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Chemotherapeutic effect of piperine solid lipid nanoparticles against benzo(a)pyrene: Induced lung cancer in mice

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Abstract
The present study was designed to investigate the mechanism-based chemo preventive effect of piperine and therapeutic effect of piperine solid lipid nanoparticles against B(a)P induced lung cancer in Swiss albino mice by estimating the status of lipid peroxidation (LPO), enzymatic (Super oxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPx)) and non-enzymatic antioxidants (Reduced glutathione (GSH), vitamin E, vitamin C) of lung tissues of control and experimental mice. Administration of B(a)P (50mg/kg, p.o) to mice resulted in increased lipid peroxidation with concomitant decrease in the levels of both enzymatic and non-enzymatic antioxidants. Pre-treatment of piperine and post-treatment of piperine solid lipid nanoparticles (100mg/kg, p.o) significantly attenuated all these changes thereby showing potent chemo preventive effect and therapeutic effect respectively.

Keywords: Piperine, lipid peroxidation, antioxidant, lung cancer, mice

1. Introduction
Lung cancer in animals is not as frequent as in man. Primary lung cancer, or tumours originating in the lung, is relatively uncommon in animals (less than 1% of all cancers, in a total number of 544 tumours from 405 dogs), although the incidence has gradually increased until recent years, in some areas the number of cases reaching over 3% [1]. Rapid industrial development in India has resulted in an increase in environmental pollution from the industries, cigarette smoking and automobiles. Tobacco smoke is established as the most important risk factor for lung cancer, because oxidative stress resulting from tobacco smoke plays a critical role in lung carcinogenesis [2]. The increased incidence of lung cancer in animals has been stimulated by the fact that these animals share man's environment and they breathe the same polluted air and similar food, mostly prepared on an industrial basis. This neoplastic condition is significantly lower in farm animals, which are sent to slaughter before they reach the "cancer age," but dogs and cats are allowed to live a full lifespan, thus are more at risk for developing lung cancer [3]. Chemoprevention is a novel approach for inhibiting, reversing or delaying carcinogenesis by using natural, dietary or synthetic agents [4]. Piperine is a major plant alkaloid present in black pepper (*Piper nigrum*) and long pepper (*Piper longum*) which is known to possess several pharmacological effects such as anti-microbial, anti-fungal, anti-inflammatory, antioxidant etc. [5]. Earlier studies have proved the cytoprotective effect of piperine on benzo(a)pyrene induced lung carcinogenesis in mice [6]. The present study was designed to investigate the antioxidant effect of piperine and its solid lipid nanoparticles in an experimental lung cancer model using lipid peroxidation, enzymatic and non-enzymatic antioxidants as biochemical end points of chemoprevention as well as therapeutic action.

2. Materials and Methods
2.1 Chemicals
Benzo(a)pyrene, piperine, reduced glutathione (GSH), 5,5'-dithio-bis(2-nitrobenzoic acid, DTNB), catalase, 2-thiobarbituric acid (TBA) were purchased from M/s. Sigma Aldrich Inc., St. Louis, MO, USA. Conventional paclitaxel was a generous gift from Cipla Ltd., Clinical Research & Development Centre, Mumbai. Glyceryl monostearate (GMS) (M/s. Alpha Chemicals Pvt. Ltd., Mumbai) and chloroform (HPLC grade from M/s. Rankem, Himachal Pradesh) were used for synthesis of nanoparticles. Lecithin soy and all other chemicals were of analytical grade procured from Himedia Pvt. Ltd. (Mumbai, India).

2.2 Animals
Male, 6-8 weeks old Swiss albino mice, weighing 20-25 g were obtained from Laboratory Animal Medicine Unit, Madhavaram Milk Colony, Chennai-51 and were housed in polypropylene cages. They were maintained in a controlled environment condition (12:12 h light/dark cycles with an ambient temperature of 25±5 ºC and humidity at 50±10%). Mice were fed with standard pellet diet and were given free access to water *ad libitum*. All the procedures with animals were conducted in accordance with the approved guidelines by the Institutional Animal Ethical Committee (No. 2345/17/DFBS/IAEC/2016).

2.3 Experimental design
Experimental animals were divided into seven groups of six mice each as follows. Group I served as vehicle control and administered corn oil orally throughout the course of the experiment. Group II animals were treated with benzo(a)pyrene at a dose rate of 50 mg/kg body weight dissolved in corn oil orally, twice a week, for 4 weeks to induce lung cancer. Group III animals were treated with standard anti-tumour drug, paclitaxel at a dose rate of 33 mg/kg body weight intraperitoneally, once a week from 10^{th} week after the first dose of B(a)P induction and continued till the end of the experiment i.e., 16^{th} week. Group IV animals were pre-treated with piperine (two weeks prior to the first dose of B(a)P induction) orally at a dose rate of 100 mg/kg body weight dissolved in corn oil, thrice a week for 16 weeks. Group V animals were treated with piperine orally at a dose rate of 100 mg/kg body weight dissolved in corn oil, once a day from 10^{th} week after the first dose of B(a)P induction and continued for 16 week. Group VI animals were treated with piperine along with B(a)P orally at a dose rate of 100 mg/kg body weight dissolved in corn oil, twice a week, for 4 weeks. Group VII animals were treated with piperine nanoparticles orally at a dose rate of 100 mg/kg body weight dissolved in milli-Q water, once a day from 10^{th} week after the first dose of B(a)P induction. The pre- and post-treated groups were used to study the chemopreventive as well as therapeutic effect of piperine.

