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ORIGINAL ARTICLE

Pyknotic Nuclei and Change in N/C Ratio of Heptocytes by Consumption of Allicin present in Garlic Extract

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ABSTRACT

Background: Garlic (allium sativum) is given as herbal medicine due to its antimalignanat effect. It has been reported that allium vegetables have marked anticancer effect against stomach and colorectal carcinomas. Organosulfur compounds in garlic activate the metabolizing enzymes and cytochrome P450s to detoxify carcinogens and other foreign compounds like lead. These components act as anticarcinogenic for gastrointestinal tract, lungs and mammary gland in experimental animals.
Aim: To evaluate the dose range of garlic (allium sativum) to induce the antitumor effect.
Study design: Experimental study
Place and duration of study: Department of Anatomy, Shaikh Zayed Postgraduate Medical Institute, Lahore from 1st October 2013 to 31st March 2014.
Methodology: Forty five Wistar albino rats of both sexes weighing between 250-350 grams were selected randomly. Two different doses of 500 and 1000 mg/kg of fresh garlic extract were given to the animals by orogastric tube for thirty days. After this period the analysis of quantitative parameters including the diameter of the hepatocytes and hapatic nucleus were recorded and nucleus to cell ratios (N/C Ratios) were calculated.
Results: The atrophic changes on the size of hepatocytes and their nucleus is noted in both experimental group B and C as compared to control group A (P<0.001).
Conclusion: There is a significant atrophic change, appearance of pyknotic nuclei indicating the apoptosis. That is leading to decrease in N/C ratio, beneficial in producing the anticarcinigen effect.
Keywords: Garlic (allium sativum), hepatocytes, atrophy, pyknotic nuclei, albino rats

INTRODUCTION

Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is the principal bioactive compound present in the garlic extract or in the raw garlic homogenate.[1] Chemically allicin is known as Allyl 2-propene thiosulphinate or diallyl thiosulphinate.[2] An enzymatic reaction resulted in producing allicin. Garlic on attacking by a microbe, crushing, cutting, chewing, damaging or its dehydration releases a vacuolar enzyme called alliinase which expeditiously lyses the cytosolic cysteine sulfoxides, alliin to form the cytotoxic and odiferous allicin. It is an oily colorless liquid which comprises 70 to 80% of the thiosulfinates.[3] Other volatile components in crushed garlic include diallylsulfide (DAS), diallyl disulfide (DADS), diallyl trisulfides (DATS), 2-vinyl dithiins and 3-vinyl dithiins.[4]

Besides above mentioned sulfur compounds, other constituents are 65% water. The dried minced garlic constitutes fructose and also contains carbohydrates, protein, fibers, and free amino acids.[2] High levels of saponins, phosphorus, zinc and potassium, moderate levels of selenium and vitamins A and C and low levels of sodium, calcium, magnesium, iron, manganese, and B-complex vitamins are also found in it. In addition, Garlic also contains rich phenolic content. Garlic contains most of the compounds which are water-soluble (97%) along with some compounds which are oil-soluble (0.15–0.7%).[4]

Aged garlic extract (AGE) is one of garlic preparation which is widely studied and used by research workers. It refers to sliced raw garlic stored in15–20% ethanol (C_2H_5OH) for 20 months. It mostly contains water soluble sulfur compounds including S-alkyl-L-cysteine and S-allylmercapto cysteine.[5]

The usage of highly concentrated garlic extract or garlic oil is expected to be toxic to heart, liver, kidneys and intestines as proved in different research studies.[2,6] It has been reported that 100 mg/kg body weight which corresponds to eating of 500 cloves or 1750 grams of raw garlic per day might induce acute toxicity in humans. Acute toxicity of garlic extracts in mice and rats have reported LD50 value greater than 32 g/kg irrespective of the route used.[7]

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MATERIALS AND METHODS

This experimental study was conducted in Department of Anatomy, Shaikh Zayed Postgraduate Medical Institute, Lahore in collaboration with Department of Zoology Quaid-e-Azam Campus, University of the Punjab Lahore. Forty five wistar albino rats of both sexes weighing between 250-350 grams were selected for this study. After 14 days of acclimatization the animals were randomly divided into three groups. Each group comprised of 15 animals. Group A (control), the animals of this group were not given garlic extracts but instead received distilled 4 ml/kg body weight of water by orogastric tube for 30 days. The other two groups B & C were experimental. Garlic extract 500 mg/kg and 1000 mg/kg was given respectively to the rats of experimental groups B & C through the orogastric tube for 30 days.

