

# Some Pharmacological and Toxicological Studies on Garlic (*Allium sativum*)

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## **Introduction**

Garlic (*Allium sativum*) is a member of lily family which is closely related to onions and chives. While it is used primarily used as a flavoring ingredient for many foods, it was also used as a stimulant for strength, endurance and as medicine in the historical past by the Chinese, Egyptians, and the Babylonians.

Present study was conducted with the purpose of investigating in some detail about the possible protective effect of garlic extract on induced gastric ulcers and also its effect on uterine smooth muscle. Furthermore, its fetotoxic and embryotoxic were also investigated.

## **Previous Works**

The epidemiological, clinical and laboratory data have proved that garlic (*Allium sativum*) contains many biologically and pharmacologically important compounds, which are beneficial to human health including cardiovascular, neoplastic, and several other diseases (Gebhart,1993; Dorant et al.,1993; El-Mofty et al.,1994; and Kieseewetter et al.,1993). Ulceration of

gastric mucosa is a major health problem. Published results of epidemiological case-control studies in China and Italy on gastric carcinoma in relation to diet suggest that consuming garlic may reduce the risk of gastric cancer (Dorant et al., 1993). Also the radical scavenging capacity of garlic had been investigated in radical generating system (Wang and Chen, 1994; Lewin and Popov, 1994 and Torok et al., 1994). Aqueous extract of garlic inhibited PGS synthesis and inhibited spontaneous rhythmic contraction of isolated sheep ureter (Ali et al., 1993). Aqeel et al., (1991), found that garlic produced reversible inhibitions on smooth muscles of aorta, trachea, intestine and isolated rabbit heart. This effect might be due to at least in part to a direct relaxant effect on smooth muscles.

### **Methods of Study**

Dialyzed garlic was prepared from freshly peeled and chopped garlic bulbs. The extract yield (w/w) was determined to be 0.83% after evaporation of representative batches of the dialysate. All doses are expressed in terms of the dry extract (Martin, et al., 1994).

Forty eight albino mice of both sex having an average weight of 20-25 gm were divided into 6 groups of 8 mice each. A control group was given the solvent (distilled water) by I.P. route in the same volume as the test group. Animals were observed during the following 6 hours for the appearance of signs of toxicity; alteration in behaviors, ataxia, convulsions, rigidity, flaccidity,

responses to external stimuli, loss of righting reflex, ptosis and piloerection. Dead animals during this period were subjected to postmortem examination. Mortality in each group were recorded after 24 hours of drug administration. Postmortem examination was carried out for evidence of any marked gross pathological lesions of the heart, lungs gastrointestinal tract, liver, kidneys, adrenals, and brain. Toxicity data were statistically analyzed following Lichfield and Wilcoxon procedure (1948). Lungs and brains were gently removed and fixed with 10% formol. The specimens were processed and embedded in paraffin. Sagittal sections were cut at 10m mickvers. Paraffin sections of the lungs were stained with haemotoxylin and eosin. Brain tissues were stained with Einarson's (193)Gallocyanice method for Nissl (Conn et al., 1965).

Dialyzed garlic and ethanol 96% were used to experiment induced ulcers in adult male albino rats (Robert,1979 and Pare and Glavin,1986). After the rats were killed, the stomachs were rapidly removed and opened to assess the gastric lesions. The lesions, hemorrhage or ulcerative were measured. The following parameters were looked for and estimated:

- The incidence of ulceration (I.U.)
- Ulcer score (U.S). The length and width of each lesion was measured and the area calculated in square millimeters.
- Ulcer Index(U.I.) = mean ulcer score of a group of animals similarly treated

x % of ulcerated animals of the group (Pauls et al., 1947).

$$\text{Preventive Index (P.I.)} = \frac{\text{U.I. control} - \text{U.I. treated}}{\text{U.I. control}} \times 100 \quad (\text{Hanoetal, 1976}).$$

Microscopic examination of sections from the gastric lesions was done and the results were recorded according to the following grading system ( Perretti et al., 1992).

Grade I: Erosion of superficial epithelial layer.

Grade II: Erosion of superficial epithelial layer and less than 1/3 of thickness of mucosa.

Grade III: Erosion or necrosis of more than 1/3 of the mucosal thickness.

Isolated fundus strips from the adult male albino rats were used in the experiments which were designed to study the effect of garlic in increasing concentrations on spontaneous tone and on contractions induced by Ach, 5-HT and bariúm chloride. Blood samples were collected from all groups in heparinized microhematocrit capillary tube using orbital sinus puncture technique for :

1. Lipid peroxides were measured in plasma.
2. Plasma nitric oxide was determined as nitrite concentrations.
3. Plasma superoxide dismutase activity was measured.
4. Plasma phospholipase-A2 activity was determined.
5. Plasma tumor necrosis factor  $\alpha$  was measured using mouse TNF- $\alpha$ , ELIZA kit.

