .001$ ). The observed increase after 24 h was significantly higher than the placebo group ( $P < .001$ ) (Table 4, Figure). However, after 6 h, the observed increase was not significantly different from the placebo ( $P = .094$ ). In the group that was administered 3 g of garlic powder, IL-12 levels after 6 h were significantly higher than the baseline level ( $P < .001$ ). After 24 h, IL-12 levels were not significantly different from the baseline or 6 h levels ( $P > .0056$ ) (Table 3). In the 3 g garlic treatment group, when time-dependent variations in IL-12 levels were compared with those of the placebo group, significant differences were observed after 6 and 24 h ( $P < .009$  and  $P = .0054$ , respectively) (Table 4). When time-dependent variations in IL-12 levels for the 1 g treatment group were compared with those of the 3 g treatment group, the observed differences were significant after 24 h, but not after 6 h ( $P < .001$  and  $P > .32$ , respectively).

#### **TNF- $\alpha$ Assay**

The median baseline urinary TNF- $\alpha$  level in the healthy participants was 13.94 pg/mg creatinine (9.31-22.82 pg/mg creatinine). Compared with baseline levels, TNF- $\alpha$  levels were not significantly different in the placebo and garlic treatment groups after 6 h and 24 h ( $P > .0056$  for each). Likewise, time-dependent variations in TNF- $\alpha$  levels between the 1 and 3 g garlic treatments groups throughout the study period were not significantly different from the placebo group ( $P > .017$

for each) (Table 5).

#### **DADS and DAS Assays**

Neither DADS nor DAS was detected in any urine samples tested using the described gas chromatography and mass spectrometry assays.

#### **Adverse Effects**

The participants tolerated both doses of dried garlic powder well. Only one participant, who was administered 1 g of garlic powder, reported mild gastrointestinal discomfort, which manifested as distension and nausea that subsided within two hours of onset. No adverse reactions were reported.

## **DISCUSSION**

Changes in urinary cytokine levels have been observed in patients with urinary tract infection<sup>(7)</sup>, urinary calculi,<sup>(8)</sup> genitourinary infections or pregnancy,<sup>(9)</sup> tubular damage caused by diabetes mellitus or obesity-related nephropathy,<sup>(10)</sup> dietary fat-induced hepatic inflammation (steatohepatitis) or inflammatory bowel disease,<sup>(11)</sup> hypertension and cardiovascular disease,<sup>(12)</sup> or drug use (e.g., anti-inflammatory drugs or cyclophosphamide).<sup>(13)</sup> These factors were assessed when screening participants, and sixty healthy volunteers were included in this study. Because the body mass index of the participants was normal and ultrasonographic evaluation did not reveal hepatosteatosis, dietary changes (particularly a low-fat diet) were not suggested.

The prophylactic and therapeutic benefits of garlic against cancer have been known since ancient times. Efforts to discover the antitumor mechanism of garlic in the modern era began with the study of Weinsberger and Persky, who demonstrated that in vitro and in vivo administration of thiosulfinate extracts from garlic inhibited the growth of malignant cells.<sup>(2)</sup> In addition, study by Marsh and colleagues demonstrated

**Table 3.** Urinary interleukin-12 levels in the study groups.

Variables	Placebo	1 g Garlic	3 g Garlic
Baseline	0 (0-4.96)	0 (0-0)	0 (0-2.90)
6th hour	3.44 (0-11.83)	4.77 (2.32-11.24)	9.87 (6.66-13.63)
24th hour	0 (0-1.43)	7.85 (5.93-14.63)	3.764 (1.36-7.42)
Baseline vs. 6th h	$P = .881$	$P < .001$	$P < .001$
Baseline vs. 24th h	$P = .101$	$P < .001$	$P = .086$
6th vs. 24th h	$P = .012$	$P = .370$	$P = .009$

Values are median (25th-75th percentile), (pg/mg urinary creatinine), n = 20.  
 $P < .01$  is considered significant due to Bonferroni correction.

**Table 4.** Comparison of time dependent variation of urinary interleukin-12 levels among the study groups.

Variables	Placebo	1 g Powder	3 g Powder	$P^*$	$P^{**}$	$P^{***}$
Baseline, 6th h	2.23 (0-9.58)	4.44 (2.32-11.24)	8.04 (4.32-11.05)	.094	.009	.320
Baseline, 24th h	0 (-4.11-0.27)	7.82 (5.92-14.63)	3.33 (-0.74-7.31)	.001	.005	.001
6-24th h	-2.59 (-9.04-0)	2.39 (-2.92-7.01)	-5.48 (-10.73-0.40)	.006	.799	.003

Values are as median (25th-75th percentile), (pg/mg urinary creatinine), n = 20.