Garlic bulbs were purchased from the local market and then its extract was obtained from PCSIR, Laboratories Complex Lahore, which was prepared by soaking garlic paste in purified water. From 25 g of raw garlic, 1 ml of garlic extract was obtained which contained approximately 90mg of allicin. Two concentrations of extract were prepared 0.2 and 0.3 g/ml corresponding to doses of 500 and 1000 mg/kg body weight of animal respectively.[8] At the end of study the rats of all groups were weighed properly before dissection and recorded. On the day 7 all the rats were euthanized by giving morphine 0.3–0.5mg/kg intraperitoneally, as an analgesic agent. The anaesthetic agent sodium pentabarbitol was administered intraperitoneally with dose of 45mg/kg. After dissection the histologic parameters recorded were inflammation and congestion in the periportal areas. The data was entered and analyzed through SPSS-20.

RESULTS

The average diameter of hepatic nucleus in group A was 9.445±0.48 µm as compared to group B and C which showed reduced value with average diameter of 7.82±0.36 and 7.64±0.34 µm respectively. The difference among groups was significant (P<0.001). The difference of group B and C was highly significant form group A (P<0.001). The diameter of hepatocyte for group A was 16.15±0.75 µm, for group B 14.18±0.84 µm and for group C13.48±0.78 µm. The difference for diameter between three groups was significant (P<0.001). The pair wise comparison revealed that the diameter of group B and C was significantly less than that of group A (P<0.001). The nucleus to cell ratio (N/C Ratio) is showing significant decline from the control group A (34.2%) to the experimental group B (32.1%) and Group C (30.4%). These results showed significant changes (P<0.001) [Tables 1-2, Figs. 1-3). Nucleo-cytoplasmic ratios were calculated by using the formula: N/CELL = nucleus area/cell area)

Table 1: Diameter of hepatic nucleus of rats (µm) and diameter of hepatocytes of rats (µm) in control and experimental groups

Group	Diameter of Nucleus	Diameter of Hepatocytes
Group A	9.45±0.48	16.15±0.75
Group B	7.82±0.36	14.18±0.84
Group C	7.64±0.34	13.48±0.87

Table 2: Comparison of diameter of hepatic nucleus of rats (µm) in control and experimental groups after administration of garlic extract (ANOVA)

	Sum of squares	DF	Mean square	F	P value
Between Groups	29.98	2	14.99	94.5	< 0.001*
Within Groups	6.66	42	0.16		
Total	36.64	44			

DF Degree of Freedom F f-test (Ratio of variances) * Significant difference (P < 0.05)

Fig. 1: Photomicrograph of liver of adult albino rat of control group A

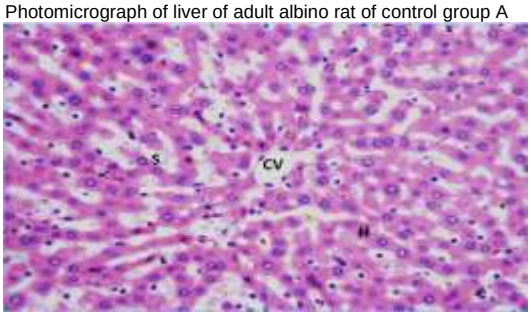


Fig. 2: Photomicrograph of liver of adult albino rat of Experimental group C showing Necrosis of hepatocytes and Pyknotic nuclei