6. Prostaglandins-E2 was measured in plasma using R & D systems PGE2 immunoassay.

Teratogenic and foetotoxic experiments were carried out by using adult albino mice and dialyzed garlic extract. In Group-I, control 24 mice received 1ml/100 g body weight of aqueous saline orally. In Group-II, treated group of 24 mice were treated with dialyzed garlic, orally once daily in a dose of 300mg/kg/day. Treatment continued until gestation day 20, inclusive. Maternal weight and urinary output were recorded every day. On gestation day 21(12 mice of each group) were killed by cervical dislocation. Preparation of 5 mm sections were made and stained with hematoxylin and eosin and toluidine blue. The slides were examined to assess potential teratogenic or fetopathic effects of in utero garlic exposure in the mice. Blood samples were taken to measure plasma glucose and total cholesterol levels. Isolated mice uterine strips, taken from the horn of uterus from control or treated dam, were mounted in a 10 ml capacity organ bath containing oxygenated De Jalon solution. The amplitude and frequency of uterine contraction was recorded for 30 minutes for both treated and control groups.

Treatment with garlic extract (300 mg/kg/day) was continued for another 2 weeks after labor for 12 mice previously treated, to follow up the growth of their fetuses in comparison to control group.

Effect of garlic extract on isolated rat anococcygeous muscle and

isolated rabbit aortic strips were also examined.

### **Results and Discussion**

Chemical analysis of dialyzed garlic showed that its major constituents are organosulfur compounds including allicin 5-oxodiallylsulfide and diallyl disulfide oxide. Selenium, a trace element common in natural garlic was detected in the dialyzed garlic at a concentration of 3 mg/l, i.e. 0.0003%.

Manifestation of acute intraperitoneal (I.P.) intoxication of mice that received variable dose levels of garlic dialysate showed signs of central nervous system depression of varying degrees that was proportional to the dose given. Postmortem examination of animals which died by the lethal dose of garlic dialysate revealed a picture of death due to respiratory depression and arrest. The I. P. L. D<sub>50</sub> of garlic dialysate for mice was 112.5 g/kg. The histopathological changes of the lungs and brain are mainly vascular; congestion of vessels with cellular exudates.

In vivo experiments of gastric lesion study, intragastric administration of 1 ml of 96% ethanol produced marked hyperemia, congestion and edema of gastric wall with large hemorrhagic linear gastric ulceration along the rugal folds. It was observed that prior to chronic treatment with garlic (300 mg/kg/day for 4 weeks), decreased the extent of damage and the degree of congestion. Its preventive index was 78.8%. It was found that the incidence

of ulceration in garlic pretreated rats was decreased significantly and the preventive index was 91.1%. Histopathological examination of the ulcerated regions in alcohol treated animals showed marked damage of gastric mucosa. According to the depth and linear length of damage, three grades were Observed (Grades I, II and III). Pretreatment of animals with garlic extract lead to decrease in both number and extent of ulceration. Cold stress was shown to cause less mucosal damage that involves only the surface columnar epithelium or upper part of gastric glands. It was observed that the pretreatment of animals with garlic extract lead to significant decrease of stress induced gastric damage.

In vitro study on isolated rat fundus strip showed that garlic concentrations of 1, 2, and 4 mg/ml produced dose related increase in basal tone of the isolated rat's fundal strip, while this increase was less with higher concentrations ( 8, 16 , 32 mg/ml ). It was observed that garlic in concentration of 16 mg/ml produced significant inhibition of contraction induced by Ach and 5-HT.

It was found that TBARS plasma levels were significantly increased by 58% in group II when compared to control ( $p < 0.001$ ). The mean values significantly decreased by 33% in group III compared to group II. Nitric oxide plasma levels in (Groups II & III), wer significantly increased ( $p < 0.001$  each), compared with the corresponding values in controls. The plasma concentrations in group II and III were increase by 275% and 192%

respectively. No significant difference was found between the levels of groups II and III. The plasma SOD activity levels in group II were significantly higher (64%) than the corresponding activity levels in control rats ( $p < 0.001$ ). Group III rats had significantly lower (27%) activity levels than group II rats ( $p < 0.05$ ). The plasma levels of TNF- $\alpha$  in group II were significantly higher than the corresponding levels of control rats. They exhibited 263% increase, ( $p < 0.001$ ). Group III rats had significantly lower levels than group II rats (68% decrease,  $p < 0.001$ ). PLA2 activity levels in group II rats were significantly lower (65% decrease) than those of control rats. In addition, they were significantly higher (178% increase) than values of group III rats ( $p < 0.001$  each). PGE2 plasma levels in both groups II and III were significantly and markedly decreased than the corresponding control levels (88% and 66% decrease respectively,  $p < 0.001$  each). Group III rats had significantly higher (186) levels of PGE2 than those levels of group II rats ( $p < 0.001$ ).

