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16. Antitumor Activity of Allicin on Lung Cancer in Benzopyrene Induced Swiss Albino Mice Model

Zeenath, Gunavathi V., Santhy, K. S.

Department of Zoology,
Avinashilingam Institute for Home Science and
Higher Education for Women, Coimbatore.

Abstract:

The present study aimed to assess the efficacy of Allicin, an important compound for its antitumor activity in benzopyrene induced lung tumour in mice model. Body weight, lung weight, various haematological parameters were analysed. Increase in body weight and reduction in lung weight were observed in Allicin treated groups. RBC, HB and platelets were seen as near normal and reduction in WBC were observed in Allicin 20 and 40mg/kg. In a WBC differential count, increase in neutrophil and a decrease in lymphocyte, monocyte, eosinophil and basophil in benzopyrene treated mice was observed after the Allicin treatment. The results of this study indicated the potential benefits and antitumor activity of Allicin on benzopyrene induced Swiss albino mice.

Keywords: Benzo(a)pyrene, Lung cancer, Allicin, and Hematological parameters.

16.1 Introduction:

Lung cancer is the major common cancer in women and men worldwide. It is a leading cause of cancer death in under developed countries. Metastatic ailment is the most common cause of lung cancer death. In modern medicine, various drugs and therapies are available in the market. But the biggest drawbacks are serious side effects and economic burden. Hence, the present study was aimed to assess the efficacy of isolated compound of Allicin for its antitumor activity in benzopyrene induced lung cancer in Swiss albino mice. Benzopyrene, a polycyclic hydrocarbon in tobacco, plays a major role in the aetiology of lung tumour. It is metabolically activated into benzopyrene 7, 8-diol-9, 10-epoxide, that reacts with DNA predominantly to form DNA adduct and progression of the disease (Mo *et al.,* 2021). The carcinogenesis involves forming of free radicals and peroxidation products, which damages many cellular macromolecules (Kim *et al.,* 2000a).

Allicin is an organo-sulphur compound that chokes malignant growth development in vitro in lung disease, hepatocellular carcinoma, melanoma, colorectal adenocarcinoma (Rajput *et al.,* 2012) and glioma cells (Li *et al.,* 2018). Allicin has been shown to be effective in killing cancer cells derived from kidney (Song *et al.,* 2015), liver, ovary, pancreas, stomach, brain (Cha *et al.,* 2012), bone and lung (Tyagi *et al.,* 2014). It can animate the invulnerable framework to deliver some bioactive anticancer elements which hinder tumour cells and can likewise invigorate the arrival of numerous cytokines which improve the resistant framework (Lichota *et al.,*2018). The present study was designed to analyze the antitumor activity of Allicin against lung cancer through the analysis of haematological parameters, body weight and lung weight.

16.2 Methods and Materials:

16.2.1 Chemicals:

2, 2- diphenyl-1-picrylhydrazyl, Benzo(a)pyrene and Allicin were purchased from Sigma Aldrich chemical company. Ferric chloride and Trichloroacetic acid (TCA) from Hi media and Merck. Ascorbic acid was procured from SDFCL (Biosar, India). All the other solvents and chemicals were of analytical grade.

16.3 *In Vivo* Studies on Benzopyrene Induced Lung Cancer in Swiss Albino Mice:

16.3.1 Experimental Animals:

Healthy male Swiss albino mice (20-25g body weight) were used for the study. The experiments were performed after the approval from the Institutional Animal Ethical Committee (AIW: IEAC.2017: ZOO: 04) and in accordance with the recommendation for the proper care and use of the laboratory animals. Animals were kept in polypropylene cages with sawdust bedding and they were maintained in a controlled environment condition of temperature and humidity on alternatively 12 h light/dark cycles. Standard pellets were given as diet and water was provided ad libitum.

16.3.2 Acute Toxicity Study:

Healthy Swiss albino mice, starved overnight, were divided in to six groups. Group I – V animals were orally fed with Allicin in increasing dose levels of 0, 25, 50, 75 and 100mg/Kg body weight. The animals were observed continuously for first 2 h for any gross change in behavioural, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again after 24 h, 48 h and 72 h for any lethality or death.

16.3.3 Tumour Induction and Drug Treatment:

Benzopyrene is a powerful carcinogenic agent and is used to induce tumours in animals. Mice were treated twice per week for 4 weeks with benzo(a)pyrene (50 mg/kg body weight dissolved in olive oil) orally. After the experimental period, the animals were fasted overnight and sacrificed, the blood was collected from the control and the experimental groups of mice; the serum was separated out for the biochemical analysis. All the vital organs were washed with ice cold saline, removed, trimmed and stored. Finally, tumour volume, and tumour weight were studied.

