U Naseema
Research Associate, CRC&N, Project Monitoring Unit, Madras Veterinary College, Chennai, Tamil Nadu, India

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

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

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

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

Piperine and its novel nano form target tumor necrosis factor-α (TNF-α) signaling in mouse model of lung tumor

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

Abstract
Preclinical studies suggest that diet rich in phytochemicals plays an important role in the prevention of the cancer. Piperine, in the dietary pepper, has been shown to exhibit various pharmacological actions. In this study, we investigated the mechanism by which piperine exhibits anti-inflammatory action against experimentally benzo(a)pyrene induced lung cancer. The expression of the tumor necrosis factor-α was up regulated in the benzo(a)pyrene group when compared to the piperine and its nanoform treated groups. We conclude that tumor necrosis factor-α enhances the tumour progression in the tumour group and piperine enhances anti-inflammatory action by down regulating the tumor necrosis factor-α expression in the mouse model.

Keywords: Benzo(a)pyrene, lung cancer, piperine, tumor necrosis factor-α

Introduction
In the veterinary literature, only a few works was carried out on primary pulmonary tumour in domestic animals. A total of 150 lung tumours, 64 were found in dogs, 33 in cattle, 12 in sheep, 8 in cats and 1 in donkeys and the most frequently diagnosed lung tumours in domestic animals were bronchogenic or bronchoalveolar adenocarcinomas (Moulton, 1978) [12]. Benzo(a)pyrene [B(a)P] is an important environmental pollutant, bio activation of B(a)P to reactive metabolites and reduced levels of antioxidants resulted in oxidative stress might be the basis for cancer (Kasala *et al.,* 2015) [6, 10]. Piperine is the dietary alkaloid extracted from the fruits and roots of *Piper nigrum* and *Piper longum* (Zheng *et al.,* 2016) [17]. However, the bioavailability of piperine in the target site is low in animals. To resolve this issue nano delivery system has been developed. We have shown previously that piperine and its nano form exhibits preventive and therapeutic effect respectively against the lung cancer by enhancing antioxidant activities and scavenging free radicals (Naseema *et al.,* 2018) [13]. The proinflammatory cytokines (TNF-α, IL-6 and IL-1β) are the major molecules involved in the inflammation-to-cancer axis. TNF-α is a cytokine in inflammatory reactions which act as an endogenous tumour promoter (Balkwill and Joffroy, 2010) [3]. This study focuses on the tumour progression activity of TNF-α and the relationship of TNF-α expression to target lung cancer therapy of piperine.

Materials and Methods
Animals: Swiss albino, male mice at the age of 6-8 months (20-25g) were purchased from the Laboratory Animal Medicine Unit, Directorate of Centre for Animal Health Studies (DCAHS), Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai-600 051 and they were maintained in a controlled environmental condition and fed with standard pellet diet and water *ad libitum.*

Chemicals: Benzo(a)pyrene [B(a)P] and piperine was 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.

Corresponding Author:
U Naseema
Research Associate, CRC&N, Project Monitoring Unit, Madras Veterinary College, Chennai, Tamil Nadu, India

Experimental design: Experimental trial was conducted as per the approved guidelines by the Institutional Animal Ethical Committee (No. 2345/17/DFBS/IAEC/2016). Mice were divided into seven groups of six mice each and administered with the drugs as follows;

Blood collection: The mice were anaesthetised by using isoflurane and blood samples were collected in plain vacutainers (BD Vacutiner, United Kingdom) from the retro-orbital sinus method before every sacrifice. The samples were allowed to clot and serum was separated by centrifuging at 1,500 rpm for 15 min.

Quantitative determination of TNF-α: The ELISA test was performed by using the RayBio®TNFα ELISA Kit as per the procedure described by manufacturer. Reagents were stored at 2-8°C and placed at room temperature for 30 min before use. At the end of experiment, serum titres were expressed in pico gram unit per millilitre (pg/mL).

