

Phytoprotective Effects of Isolongifolene Nanoparticles (ICN-K04) against Benzo (a) pyrene - Induced Lung Cancer in Sprague Dawley Rats

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DOI: 10.67224/ioasdjmps.2026.v03i04.001

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ABSTRACT	Original Research Article
Benzo (a) pyrene (B(a)P) is a potent chemical carcinogen associated with lung carcinogenesis and cellular damage. The current research has demonstrated the effects of 5-Fluorouracil (5-FU), Chitosan Nanoparticles, Isolongifolene and ICN-K04. Animals were divided into Six groups: Control, B(a)P, B(a)P + 5-FU, Chitosan nanoparticles, Isolongifolene and ICN-K04. Body weight, Tumor incidence, Lung weight and Pulmonary ultrastructure were assessed. B(a)P-treated animals showed progressive body weight loss and 100% tumor incidence. Tumor incidence decreased to 37.5%, 75%, 25%, and 0% following 5-FU, chitosan nanoparticles, isolongifolene, and ICN-K04 treatment, respectively. Lung weight was significantly reduced in the 5-FU- and ICN-K04-treated groups. TEM analysis showed lipid accumulation, nuclear abnormalities, and mitochondrial swelling in B (a)P-treated animals. Isolongifolene and ICN-K04 treatment showed comparatively preserved cellular ultrastructure and reduced lipid accumulation. Overall, isolongifolene and ICN-K04 showed protective effects against B(a)P-induced lung carcinogenesis under the experimental conditions. Keywords: Isolongifolene, Chitosan nanoparticles, Lung Cancer, ICN-K04 and TEM.	Article History Received: 15-07-2026 Accepted: 07-09-2026 Published: 01-10-2026
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INTRODUCTION

Novel drugs entering the oncology market has slowed in recent years, there has been a significant shift toward exploring new therapeutic approaches. The cancer-related deaths prompted the pharmaceutical industry has turned its attention to investigate a new class of therapeutic agents known as anti-cancer and anti-tumor agents.

The anticancer potential of a new formulation using *in vivo* experimental models under controlled conditions was evaluated. Preventing or reducing the formation of carcinogen-induced tumors in animal models is considered an effective approach for analyzing anticancer activity (Manimaran *et al.*, 2024). Well-designed animal models make it possible to determine the effective dose of a new formulation and allow for the precise quantification of its antitumor effects. Despite the complexity of lung cancer biology, its molecular studies

are becoming increasingly well understood (Salgia *et al.*, 2011).

Benzo(a)pyrene is considered a prototype toxic polycyclic aromatic hydrocarbon (PAH), a classic DNA-damaging agent, and a potent carcinogen. Benzo(a)pyrene formed by incomplete combustion of organic materials such as tobacco, coal tar, wood, organic fuels, grilled or smoke foods (Zhang *et al.*, 2021a). It is classified as Group 1 carcinogen by International Agency for Research on Cancer (IARC) (Amadou *et al.*, 2021). It has been identified as a major risk factor for inducing Lung cancer (Hepato cellular carcinoma). Nanoparticles are extremely small and capable of penetrating cells to reach intracellular components—such as proteins and DNA—within pathological tissues (Petros and DeSimone, 2010).

The use of plant-derived compounds in the treatment of various diseases, particularly cancer, has

expanded significantly. These compounds are commonly referred to as phytochemicals. Despite the diversity of their applications, their clinical use remains limited due to insufficient evidence and low bioavailability. Phytochemicals have the tendency to suppress the survival and proliferation of malignant cells (Yan *et al*, 2018), as well as diminishing the invasiveness and vascularization of tumors cells (Lu *et al*, 2018a). The safety and efficacy profiles of many plant-derived compounds, as well as their rates of assimilation and absorption through the digestive system into the bloodstream, remain poorly understood. There is therefore an increasing need to develop alternative approaches for the effective use of these phytochemicals and to explore their potential as chemotherapeutic and chemopreventive agents.

This essential requirement has sparked considerable interest among researchers in the development of innovative and advanced drug delivery systems capable of overcoming the limitations associated with conventional approaches. In recent years, numerous studies have reported on *in vitro* and *in vivo* experiments involving various biocompatible and biodegradable nanoparticles designed to encapsulate and deliver phytochemicals to the target cells.