Mice from each group were euthanized after 16^{th} week by cervical decapitation under inhalation anaesthesia. A 10 % homogenate of the lung tissue was prepared in 0.01M phosphate buffer (pH 7.4). The homogenate was centrifuged at a speed of 2,500 rpm for 30 min in a refrigerated high-speed centrifuge at 4 ºC and the resultant supernatant were used for further analysis.

2.4 Antioxidant analysis
The following antioxidant estimations were carried out in lung homogenate samples. Lipid peroxidation assay was determined as thiobarbituric acid reactive substances (TBARs) [7]. Antioxidant profile like Super oxide dismutase (SOD) [8], catalase (CAT) [9], glutathione peroxidase (GPx) [10], reduced glutathione (GSH) [11], vitamin E [12] and vitamin C [13] were estimated.

2.5 Statistical analysis
For statistical analysis, one-way analysis of variance (ANOVA) was used, followed by the Duncan's multiple range test. Analysis was performed by using IBM SPSS software version 20 for windows. The differences among the groups were considered significant when $p<0.05$.

3. Results
Table I shows the levels of lipid peroxidation, cellular antioxidant enzymes such as SOD, CAT and GPx and non-enzymatic antioxidants (GSH, vitamin E and C) estimated in lung tissues of various groups.

The degree of LPO in the lungs of control and experimental groups of animals was analyzed for oxidative stress. A significant increase ($p<0.01$) in the level of lipid peroxidation were observed in the tumour bearing (Group II) and piperine along with B(a)P treated animals (Group VI) when compared to vehicle control and standard drug control animals (Group I and Group III). Piperine pre (Group IV), PIP-SLNPs (Group VII) and post-treated animals showed a significant ($p<0.01$) decrease in the levels of lipid peroxides when compared with tumour bearing (Group II) animals.

The activity of enzymatic antioxidants such as SOD, CAT and GPx and non-enzymatic antioxidants such as GSH, vitamin E and vitamin C were found to be significantly ($p<0.01$) reduced in B(a)P induced lung cancer bearing (Group II) and piperine along with B(a)P treated (Group VI) animals when compared to control animals (Group I). Paclitaxel (Group III) treated animals significantly ($p<0.01$) increased (or) restored both enzymatic and non-enzymatic antioxidants to near normal levels when compared to B(a)P treated tumour bearing (Group II) animals. Piperine pre (Group IV), PIP-SLNPs (Group VII) and post-treated (Group V) animals showed significant ($p<0.01$) increase in the levels of both enzymatic and non-enzymatic antioxidants when compared with tumour bearing (Group II) animals.

Table 1: Effect of piperine and its nanoparticles on lipid peroxidation, enzymatic and non-enzymatic antioxidants (Mean ± SE) induced by benzo(a)pyrene in lung of Swiss albino mice (n=6)

Analysis	Experimental groups						
	I	II	III	IV	V	VI	VII
LPO (nmoles of MDA released/mg protein)	0.57^{a}±0.02	1.20^{d}±0.08	0.61^{a}±0.03	0.65^{ab}±0.03	0.76^{b}±0.02	0.91^{c}±0.02	0.64^{ab}±0.03
SOD (units/min/mg protein)	4.42^{c}±0.16	3.45^{a}±0.09	4.49^{c}±0.08	5.26^{d}±0.04	4.42^{c}±0.04	3.99^{b}±0.07	4.65^{c}±0.09
CAT (µmoles of H_2O_2 consumed/min/mg protein)	244.5^{b}±3.93	143.2^{a}±5.64	255.3^{b}±6.49	259.5^{b}±9.09	245.6^{b}±6.26	149.7^{a}±4.12	257.5^{b}±6.37
GPX (µmoles of GSH oxidized/min/mg protein)	41.0^{b}±0.83	22.59^{a}±0.97	46.87^{c}±0.44	51.12^{d}±1.82	41.8^{b}±0.45	23.5^{a}±0.83	46.24^{c}±0.48
GSH (µg/mg protein)	1.22^{c}±0.06	0.88^{a}±0.02	1.44^{e}±0.01	1.54^{f}±0.03	1.35^{d}±0.02	1.06^{b}±0.02	1.43^{de}±0.01
Vitamin E (µg/mg protein)	0.49^{c}±0.02	0.30^{a}±0.02	0.58^{d}±0.01	0.65^{e}±0.01	0.50^{c}±0.02	0.39^{b}±0.01	0.58^{d}±0.02
Vitamin C (µg/mg protein)	0.38^{c}±0.02	0.23^{a}±0.01	0.46^{e}±0.02	0.53^{f}±0.02	0.44^{d}±0.01	0.29^{b}±0.01	0.47^{e}±0.02