16.3.4 Experimental Design:

Animals are grouped and treatment was given as follows,

- **Group I:** Normal Control {mice were given normal olive oil orally for 16 weeks}.

- **Group II:** Benzopyrene (50mg/Kg) {mice were treated twice per week for 4 weeks with benzo(a)pyrene (50 mg/kg body weight dissolved in olive oil) orally and then continued with or without the vehicle for additional 12 weeks}
- **Group III:** Benzopyrene + Paclitaxel (5mg/Kg) {mice were treated twice per week for 4 weeks with B(a)p (as in Group II). Subsequently, they were treated with paclitaxel (5 mg/kg body weight dissolved in olive oil orally) daily for 12 weeks}
- **Group IV:** Benzopyrene + Allicin (20mg/Kg) {mice were treated with B(a)p (as in Group II) for 4 weeks and subsequently, they were treated with Allicin 20mg/kg and continued for 12 weeks}
- **Group V:** Benzopyrene + Allicin (40mg/Kg) {mice were treated with B(a)p (as in Group II) for 4 weeks and subsequently, they were treated with Allicin 40mg/kg and continued for 12 weeks}
- **Group VI:** Allicin alone (20mg/Kg) {mice were treated with Allicin alone 20mg/kg for 16 weeks to find out cytotoxicity, if any, induced by Allicin}.

16.4 Body Weight and Lung Weight Analysis:

A careful record of body weight of all the animals belonging to normal control and different treatment groups was kept throughout the study. The animals were weighed at the beginning of the experiment and then weekly, and finally before sacrifice. At the end of the study, lungs were excised from the mice, washed in normal saline and the weights were measured using digital weighing balance.

16.5 Haematological Analysis:

Haemoglobin (Hb), Red blood cell (RBC), White blood cell (WBC) and platelet (PLT) count were estimated using an auto haematology analyzer (BC-2800 Vet, Mindray Medical Instrumentation, China). Differential leukocyte counts were determined from the blood smears stained with Leishman-Giemsa stain.

16.6 Statistical Analysis:

All numerical data is presented as the mean value ± standard deviation. All the *in vitro* experiments were done in triplicate, and the experiments were repeated at least thrice. The statistical software SPSS version 17.0 was used for the analysis. p value <0.01 was considered significant. *In vivo* antitumor activity of Allicin was determined by applying One-way ANOVA followed by Duncan's multiple range test. Statistical difference was considered significant if p value was less than 0.05 and 0.01.

16.7 Result and Discussion:

16.8 Acute Toxicity Study:

The results of acute toxicity study of Allicin are presented in Table 16.a. No mortality or change in body weight was observed in mice at a dose level of Allicin 0, 25, 50 and 75 mg/Kg body weight. Some clinical signs such as tremors, pilo erection and abdominal

breathing were observed after the oral dosing of 100 mg/Kg but no mortality or change in body weight was observed. These observations indicated that the calculated LD_{50} value (Dixons likelihood method) for the oral doses of the Allicin was found to be more than 100 mg/Kg body weight, accordingly 20 and 40 mg/Kg body weight were taken as low and high dose of Allicin for the experiment.

Table 16.a: Clinical signs of toxicity observed during acute oral toxicity study of Allicin

Dose of Allicin (mg/Kg b.wt)	Latency	Symptoms
0	-	None
25	-	None
50	-	None
75	-	None
100	-	Tremor, Piloerection, abdominal breathing

16.9 Effect on Body Weight and Tumor Growth Analysis:

Table 16.2 depicts the body and lung weight of animals in various treatment groups. Throughout the experimental period, except Group I (Control) all the animals decreased body weight. After tumour induction, at the end of the 16th week, when compared with tumour bearing group II (14.27 ± 1.24mg), the treatment groups III, IV and V (positive control and Allicin 20 and 40mg/Kg) showed increase in body weight 24.50 ± 1.99, 23.66 ± 0.98 and 24.08 ± 1.02 respectively. Allicin alone (20mg/Kg) treated group recorded a weight of 24.42 ± 1.34 when compared with normal control (25.55 ± 1.89) indicates that Allicin has no significant adverse effects on the normal weight. Accordingly, the lung weight which was found to be high in the Group II (342.05±17.30mg) mice was significantly ($p<0.05$) decreased by the paclitaxel treatment and Allicin treatment (20 and 40mg/Kg) (Table 16.b).