Initially, several plants were selected and analyzed for their chemical properties to screen for the presence of isolongifolene. The results showed that many of these plants contained isolongifolene at varying concentrations. Among them, *Hydrocotyle javanica* and *Lantana camara* exhibited relatively high isolongifolene levels—5.10% and 7.79%, respectively. Isolongifolene is a sesquiterpene hydrocarbon with a longifolene-type skeleton; it has been identified in the essential oils of Pine cones and Japanese cedar (Shieh *et al.*, 1981; Kurose *et al.*, 2007).

Isolongifolene is a commercially available Sesquiterpenoid. It is widely used as a fragrance ingredient in cosmetics, perfumes, room sprays, detergents, deodorants and fabrics. It exhibits characteristic woody and amber olfactory smell. The present study was therefore conducted to evaluate the anti-carcinogenic potential of isolongifolene nanoparticle against benzo(a)pyrene-induced lung cancer in Sprague Dawley (*Rattus norvegicus*) rats.

MATERIALS AND METHODS

Materials

Isolongifolene was purchased from sigma Aldrich, Gelatin was obtained from Central Drug House (Delhi, India), sodium alginate, chitosan (Medium molecular weight), sodium tripolyphosphate were purchased from Genei, Bangalore, India. All other chemicals used were of analytical grade.

Preparation of Isolongifolene- chitosan nanoparticles

Isolongifolene Chitosan nanoparticles (ICN) were prepared by ionic crosslinking of chitosan with sodium tripolyphosphate (TPP) anions as given by Manimaran *et al.*, 2022. About 2 mg/mL of Chitosan was dissolved in 0.25%v/v acetic acid with constant stirring at 10°C for 12 hrs. 0.75% w/v of TPP (aqueous solution) was added (2:1 ratio) into chitosan solution containing 100 µg/mL of isolongifolene (dissolved in methanol) with continuous stirring for 6 hrs at 4°C. The resultant dispersion was centrifuged at 13000×g for 20 min at 4°C. The supernatant was then removed and Isolongifolene Chitosan Nanoparticles (ICN) were collected and stored at -55 °C.

Experimental animal protocol

Sprague Dawley (*Rattus norvegicus*) rats obtained from National Institute of Nutrition, Hyderabad, India, and were housed in sterilised polypropylene cages. They were maintained under standard laboratory conditions of controlled atmospheric parameters (12:12 h light/dark cycles with an ambient temperature of 25 ± 5°C and humidity at 50 ± 10%). Rats were fed with standard pellet diet and were given free access to water ad libitum. All the procedures with animals were strictly conducted in accordance with approved guidelines of IAEC (Institutional Animal Ethical Committee) regulated, constituted and monitored by the CCSEA (Committee for Control and Supervision of Experiments on Animals), Department of Animal Husbandry and Dairying (DAHD), Ministry of Fisheries, Animal Husbandry and Dairying, Government of India. The Animal ethical committee approval number obtained was 1085/ac/07/PU-IEAC/ 2012/04.

The animals were randomly sorted into six groups (n=8 per group). The treatment regimen was performed by administering the optimum dose of Isolongifolene was mentioned below by schematic representations.

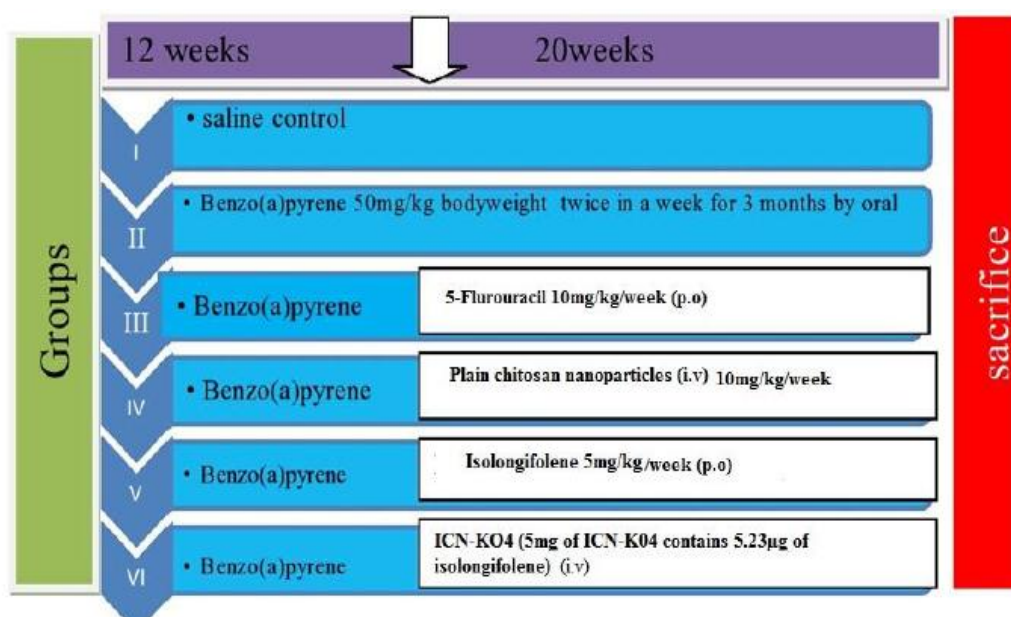


Figure 1: Protocol for experimental regimen.

