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U Naseema
Ph.D-Scholar, Department of Veterinary Pathology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Ganne Venkata Sudhakar Rao
Professor & Head, Department of Veterinary Pathology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

N Pazhanivel
Professor, Department of Veterinary Pathology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

V Pandiyan
Professor & Head, Department of Veterinary Biochemistry, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

P Sriram
Professor & Head, Department of Veterinary Pharmacology & Toxicology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Correspondence
U Naseema
Ph.D-Scholar, Department of Veterinary Pathology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Effect of piperine solid lipid nanoparticle on haematological alterations in Benzo(A) pyrene induced lung cancer in mice

U Naseema, Ganne Venkata Sudhakar Rao, N Pazhanivel, V Pandiyan and P Sriram

Abstract
The present study was aimed to evaluate the anticancer potential of piperine solid lipid nanoparticle against benzo(a)pyrene induced lung cancer by analysing the haematological changes in experimental mice. Benzo(a)pyrene was administered orally (50 mg/kg body weight) to induce lung cancer in Swiss albino mice. The levels of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), lymphocytes and platelets (PLT) were lower while the levels of total leukocyte count (TLC) and neutrophils were higher in the mice administered with benzo(a)pyrene alone and in combination with piperine. Post-treatment with piperine solid lipid nanoparticles (100mg/kg body weight) significantly reversed all these changes. The results of this study indicated the potential benefits of piperine and its nanoparticle in cancer treatment in Swiss albino mice.

Keywords: Lung cancer, mice, piperine, nanoparticle, haematological alterations

1. Introduction
Lung cancer is a serious health problem both in human and animals especially in most developed countries and incidence rate is gradually increasing every year because of the rapid industrial development that resulted in an increase in environmental pollution from the industries, cigarette smoking and automobiles. Tobacco smoke contains more than 60 polycyclic aromatic hydrocarbon (PAH), which plays a critical role in lung carcinogenesis [1]. Benzo (a) pyrene (BaP) is a PAH and its metabolites can bind to DNA, forming BaP-DNA adducts which interfere with or alter DNA replication and might be associated with an increased risk of several forms of cancer [2]. Piperine is a pungent alkaloid present in black (*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 [3]. The present study was designed to analyze the anticancer effect of piperine solid lipid nanoparticles against lung cancer through the analysis of haematological parameters.

2. Materials and Methods
2.1 Chemicals
Benzo(a)pyrene (B(a)P) and piperine 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. Haematological analyses were done by using diagnostic kits (Agappe Diagnostics Ltd., Kerala).

2.2 Animals
Six-to eight weeks old, male Swiss albino mice, weighing 20-25 g were obtained from Laboratory Animal Medicine Unit, Madhavaram Milk Colony, Chennai-600 051 and were housed in polypropylene cages. They were maintained in a controlled environmental condition 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
Mice were divided into seven groups of six animals each as follows: Group I animals (vehicle control) were administered with corn oil orally throughout the experiment.

Mice in all the groups (except Group I) were administered B (a) P (50 mg/kg body weight) orally, twice a week, for 4 weeks. After the last dose of B (a) P administration, Group III animals were treated with standard anticancer drug, paclitaxel (33 mg/kg body weight) intraperitoneally, once a week. Group IV animals were pre-treated with piperine, thrice a week, orally (100 mg/kg body weight), starting two weeks prior to the first dose of B (a) P administration and continued till the end of the trial. Group V and Group VII animals were post-treated, orally, with piperine and piperine solid lipid nanoparticles (SLNP) respectively, at a dose rate of 100 mg/kg body weight, once a day, starting the day after the last dose of B (a) P administration and continued till the end of experiment. Group VI animals were administered B (a) P (50 mg/kg body weight) along with piperine (100 mg/kg body weight) orally, twice a week, for 4 weeks. At the end of experiment, blood samples were collected from retro-orbital sinus, into the EDTA-3K vacutainer tubes.

2.4 Haematological analysis

Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total erythrocyte count (TLC) and platelet (PLT) count were estimated by 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.

2.5 Statistical analysis

The data obtained from different parameters were subjected to one-way analysis of variance (ANOVA) using SPSS (Version 16 for windows) statistical software.

3. Results and Discussion

Effect of benzo (a) pyrene and piperine solid lipid nanoparticle on haematological values and the percentage of differential count in control and experimental group of animals are depicted in Table 1.

In the present study, the levels of haemoglobin (Hb), packed cell volume (PCV)and total erythrocyte count (TEC) were significantly (p<0.01) reduced in benzo(a)pyrene (BaP) (Group II) and piperine along with benzo(a)pyrene treated (Group VI) animals indicating anaemia which was normocytic and normochromic. Anaemia might occur due to the free radicals resulting from B(a)P metabolism which leads to direct liver injury and free radicals liberated from the liver into the circulation affecting the erythrocytic membranes leading to the disturbed haematopoiesis, destruction of erythrocytes and reduction in the rate of their formation and/or their enhanced removal from the circulation [4]. The anaemia results in hypoxia which might accelerate the malignant progression, spreading of cancer cells and increased potential for local invasiveness [5].