Table 16.b: Effect of Allicin on body weight and lung weight of treated groups of Swiss albino mice

Parameters	Body weight (g)	Lung weight (mg)
Normal control	25.55 ± 1.89^{a}	223.58 ± 15.82^{b}
Benzopyrene (50mg/Kg)	14.27 ± 1.24^{b}	342.05 ± 17.30^{a}
Benzopyrene(50mg/Kg) + Paclitaxel (5mg/Kg)	24.50 ± 1.19^{a}	276.59 ± 8.62^{b}
Benzopyrene(50mg/Kg) + Allicin (20mg/ Kg)	23.66 ± 0.98^{a}	261.8 ± 8.72^{b}
Benzopyrene(50mg/Kg) + Allicin (40mg/ Kg)	24.08 ± 1.02^{a}	255.8 ± 9.25^{b}
Allicin alone (20mg/Kg)	24.42 ± 1.34^{a}	249.6 ± 9.63^{b}
F-test	**	**

Parameters	Body weight (g)	Lung weight (mg)
Sed	0.83	7.65
CD ($p< 0.05$)	2.18	20.06

Values are expressed as the mean ± S.E.M (n=6); Statistical significance (p) calculated by one way ANOVA followed by Duncan's multiple range test. Significant levels were ** $p < 0.01$; * $p < 0.05$; NS –Non significant.

16.10 Effect of Allicin on Haematological Parameters:

In our present study, Benzopyrene induced mice showed reduction in haemoglobin and RBC count, when compared to control group, which is an indication of anaemia. Anaemia might occur due to the free radicals resulting from benzopyrene metabolism which leads to direct liver injury.

The free radicals liberated from the liver into the circulation affect the erythrocytic membranes leading to the disturbed haematopoiesis, destruction of erythrocytes and reduction in the rate of their formation and their enhanced removal from the circulation (Nithya *et al.,* 2014).

White blood cells, lymphocytes and neutrophils play a crucial role in the systemic inflammatory response, often observed in cancer patients. Increase in WBC count and alterations in differential count (lymphocytes, monocytes, Eosinophils, Basophils and neutrophils) have been suggested as one of the hallmarks of carcinogenesis.

In the current study the lung cancer bearing animals showed elevated WBC count and neutrophil count with reduced lymphocyte, monocyte, Eosinophils and Basophils in Benzopyrene induced Swiss albino mice. In a differential count of WBC, a significant ($p< 0.05$) decrease in monocyte, Lymphocytes, basophils and eosinophil and an increase in Nutrophilsin benzopyrene induced Swiss albino mice were observed (Sreejaya and Santhy, 2014).

Lymphocytes were significantly reduced in number, in response to stressful condition. Moreover, lymphocytes migrate to the site of inflammations which may be due to toxic effect of benzopyrene (Nithya *et al.,* 2014). This was in accordance with the work of Saeed *et al.,* (2011) who attributed the increase in the WBC count to antioxidant activity of vitamin E and tocopherol quinone. Similarly, Allicin could increase the WBC count, due to its role in free radical scavenging (Hajzadeh *et al.,*2008).

Lymphocytes play akey role in all immune responses and are always directed against the specific foreign antigens (toxins). Lymphocytes were significantly decreased in number in response to stressful condition. In addition, lymphocytes migrate to the site of inflammations which may be resulting due to toxic effect of B(a)P. We observed a significant difference in circulating WBC after Allicin treatment and no significant difference was observed between control and Allicin alone treated animals (Table 16.c).

Table 16.c: Effect of Allicin on haematological parameters

Parameters	Haemoglobin (g/dl)	RBC ($x10^6$ cells/µl)	WBC ($x10^3$ cells/µl)	Neutrophils (%)	Monocytes (%)	Lymphocytes (%)	Eosinophils (%)	Basophils (%)	Platelets ($x10^3$ cells/µl)
Normal control	12.67 ± 0.88a	6.06 ± 0.61a	6.72 ± 1.16b	29.52 ± 1.93a	0.50 ± 0.08a	68.59 ± 1.56b	0.73 ± 0.15a	2.41 ± 0.04a	405.4 ± 9.47a
Benzopyrene (50mg/kg)	8.97 ± 0.69b	3.77 ± 0.82b	12.78 ± 0.99a	47.12 ± 1.56b	0.17 ± 0.05a	38.23 ± 1.71a	0.26 ± 0.14b	1.52 ± 0.02b	367.6 ± 16.00b
Benzopyrene(50mg/kg) + Paclitaxel (5mgmkg)	11.30 ± 0.96a	5.08 ± 0.82a	10.79 ± 1.07b	44.69 ± 1.10a	0.24 ± 0.03b	64.60 ± 1.68b	0.72 ± 0.17a	2.22 ± 0.01a	376.4 ± 15.78a
Benzopyrene(50mg/kg) + Allicin (20mg/kg)	11.56 ± 0.84a	4.86 ± 0.81a	11.00 ± 0.80b	44.80 ± 1.97a	1.41 ± 0.06b	67.24 ± 1.45b	0.66 ± 0.15a	2.17 ± 0.03a	378.2 ± 12.13a
Benzopyrene(50mg/kg) + Allicin (40mg/kg)	11.86 ± 0.90a	4.97 ± 0.58a	12.30 ± 0.71b	46.58 ± 1.51a	0.44 ± 0.09b	67.36 ± 1.66b	0.68 ± 0.12a	2.20 ± 0.05a	377.6 ± 16.30a
Allicin alone (20mg/kg)	11.47 ± 0.54a	4.82 ± 0.84a	10.73 ± 0.86a	44.53 ± 1.14a	1.26 ± 0.07b	66.43 ± 1.70a	0.55 ± 0.15a	2.03 ± 0.06a	376.2 ± 16.17a
F- test	**	**	**	**	*	**	**	NS	**
SED CD($p<0.05$)	0.5171.355	0.4801.258	0.6011.574	0.9962.609	0.1050.277	2.8917.575	0.097 0.255	0.275 0.722	9.20 24.108