TNF- $\alpha$  plasma levels were significantly increase (269%) in IV in comparison to both control (269%) and group V (67%) levels ( $p < 0.001$  each). Plasma PLA2 activity was significantly increased in both IV and V groups in comparison to group I. The levels were increased by 31% and 47%. The levels of PGE2 in plasma of both of groups IV and V were significantly higher in group V than in group IV values.



A significant maternal increase in weight gain in garlic pretreated group (55.3± 1.73 versus 44.5± 1.44) was observed. The mean weight per fetus and placenta weight were significantly increased in garlic treated group by 39.65% and 16.6% respectively. Moreover, the mean average number of fetuses/mother was significantly increased in garlic treated group by 22.2%. There was significant increase in fetal weight by 19.8% at the end of period of observation (at day 14<sup>th</sup>).

Histopathological examination of the endometrium of pregnant animals pretreated with garlic showed an increase in height of secretory epithelium, marked dilation of endometrial glands compared to control group. Tissues from fetuses of pretreated animals showed features of enhanced maturation and differentiation.

Garlic in concentrations of 1, and 2 mg/ml produced a dose related decrease in spontaneous rhythmic uterine contraction of isolated pregnant uterine strips. However, garlic in concentration of 4 mg/ml has no effect.

Garlic in concentration of 1, 2, and 4 mg/ml produced dose related showed increase in basal tone of the isolated rat anococcygeous muscle. However, it produced inhibition of Ach induced contraction. Garlic in concentrations of 1, 2, 4 and 8 mg/ml produced a dose related increase in basal tone of isolated rabbit aortic strips. Also, 1 and 2 mg/ml produced dose related potentiation of N.adr. induced contraction (1mg/ml), meanwhile, 4 and 8 mg/ml inhibited N.adr. contraction.

Present study demonstrated that, intraperitoneal injection of variable dose levels of garlic dialysate in mice produced varying degrees of central nervous depression in form of sedation and loss of pain sensation that was proportional to the dose given. On increasing the dose of garlic dialysate up to 150 g/kg body weight, animals developed peripheral and central cyanosis, respiration became irregular, mostly abdominal. Convulsions of tonic type with occasional gasps terminating in respiratory arrest after 30 minutes of injection.. Postmortem examination of animals died by the lethal doses of garlic dialysate showed a picture of death due to respiratory arrest. There is hemorrhage and excess fluid in body cavities. All the organs showed severe congestion.

The data of histopathological study on lungs and brain an previous study by Joseph et al.,(1989), showed that the cause of death appear to be acute pulmonary edema. Recently, garlic preparation containing 1.3% allicin in a modest dose (14 mg of allicin) was shown to cause significant decrease in diastolic blood pressure in severely hypertensive patients. Although the mechanism by which allicin dilated the pulmonary vascular bed is uncertain.

Present study also demonstrated that all animals subjected to intragastric administration of 1 ml 96% ethanol or cold restrained stress induced ulcer, showed ulcerative lesions with mucosal damage. Biochemical

results revealed marked activation of lipid peroxidation process with significant increase in superoxide dismutase activity (SOD), nitric oxide (NO) and tumor necrosis factor- $\alpha$ , (TNF- $\alpha$ ) in both groups of rat with induced ulcer.

There is a significant increase in plasma level of NO in ethanol induced than in stress induced ulcer (275% vs 234%), with significant decrease in PGE2 level which also was more evident in ethanol induced ulcer (88% vs 73%) in comparison to control groups. Stadler et al., (1993) found that increased level of NO inhibits the synthesis of IL-6, PGE2 and thromboxane in macrophages (in injured tissue). This could be a factor that interprets the observed decrease in PGE2 in rats with induced ulcer. In the present work the plasma levels of TNF- $\alpha$  and IL-1 have been shown to stimulate PLA2 activity (Winkler et al., 1993). Neutrophil, monocytes and macrophages are important sources of secretory PLA2 in gastric disorders (Waite, 1987). However, in the present study PLA2 levels were significantly lower in group II than the corresponding levels in controls. This may be attributed to the severity of ethanol induced gastric lesions.

Moreover, this study was conducted to assess any fetotoxic effects of in utero garlic exposure in rats. The effect of garlic administration on isolated rat uterine strips was investigated. Observation of pregnant rats during the period of gestation revealed significant maternal weight gain with marked increase in size of gravid uterus. Results of isolated rat uterine strips taken from pretreated rats showed significant increase in frequency and

amplitude of spontaneous contraction. Also there was a significant increase in the mean weight of foetus, placenta and the mean average number of fetuses/mother in garlic treated group.