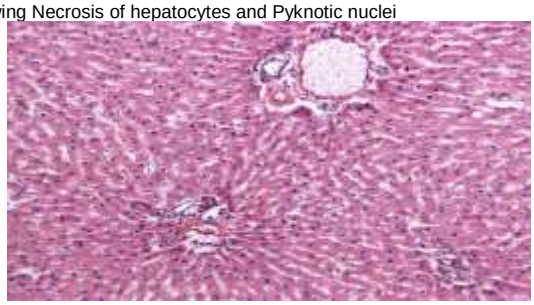
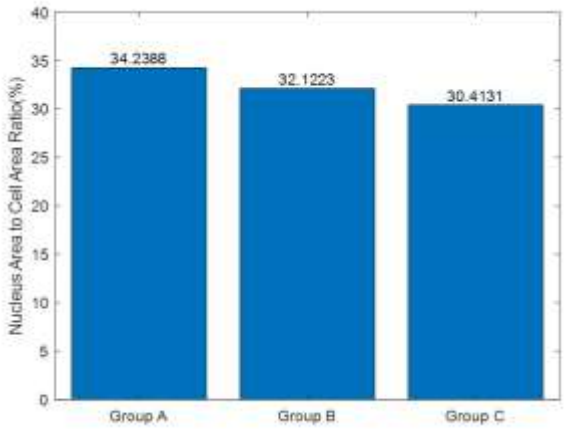


Fig. 3: Comparison of hepatic nucleus to area of hepatocyte ratio (N/C Ratio) of rats in control and experimental groups after administration of garlic extract



DISCUSSION

Garlic has its most popular use in our daily diet. It has medicinal importance for its multiple beneficial and protective effects. This study also reflects it beneficial effect as anticancer property by enhancing the apoptosis and cell death. There was decrease in diameter of hepatocyte of animals in experimental groups B and C as compared to control group A (Table:1, Fig:2). The atrophic changes after garlic administration would be due to the irregularities in blood circulation and sinusoidal congestion. That would be suggestive of ischemic atrophy.[9] These findings of atrophied hepatocytes and hepatic lobule are in accordance with the findings of Manal, who also noted the atrophy of hepatocytes after administration of 10% dried garlic powder.[10]

The average diameter of nucleus of hepatocytes in experimental group B and C was reduced as compared to control group A. The difference among groups was statistically significant (P<0.001, Tables 1-2). Such nuclear shrinkage with increased basophilia is suggesting the pyknotic nuclei in the hepatocytes undergoing necrosis.[11] In this study many binucleated nuclei were also observed (Figs. 1-2). The increase in the number of binucleated cells would most likely thought to be due to inhibition of cell division process during cellular injury.[12] These findings coincide with the histological changes observed by Rehman and Banerjee in their research on albino rats that showed hepatocellular necrosis in the animals due to higher doses of garlic[2,6].

The nucleus to cell ratio (N/C Ratio) is reduced showing significant change among the control group A and the experimental group B & C Group C (Fig. 3). It is the parameter indicating increase in the apoptosis and cell death.[13] These results are in support of the anticancer effect of the allicin. It was reported in a previous study that allicin has potential to suppresses the growth of various tumor cells. In particular, it targets many signaling pathways associated with cancer development.[14] The organosulfur compounds in garlic activate the metabolizing enzymes and cytochrome P450 to detoxify the carcinogens.[15]

Allicin may induce cell apoptosis, cell cycle arrest and inhibit proliferation. It was seen in a study that allicin can mediate the apoptosis of cancer cells by activating caspase-3, −8 and −9,[16] and also could activate autophagy of human liver cancer cells and induce cell death through apoptosis[15],[16]

It has been confirmed that it does not affect the growth of normal intestinal cells when inducing apoptosis of gastric cancer cells.[17,18] Secondary metabolites of allicin also play antitumor roles by inhibiting tumor cell proliferation and inducing apoptosis[18].

CONCLUSION

Although the garlic extract at higher doses has the potential antitumor effect and could induce toxicity, so it may be used as the combination therapy, which is a pharmaceutical regimen that would be helpful to suppress the tumor cell proliferation. Therefore, that would be beneficial to use more than one drug to cure a disease and obtain higher response as compared to the single treatment. For instance, the synergistic activity of two antineoplastic agents allows to reach the maximum antitumor effect using a lower concentration of each compound and thus to limit the probability of inducing toxicity. This strategy is also common to overcome cancer chemoresistance that can arise from monotherapy.

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