Healthy Sprague Dawley (SD) rats were divided into six groups. Each group consists of eight animals. And the treatment details of six groups given below:

Group 1: Control SD rats treated with Saline alone (P.O).

Group 2: Negative control SD rats were treated with Benzo(a)pyrene 50mg/kg body Weight (P.O) for 3 months.

Group 3: SD Rats were treated with Benzo (a) pyrene and after 3 months of PIP (Post Induction Period) onwards rats treated with 10 mg/kg/week of 5-Flurouracil (P.O route).

Group 4: SD Rats were treated with Benzo (a) pyrene and plain chitosan 10 mg/kg/day (I.V) after 3 months of PIP

Group 5: SD Rats were treated with Benzo (a) pyrene and Isolongifolene 250 mg/kg/day (P.O) after 3 months of PIP;

Group 6: SD Rats were treated with Benzo (a) pyrene and after one month of PIP the animals treated with ICN-K04 5mg/kg/week (I.V)

At the end of experiment (150 days), animals were sacrificed with an excess of diethyl ether anesthesia, the lung tissues were dissected, processed and analyzed for further studies.

Body weight and Lung weight changes

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

Ultrastructure analysis using Transmission Electron Microscopy (TEM)

After fixation, lung sections were cut into approximately 1 mM cubes and post fixed in 1% Osmium tetroxide for 2hrs at 4°C. Then, cubes were dehydrated in series of ethanol and treated twice with Propylene oxide for 10 min (RT). Further, tissues were infiltrated with EPON mixture and Propylene oxide (1:1 v/v for 2hrs at RT) and embedded in EPON mixture containing Taab/812, followed by polymerization at 60°C for 24 hrs. Ultra microtome was used to cut tissues at 0.5µm sections and stained with 0.5% toluidine blue to view the cells. Then the 60nm ultra-thin sections were cut and mounted on copper grids (300 mesh). The sections were double stained with Uranyl acetate and Lead citrate and then examined by Transmission Electron Microscope (Philips CM10) and photographed.

Statistical analysis

All data were expressed as Mean $\pm$ Standard Error Mean (SEM) of number of experiments. One-way analysis of variance (ANOVA) using Graphpad PRISM (Version-5.01, USA).

RESULTS

Body weight

The initial and final body weights were observed which was shown in Figure 1. Body weight of the Control and Experimental animal shows that there were no significant changes occurred initially. But eventually, there was a sharp decline in the body weight in experimental groups when compared to control groups. However, significant weight variations were observed among the different groups. The tumor incidence of each treatment group and lung weight was measured which was shown in Table 6 and Figure 2. At the end of the treatment, 5-Flurouracil, Isolongifolene and ICN-K04 (Group 3, 5 and 6) treated group's shows

minimal tumor induction. However, the lung weight is significantly decreased in 5-Flurouracil and ICN-K04

when compared to Bezo (a) pyrene and Chitosan treated (Group 2 and 4) animals.

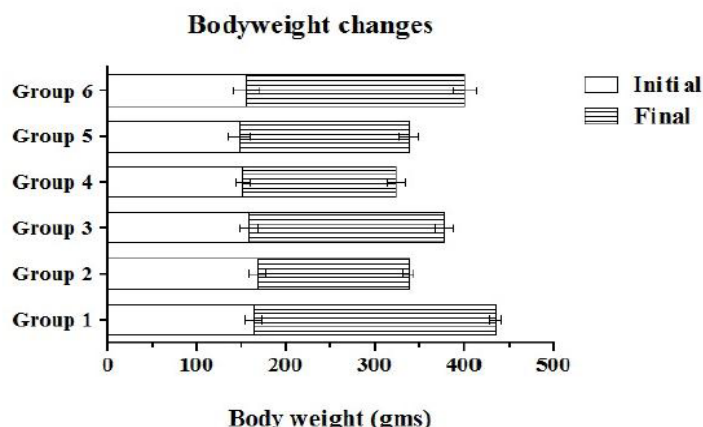


Figure 2: Body weight changes of the Control and Experimental animals. Treatment –Group 1: Saline, Group 2: Benzo (a) pyrene, Group 3: Benzo (a) pyrene+5-Flurouracil, Group 4: Benzo(a)pyrene + Chitosan nanoparticles, Group 5: Benzo(a)pyrene + Isolongifolene, Group 6: Beno(a)pyrene +ICN-K04.