\* 1 g powder vs. placebo, where  $P < .01$  is considered as significant due to Bonferroni correction.

\*\* 3 g powder vs. placebo.

\*\*\* 1 g vs. 3 g powder.

in a rat transitional cell carcinoma model that intravesical administration of garlic extracts caused tumor volume regression comparable to BCG (Bacillus Calmette-Guerin) instillation, tumor necrosis and lymphocyte and macrophage infiltration.<sup>(3)</sup> The accumulated data suggesting that the anti-tumor effect of garlic may be related to immune stimulation prompted us to evaluate the activation of immune responses within the urothelium of healthy humans after oral intake of garlic. Urinary cytokine levels were chosen as the main outcome of the study because of the well-described increases in urinary cytokine levels following oral administration of the immunostimulatory agents bropirimine, BCG, keyhole limpet hemocyanin and corynebacterium parvum. We studied the kinetics of urinary IL-8, IL-12 and TNF- $\alpha$  after oral administration of dehydrated garlic powder. We chose these cytokines because tumor recurrence is associated with TNF- $\alpha$  and IL-8 levels, and IL-12 has antitumor activity in in vivo models of bladder cancer.<sup>(14-17)</sup>

The sources of IL-8 in the bladder are the transitional epithelium, endothelial cells, mast cells, neutrophils, T-lymphocytes and macrophages.<sup>(18)</sup> Previous studies assessing urinary lev-

els of IL-8 in BCG-instilled human bladder transitional cell carcinoma reported contradicting results. Rabinowits and colleagues demonstrated no variation in IL-8 levels between patients with tumor recurrence and remission after 6 cycles of BCG instillation.<sup>(19)</sup> In contrast, Sheryka and colleagues demonstrated reduced IL-8 levels during remission.<sup>(20)</sup> In our study, the 1 g and 3 g dried garlic powder treatment groups demonstrated no differences in urinary IL-8 levels when compared to baseline levels or placebo levels (Table 2).

IL-12, a bioactive cytokine produced by macrophages, dendritic cells, T-cells and natural killer (NK) cells, plays a key role in the differentiation of naïve T cells into Th-1 cells. IL-12 is a potent stimulator of interferon gamma (INF- $\gamma$ ) production in T-helper and NK cells. Urinary IL-12 levels have been reported to increase after intravesical BCG instillation.<sup>(21)</sup> In our study, 6 h and 24 h after oral intake of a single dose of 1 g of garlic powder, we observed significant increases in urinary IL-12 levels compared to baseline levels. In the 3 g treatment group, significant increases were observed 6 h after administration of garlic powder. A decrease in median IL-12 levels 24 h after intake of 3 g of garlic and an increase

**Table 5.** Comparison of time dependent variation of urinary tumor necrosis factor- $\alpha$  levels among the study groups.

Variables	Placebo	1 g Powder	3 g Powder	P*	P**	P***
Baseline, 6th h	2.10 (-3.25-7.33)	2.73 (-2.11-7.43)	4.08 (-1.38-14.42)	.694	.287	.500
Baseline, 24th h	-0.11 (-3.13-7.35)	0.73 (-8.61-7.73)	-1.57 (-6.57-7.17)	.912	.603	.682
6-24th h	-3.25 (-15.33-5.19)	-1.49 (-17.03-3.90)	-5.77 (-19.93-7.52)	.723	.782	.938

Values are as median (25th-75th percentile), (pg/mg urinary creatinine), n = 20.

\* 1 g powder vs. placebo, where  $P < .01$  is considered as significant due to Bonferroni correction.

\*\* 3 g powder vs. placebo.

\*\*\* 1 g vs. 3 g powder.

in median IL-12 levels 6 h after intake of the placebo may have adversely affected our analysis of significance (Tables 3 and 4) (Figure). We believe these effects were due to extreme values that altered the normal distribution because of our relatively small sample size. Nevertheless, our results strongly encourage further studies assessing the importance of urinary IL-12 levels, particularly studies that have a larger number of participants and studies that administer garlic for a longer period of time.