The difference in the effect of garlic on smooth muscles observed in the current study may depend largely upon the type of smooth muscle tissues, the contractile agent and garlic concentrations. The mechanism of this effect may be due to nature of PGs released and this needs further investigation.

### **Conclusion and Recommendation**

The results of the present work clearly indicate the rationale of the use of garlic (*allium sativum*) plant both as food and medicine. It can be used as a cytoprotective agent against gastric ulceration, in hyperlipidemia, in prophylaxis of coronary artery diseases and possibly as aphrodisiac due to its effect on increasing the release of nitric oxide.

It is recommended to investigate further in more details, the beneficial effects of garlic as antioxidant and for its nitric oxide releasing effects. As administration of garlic during pregnancy and lactation produced significant increase in the weight of offsprings and enhanced maturation and differentiation of foeti skin, bones and cartilage. Therefore, estimation of growth factors is recommended. However, due to the toxicity shown in mice and rats, the consumption of garlic in excessive amounts is not

recommended. It should be used in moderation.

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## دراسات سمية وفارماكولوجية على نبات الثوم

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**المستخلص :** نبات الثوم عشب طبي استخدمه الإنسان في شتى أنحاء العالم منذ آلاف السنين ولازال يستخدم حتى الآن لفوائده العلاجية العديدة في أمراض ارتفاع ضغط الدم والسكر والسرطان. وفي هذه الدراسة تم تحضير خلاصة الثوم وتحليل مكوناتها ودراسة السمية الحادة لهذه الخلاصة. ولقد أثبتت النتائج التي أجريت على الفئران البيضاء أن استخدامه آمن حتى جرعات كبيرة تصل إلى ٢١٨ جرام/ كيلو جرام.

وقد تم أيضا تقييم المفعول الواقي لخلاصة الثوم ضد تقرح المعدة التجريبي المستحدث من الجرذان بواسطة الإجهاد النفسي أو خلاصة الكحول المركزة. وقد لوحظ انخفاض ملحوظ في عدد القرع المتكونة في معدة الجرذان التي أعطيت خلاصة الثوم عن طريق الفم في جرعة قدرها ٣٠٠ مجم/كجم لمدة أربع أسابيع بالمقارنة بالمجموعة الضابطة. كذلك استطاع أن يثبت مفعول إرتخائي على التوتر الأساسي لنسيج المعدة المعزولة وأن يضاد المفعول الانقباضي ككل من خلايا الكولين والسيروتونين وكلوريد الباربيوم. كما أظهرت النتائج البيوكيميائية على أن المعالجة بخلاصة الثوم قد حافظت على مستويات كل من فوق أكاسيد الدهون وأنزيم فوق أكسيد الديسموتاز والفسفوليباز ٢ وأعمال الورم النخري عند معدلاتها الطبيعية. مما يدل على أنه قد أخرج عمليات الأكسدة في الجسم. وبالإضافة إلى ذلك فقد وجد زيادة جوهرية في مستويات كلاً من أكسيد النيتروز والبروستاجلاندين 2E في مصلى الجرذان المعالجة.

وأيضا تم تقييم التأثيرات الماسخة أو السامة على أجنة الفئران الحوامل بالتعرض المزمن لخلاصة الثوم حيث تم إعطاء خلاصة الثوم بالفم للفئران (٣٠٠ مجم/كجم) لمدة عشرين يوما من بداية الحمل. وقد أظهرت النتائج زيادة ذات دلالة إحصائية في وزن الفئران الحوامل في المجموعة المعالجة. وأوضحت النتائج عدم وجود تأثيرات سامة على الأجنة بل لوحظ زيادة في متوسط عدد

الأجنة كل أم في المجموعة المعالجة كذلك أثبتت النتائج الإحصائية زيادة في وزن الأجنة وزيادة في وزن المشيمة. كما لوحظ أيضا زيادة نمو أجنة الفئران المعالجة خلال فترة ٢١ يوما بعد الولادة.

كما أظهرت النتائج التي أجريت على أنسجة الرحم المعزولة من الفئران الحوامل أن لخلاصة الثوم تأثير مثبط ذو دلالة إحصائية على انقباضات الرحم.

ونستخلص من هذا البحث أن استخدام الثوم آمن وأن الحماية التي توفرها خلاصة الثوم ضد تقرح المعدة قد نتج بسبب العوامل المضادة للأكسدة وتوسيع الأوعية الدموية وزيادة تصنيع البروستاجلاندين والتي بدورها تؤدي إلى زيادة تدفق الدم والأكسجين والذي أدى أيضا إلى زيادة نمو أجنة الفئران وكذلك ارتخاء العضلات الملساء.