Means with different superscript within a row differ from each other ($p<0.01$)- One-way ANOVA-Duncan test ; ** – $p<0.01$

4. Discussion

The incidence of lung cancer is currently on the raise as a cause of cancer mortality all over the world both in human and animals. Recent report concluded that high dietary intake of alkaloids reduces further risk of developing lung cancer [14]. Piperine, a dietary alkaloid exhibits anti-oxidant properties, both *in vitro* and *in vivo* conditions. Hence, in the present study we investigated the chemopreventive and chemotherapeutic role of piperine and its nanoparticles against B(a)P-induced oxidative damage in Swiss albino mice. B(a)P is the powerful carcinogen with an ability to produce large quantities of free radicals, which in turn interact with the membrane lipids and consequently induce lipid peroxides [15].

In the present study, piperine and its solid lipid nanoparticles given by oral route at a dose rate of 100 mg/kg body weight which enhanced both enzymatic and non-enzymatic antioxidants and suppressed lipid peroxidation in pre- and post-treated animals. A significant raise in the levels of LPO was observed in the lungs of cancer bearing animals which is accordance with the previous report [4], whereas treatment with piperine caused significant decrease in levels of lipid peroxides. This confirms that piperine inhibits LPO thereby preventing the formation of lipid peroxides which are engaged in carcinogenesis. These observations clearly confirm the antioxidant and free-radical scavenging activity of piperine in reducing the deleterious effects of B(a)P.

Antioxidant enzymes are most powerful free radical scavengers and act as inhibitors of oxidative damage induced carcinogenesis [16]. Enzymatic antioxidants like SOD, CAT and GPx play an important role as a protective enzyme against lipid peroxidation in serum and tissues. In our study, the lung cancer bearing animals showed decrease in the activities of all enzymatic antioxidants i.e., SOD, CAT and GPx. Piperine treatment significantly increased all above enzymatic antioxidants in pre-treated and nanotreated animals than post-treated animals. This result confirmed the ability of piperine to protect lung from oxidative damage by raising the levels of enzymatic antioxidants.

The reduced glutathione (GSH) which is a non-enzymatic antioxidant is an important cellular reductant which provides protection against attack by free radicals, peroxides and other toxic compounds [17]. The reduced GSH in tissues maintains the cellular levels of other non-enzymatic antioxidants like vitamin E and C in active form. In the present study, B(a)P-treated animals showed a significant decrease in the activities of non-enzymatic antioxidants (GSH, vitamin E and C), this might be due to raised need of the cells to counteract the increased oxidative stress and also for quenching enormous free radicals produced during carcinogenesis. However, treatment with piperine caused a significant increase in the levels of these non-enzymatic antioxidants, which suggest that free radical scavenging ability of piperine and its protective effect against B(a)P-induced lung carcinogenesis. Lipid peroxidation and antioxidant profile are more pronounced in the pre-treated animals and nanotreated animals when compared to the post-treated animals, probably because piperine in pre-treated animals, inhibits the initiation of B(a)P activation (or) detoxification process [18] and nanopiperine increases the bioavailability of the drug in the target site in animals which might be result in more inhibition of oxidative damage (or) detoxification process. Standard drug treated animals effectively restored to normal or increased some of the antioxidants profile when compared to tumour bearing animals which might be due to its potent anticancer activity.

Piperine along with B(a)P treated (Group VI) animals showed lipid peroxidation and both enzymatic and non-enzymatic antioxidants similar to tumour bearing (Group II) animals. Piperine enhances the bioavailability of many drugs by increasing the absorption from the intestine, suppressing the drug metabolism by the body in the pulmonary and hepatic tissues via inhibiting CYP3A4 and P-glycoprotein [19, 20]. Hence, piperine increases bioavailability of the B(a)P, which leads to more production of free radicals ultimately resulting in higher lipid peroxidation and reduced antioxidant profile.

5. Conclusions

The present study demonstrated that the piperine and its solid lipid nanoparticles possess potent free radical scavenging and antioxidant activities. From the results, it is evident that piperine is capable of protecting the lungs against oxidative damage. Overall, these findings confirmed the chemo preventive potential of piperine and therapeutic potential of piperine solid lipid nanoparticles against B(a)P induced lung cancer in Swiss albino mice.

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