Intravesically instilled BCG and garlic extract are known to induce TNF- $\alpha$  production by macrophages and NK cells. Maki and colleagues and Shintani and colleagues measured urinary levels of TNF- $\alpha$  after BCG instillation in bladder tumor patients and reported increased levels in responders.<sup>(22,23)</sup> In contrast to these studies, we chose to administer garlic orally to determine the importance of garlic in a normal diet and to investigate the possible protective effect of garlic in the urinary system. We demonstrated that a single oral dose of garlic powder has no effect on urinary TNF- $\alpha$  levels (Table 5). However, because the study period was restricted to 24 h, late responses to oral garlic intake may have been missed.

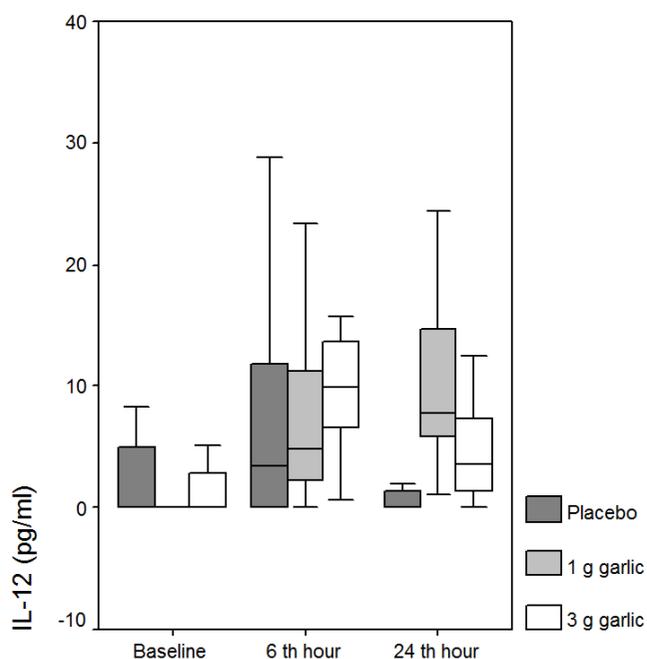
Fresh raw garlic consists of 85% water. During the dehydration process, only water is removed from the garlic. To date, the recommended doses of fresh and processed garlic have not been standardized for clinical studies. In 1988, the German Commission E Monograph advised 4 g of fresh garlic for maximum daily oral intake without evidence-based referral.<sup>(24)</sup> In their study assessing the immunostimulatory effects of garlic in humans, Abdullah and colleagues and Kandil and colleagues used orally administered aged garlic extract

in amounts ranging from 1.8 g to 10 g, within safety limits.<sup>(25,26)</sup> In our study we empirically chose a single dose of 1 g or 3 g of garlic powder, which corresponds to approximately 8.75 g (2 cloves) and 26.25 g (7 cloves) of fresh garlic, respectively, based on traditional doses for daily supplementation. Depending on the amount and duration of consumption, various adverse effects of garlic have been described, including as diarrhea, bronchial asthma, contact dermatitis and hepatotoxicity.<sup>(27-30)</sup> Organosulfur constituents of garlic inhibit lipooxygenase and cyclooxygenase enzyme activities in the gastric mucosal membrane. Prostanoid compounds in garlic are reported to cause reflux and esophagitis through esophageal sphincter relaxation.<sup>(31)</sup> Our participants tolerated a single dose of 3 g of garlic powder quite well. Only one participant in the 1 g garlic powder treatment group suffered from dyspepsia, which subsided within two hours.

DADS and DAS, the oil-soluble organic sulfur constituents of garlic to which some of the biological effects of garlic have been attributed, were not detected in the urine of any of our participants. This outcome is inconsistent with the findings of Amagase and colleagues and Germain and colleagues, who demonstrated that the absence of urinary DAS and DADS activity was due to rapid and extensive hepatic metabolism by the enzyme alliinase, which converts alliin to allicin, and with findings of Lawson and colleagues, who reported that more than half of the alliin precursor for DADS and DAS was lost during the dehydration of fresh garlic.<sup>(32-34)</sup>

## CONCLUSION

We observed a significant increase in IL-12, a potent stimulator of Th-1 immune responses, following oral intake of garlic



**Figure.** Urinary interleukin-12 levels in the study groups.

in doses representative of traditional daily supplementation doses. These results encourage further studies investigating the immunostimulatory effects of garlic in the urinary system. In addition, other forms of garlic supplementation, such as essential oil, oil macerate, and aged extract should be included in future studies assessing the efficacy of long-term garlic consumption on a wide spectrum of parameters.

## CONFLICT OF INTEREST

None declared.

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