Table 1: Tumor incidence of the control and experimental animals (%)

S.No	Groups (n=8)	No of tumor incurring SD rat	Tumor incidence (%)
1	Control	0	0
2	Benzo (a) Pyrene	8	100
3	B(a)P + 5-FU	3	37.5
4	B(a)P + Chitosan	6	75
5	B(a)P + Isolongifolene	2	25
6	B(a)P + ICN-K04	0	0

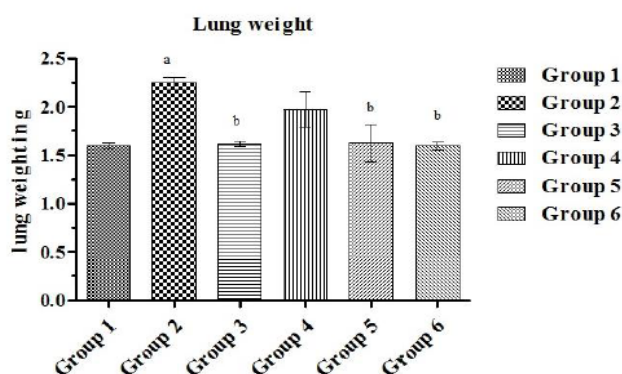


Figure 3: Lung weight changes of the control and experimental animals. Treatment: Group 1: Saline, Group 2: Benzo(a)pyrene, Group 3: Benzo(a)pyrene + 5- Flurouracil, Group 4: Benzo(a)pyrene + chitosan nanoparticles, Group 5: Benzo (a)pyrene + Isolongifolene, Group 6: Benzo (a)pyrene + ICN-K04. The values are statistically significant at $P<0.05$ by ANOVA test. a..BaP vs. control. b.differ from BaP.

TEM analysis of dissected Lung

Morphological changes were analysed by Transmission electron microscope (TEM) and the observations were showed in Figure 4. The lung from control animals revealed a normal architecture (Group 1) as normal mitochondria and nucleus. In lung cancer bearing animals (Group 2 and Group 4) showed a closely

packed by lipid droplets and a typical arrangements of nucleus and swelling of mitochondria where as in isolongifolene and ICN-K04 (Group 5 and Group 6) mitochondria are increased in number as well as multi cells are present which accompanied by metabolism of cells.