Values are expressed as the mean ± S.E.M (n=6); Statistical significance (p) calculated by one way ANOVA followed by Duncan's multiple range test. Significant levels were ** $p < 0.01$; * $p < 0.05$; NS –Non significant.

16.11 Conclusion:

In the current study, significant increase in the activities of these membrane integrity enzymes in Allicin treated animals indicate the protective role of Allicin. From the above results, it can be inferred that Allicin possess significant anticancer effect through its role in prevention of erythrocyte membrane damage and restoration of membrane integrity.

16.12 Reference:

1. Cha, J.H., Choi, Y.J., Cha, S.H., Choi, C.H. and Cho, W.H., 2012. Allicin inhibits cell growth and induces apoptosis in U87MG human glioblastoma cells through an ERK-dependent pathway. *Oncology reports*, 28(1):41-48.
2. HAJZADEH, M., MOHAMMADIAN, N., RAHMANI, Z. and BEHNAM, R.F., 2008. Effect of thymoquinone on ethylene glycol-induced kidney calculi in rats.
3. Kim, H.S., Kwack, S.J. and Lee, B.M., 2000. Lipid peroxidation, antioxidant enzymes, and benzo [a] pyrene-quinones in the blood of rats treated with benzo [a] pyrene. *Chemico-biological interactions*, 127(2):139-150.
4. Li, C., Jing, H., Ma, G. and Liang, P., 2018. Allicin induces apoptosis through activation of both intrinsic and extrinsic pathways in glioma cells. *Molecular medicine reports*, 17(4):5976-5981.
5. Lichota, A. and Gwozdzinski, K., 2018. Anticancer activity of natural compounds from plant and marine environment. *International journal of molecular sciences*, 19(11):3533.

6. Mo, J., Au, D.W.T., Guo, J., Winkler, C., Kong, R.Y.C. and Seemann, F., 2021. Benzo [a] pyrene osteotoxicity and the regulatory roles of genetic and epigenetic factors: A review. *Critical Reviews in Environmental Science and Technology*, 1-39.
7. Nithya, G., Mani, R. and Sakthisekaran, D., 2014. Oral administration of thymoquinone attenuates benzo (a) pyrene induced lung carcinogenesis in male Swiss albino mice. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(7):260-263.
8. Rajput, S. and Mandal, M., 2012. Antitumor promoting potential of selected phytochemicals derived from spices: a review. *European Journal of Cancer Prevention*, 21(2):205-215.
9. Saeed, M., Villarroel, M., Reisner, A.T., Clifford, G., Lehman, L.W., Moody, G., Heldt, T., Kyaw, T.H., Moody, B. and Mark, R.G., 2011. Multiparameter Intelligent Monitoring in Intensive Care II (MIMIC-II): a public-access intensive care unit database. *Critical care medicine*, 39(5):952.
10. Song, B., Shu, Y., Cui, T. and Fu, P., 2015. Allicin inhibits human renal clear cell carcinoma progression via suppressing HIF pathway. *International journal of clinical and experimental medicine*, 8(11):20573-20580.
11. Sreejaya, S.B. and Santhy, K.S., 2014. Evaluation of Antitumor properties of Rhizome of Acorus calamus L using DALTON'S Ascites Lymphoma Bearing Swiss Albino mice. *Int J Pharm Bio Sci*, 5(4):119 – 125.
12. Tyagi, G., Pradhan, S., Srivastava, T. and Mehrotra, R., 2014. Nucleic acid binding properties of allicin: Spectroscopic analysis and estimation of anti-tumor potential. *Biochimicaet Biophysica Acta (BBA)-General Subjects*, 1840(1):350-356.