In the current study, animals administered with B(a)P alone and in combination with piperine showed significant (p<0.01) elevation of total leukocyte count (TLC) and neutrophil count with reduced lymphocyte count. Ganger *et al.* [6] suggested that an increase in TLC and alterations in the differential count (lymphocytes and neutrophils) were considered as the hallmarks of carcinogenesis.

In the present study, B(a)P alone and in combination with piperine administered animals showed significant (p<0.01) reduction of platelet (PLT) count. Thrombocytopoenia might be due to the immune-mediated destruction of platelets or bone marrow involvement or by effect of free radicals formation.

Our results in the present study are also in accordance with the reports of Anandakumar *et al.* [7] and Nithya *et al.* [4] who attributed the reduction in Hb, TEC, PCV, lymphocyte and elevation of TLC, neutrophil count in B(a)P treated animals.

Eosinophil, basophil and monocyte values showed no significant difference between the control and experimental group of animals.

Piperine supplementation considerably prevented all the above haematological changes and restoration of values is more pronounced in the pre-treated (Group IV) animals and nano-treated (Group VII) animals when compared to the post-treated (Group V) animals, probably because in pre-treated animals, it inhibits the activation (or) detoxification process of B(a)P [8] and nano-piperine increases the bioavailability of the drug in the target site in animals which might have protected the tissues from hypoxia and reduced the extent of carcinogenesis. Paclitaxel (Group III) treated animals effectively restored to normal haematological profile when compared to B(a)P treated animals which might be due to its potent anticancer activity.

B(a)P in combination with piperine treated (Group VI) animals showed haematological changes similar to B(a)P alone treated (Group II) animals. Piperine enhances the bioavailability of 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 [9]. Hence, piperine increases bioavailability of the B(a)P, which leads to more production of free radicals ultimately resulting in alterations in the haematological profile.

Table 1: Effect of benzo(a)pyrene and piperine solid lipid nanoparticle on haematological values (Mean±SE) in control and experimental group of animals (n=6)

Parameters	Experimental groups						
	I	II	III	IV	V	VI	VII
Hb (g/dL)	12.03^{c} ±0.18	7.29^{a} ±0.27	11.78^{c} ±0.22	11.69^{c} ±0.17	8.92^{b} ±0.23	7.34^{a} ±0.27	11.65^{c} ±0.17
TEC ($\times10^{6}$/µL)	7.04^{c} ±0.57	3.63^{a} ±0.17	7.00^{c} ±0.15	7.03^{c} ±0.14	4.93^{b} ±0.19	3.78^{a} ±0.17	6.92^{c} ±0.17
PCV (%)	38.17^{c} ±2.96	18.45^{a} ±1.37	36.61^{c} ±2.50	36.42^{c} ±2.16	28.48^{b} ±1.39	19.35^{a} ±1.54	35.72^{c} ±2.19
TLC ($\times10^{3}$/µL)	5.33^{a} ±0.18	12.38^{c} ±0.19	5.53^{a} ±0.28	5.59^{a} ±0.25	9.09^{b} ±0.37	11.74^{c} ±0.26	5.65^{a} ±0.32
Neutrophils (%)	19.60^{a} ±0.63	51.04^{d} ±0.78	20.38^{a} ±0.67	21.22^{a} ±0.63	39.43^{b} ±1.79	47.00^{c} ±1.84	21.21^{a} ±0.99
Eosinophils (%)	4.62 ±2.94	4.95 ±3.01	4.47 ±3.02	3.95 ±2.90	4.88 ±3.07	4.37 ±2.98	5.08 ±3.09

Basophils (%)	2.75 ±0.90	2.52 ±0.86	1.98 ±0.72	2.10 ±0.74	1.22 ±0.42	1.80 ±0.64	2.10 ±0.87
Monocytes (%)	4.43 ±0.86	4.05 ±0.74	4.07 ±0.75	4.05 ±0.73	4.02 ±0.65	4.13 ±0.56	3.85 ±0.56
Lymphocytes (%)	75.92^{b} ±3.88	29.68^{a} ±0.76	77.03^{b} ±4.53	77.35^{b} ±3.83	70.55^{b} ±4.29	32.36^{a} ±1.45	74.16^{b} ±4.32
PLT ($\times 10^3/\mu L$)	523.83^{b} ±98.26	210.83^{a} ±24.81	520.67^{b} ±56.29	479.83^{b} ±46.55	482.00^{b} ±49.29	235.67^{a} ±27.04	475.33^{b} ±57.54

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

4. Conclusions

Based on the above haematological studies it may be suggested that piperine solid lipid nanoparticles can be used as a promising chemotherapeutic agent against benzo (a) pyrene induced lung cancer in mice. From these results and the results of our previous studies, it is concluded that piperine and its solid lipid nanoparticles exert a protective effect in the treatment of lung cancer.

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