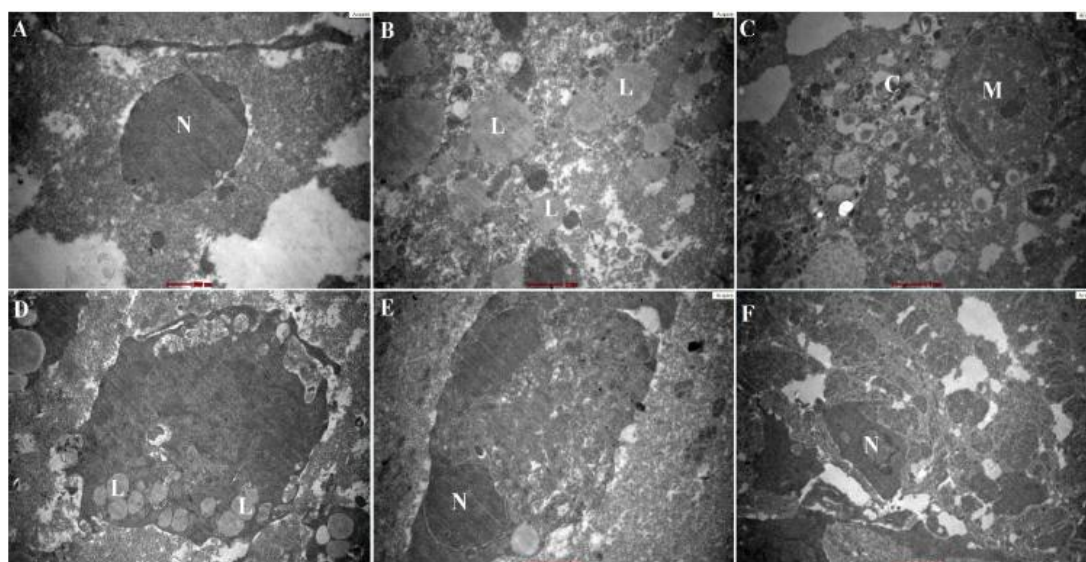


Figure 4: Electron microscopic observation of Lung tissue. (A) Normal morphological pattern of cells Group 1. Hyper accumulation of lipid drops samples from Group 2(B) and 4 (D) respectively. Minimal accumulation of lipid drops of the cellular oraganells and multi cells remains stable in Group 3 (C) and 5(E). No significant variations and further multiplication of multi cells in Group 6(F). Treatment: Group 1: Saline, Group 2: Benzo(a)pyrene, Group 3: Benzo(a)pyrene+5- Flurouracil, Group 4: Benzo(a)pyrene + Chitosan nanoparticles, Group 5: Benzo(a)pyrene+Isolongifolene, Group 6: Benzo(a)pyrene + ICN-K04.

DISCUSSION

Better stability, high safety and efficacy profile and comparatively easy preparation method, and providing versatile routes of administration have turned the chitosan nanoparticles to be more successful, highly admirable and appreciable. Their sub-micron size not they are well apt for oral application and also applicable for mucosal routes of administration as well as parental administration because of their best property of biocompatibility and biodegradability (Manimaran and Vasan, 2024).

As a new drug delivery system, they have attracted increasing attention for their wide range of applications. This study deals on ICN-K04, including its preparation methods, modification, and applications. Biological substances have cleared from the blood stream according to their size. Small particles (1 - 30 nm) were rapidly cleared by the kidney. Carriers having size larger than 30 nm have cleared by reticuloendothelial system (RES), which included macrophages located in the liver and spleen (Gaumet *et al.*, 2008). Based on the age, sex and genetic in different individuals, to determine the efficacy and toxicity of drug carriers predisposition affect rate of their clearance is not an easy task (Igarashi *et al.*, 2008). Whether nanocarriers would take up by the macrophages or not, depended on opsonisation by the innate immune system (Owens and Peppas, 2006). Surface properties of nanocarriers could affect rate of their clearance by RES. A useful method that has helped large particles escape from opsonization has developed in Rutgers university in the 1960s (Owens and Peppas, 2006).

In order to be effectively delivered towards the target tissues, carriers should be able to remain in the blood circulation for adequate duration of time. Unmodified conventional carriers have used in drug delivery systems have cleared from blood stream by RES depending on their size and surface characteristics (Moghimi *et al.*, 2001). Therefore, the fate of injected carriers could be controlled by modulation of their size as well as surface characteristics.

Particle size was the most important characteristics of carrier systems. It has determined the *in vivo* distribution, biological fate, toxicity and the targeting ability of carrier systems (Mohanraj, 2007). The size of nanoparticles have used in drug delivery systems should be large enough to prevent from leakage into the blood capillaries. On the other hand, it should be small enough to escape from macrophages located in lung alveoli (Cho *et al.*, 2008).

A potent chemical carcinogen present in tobacco smoke and environmental pollution causes lung cancer in humans and in experimental systems is the Benzo (a) pyrene. The radical cationic forms of B(a)P may be involved in both the mechanism and metabolic activation leading to the formation of DNA adducts, which are key components for tumor initiation process (Sullivan, 1985).

The present results revealed that the final body weight of the B(a)P administered animals was significantly lowered than that of control group, while the lung weight, tumor incidence was significantly increased. Treatment with ICN-K04 increased the final body weight and prominently decreased lung weight and

tumor incidence in group when compared to the group administered with B(a) P.

TEM allows observation of organelles in the cytoplasm. Since the ICN-K04 has an influencing effect on mitochondria one of the possible mechanisms of inducing apoptosis may be due to release of cytochrome C, mediating intrinsic apoptotic cascades.

CONCLUSION

Lung cancer is one of most common and serious type of cancer and the etiology remains unknown. Current lung cancer therapy is chemotherapy and targeted therapy which improves the survival rate based on the diagnosed stage as well as the therapy has limited owes and adverse side effects. Hence, newer treatments may be a good option for lung cancer. Therefore, a compound with strong anticancer property will pave betterment for the treatment of lung cancer.

Conflicts of interest

Authors declare that they have no conflict of interest concerning this article.

Acknowledgments

We thank Prof. Dr. N. Elangovan., Professor and Head, Department of Biotechnology, Periyar University, Salem-636011, Tamil Nadu, India for discussion on cancer biology studies.

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