Statistical analysis: To test the hypothesis, the One-way Analysis of Variance (ANOVA), followed by Duncan's multiple range test applied by IBM SPSS software version 20 windows. The significant differences ($p < 0.01$) among the groups were highly considered.

Results and Discussion
Inflammation plays a central role in cancer progression. TNF-α acted as a double-dealer in the studies that could be either pro or anti-tumorigenic. On one hand, TNF-α acted as an endogenous tumour promoter to bridge inflammation and carcinogenesis by stimulation of cancer cell's proliferation, angiogenesis, growth, invasion and metastasis. On the other hand, TNF-α could be a cancer killer by inducing apoptosis through inhibiting NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells) which extracts potential cancer therapeutic effect (Wang and Lin, 2008) [16].

In this study, the effect of piperine on the levels of TNF-α (pg/mL) concentration in experimental mice groups were depicted in Table I. Here we compared the means of TNF-α concentration among the B(a)P, treated and control groups. B(a)P induced lung cancer animals (group II) exhibited significant increase ($p < 0.01$) in the level of TNF-α concentration when compared to that of control animals (group I). These findings concurred with the previous reports of Anandakumar *et al.* (2012) [1]; Bodduluru *et al.* (2015) [6, 10]; Bodduluru *et al.* (2016) [7] who explained that it might be due to enhanced activity of NF-κB during lung carcinogenesis. Several preclinical studies also suggested that TNF-α mainly produced by cancers which act as an endogenous tumour promoter (Tselepis *et al.,* 2002; Ben-Baruch, 2003; Balkwill and Joffroy, 2010) [15, 4, 3]. Proliferating tumour cells, their surrounding host stromal cells and tumour-infiltrating inflammatory/immune cells create a tumour microenvironment (Ariztia *et al.,* 2006) [2]. It also formed by recruitment of fibroblasts, migration of immune cells, matrix remodelling and new vascularization (Junttila and de Sauvage, 2013) [9]. Within microenvironment, proinflammatory mediators participated in a complex inflammatory signaling that facilitates extravasation of tumour cells through the stroma, thereby fostering tumour progression (Philip *et al.,* 2004) [14].

Table 1: Mean (±S.E) TNF-α quantification (pg/mL) in serum of mice in different groups by ELISA (n=6)

Treatment groups	Control	B(a)P	B(a)P+ Paclitaxel	Piperine + B(a)P	B(a)P+ Piperine	B(a)P& Piperine	B(a)P+ PIP-SLNPs
TNF-α	22.71[a]±1.38	85.46[e]±3.21	43.97[b]±3.06	58.79[c]±1.73	60.07[c]±1.91	74.31[d]±2.55	58.04[c]±1.29

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

Our results also demonstrated that the significant ($p < 0.01$) reduction in the levels of TNF-α expression upon treatment with piperine (group IV, V and VII). Piperine is a natural phytochemical that possesses numerous physiological actions, as induced by its anti-inflammatory action. The mechanism underlying these effect include the inhibition of tumour necrosis factor-α signaling pathway. Compared to control and paclitaxel (group III), the TNF-α concentration in treated groups showed significantly ($p < 0.01$) increased but lower than B(a)P group and B(a)P in combination with piperine treated group (VI) which was well-correlated with the extent of chemotherapy response. From these results, TNF-α might be a target for the lung cancer therapy. These findings are in agreement with the reports of Ferrajoli *et al.* (2002) [8]; Michalski *et al.* (2004) [11]; Berberoglu *et al.* (2004) [5] who suggested that serum TNF level could be an indicator for chemotherapy response and prognosis.

Conclusion
The present study demonstrated that TNF-α plays a critical role in tumour progression and piperine is capable of protecting the lungs against inflammatory stress which represents the therapeutic effect of piperine against B(a)P induced lung cancer in Swiss albino mice. Although further experiments with other diagnostic aids will be needed to clarify this therapeutic effect of piperine.

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