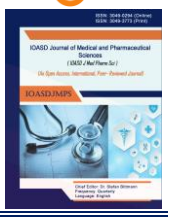
OPEN ACCESS

IOASD Journal of Medical and Pharmaceutical Sciences

IOASD J Med Pharm Sci, Vol-3, Iss-4, Oct-Dec., 2026
Frequency: Quarterly
ISSN: 3049-0294 (Online)
ISSN: 3049-3773 (Print)
Homepage: https://ioasdpublisher.com/ioasdjmps



Modulatory Effects of ICN-K04 against B (a) P Induced Haematological Alterations, Oxidative Stress and Mitochondrial Dysfunction in Sprague Dawley Rats

Kalimuthu Tulasi Ramakrishnan[1], Kowsalya Rengasamy[2], and Dharmar Manimaran[3*]

[1]Department of Biotechnology, K S Rangasamy College of Arts and Science (Autonomous), KSR Kalvi Nagar, Tiruchengode, Namakkal - 637 215, Tamil Nadu, India.

[2]Department of Biotechnology, School of Biosciences, Periyar University, Salem - 636 011, Tamil Nadu, India.

[3]Department of Animal Nutrition, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal - 637002, Tamil Nadu, India.

Corresponding Author: Dharmar Manimaran

Department of Animal Nutrition, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal - 637002, Tamil Nadu, India | Email: manimaranbiotech2011@gmail.com

DOI: 10.67224/ioasdjmps.2026.v03i04.010

ABSTRACT

Benzo (a) pyrene [B(a)P], a polycyclic aromatic hydrocarbon, induces systemic toxicity primarily through oxidative stress. This study investigated the protective effects of 5-Fluorouracil (5-FU), chitosan nanoparticles, isolongifolene, and isolongifolene-loaded chitosan nanoparticles (ICN-K04) against B (a) P-induced alterations in haematological parameters, antioxidant status, and mitochondrial enzyme activities in Sprague-Dawley rats. Animals were divided into six experimental groups: control, B (a) P, B (a) P + 5-FU, chitosan nanoparticles, isolongifolene, and ICN-K04. Haematological parameters, antioxidant enzymes, reduced glutathione, vitamins A, C, and E, and mitochondrial enzymes were evaluated using standard biochemical methods. B (a) P exposure significantly reduced hematological indices, antioxidant defenses, and mitochondrial enzyme activities compared with controls. Treatment with 5-FU, isolongifolene, and ICN-K04 improved several altered parameters, with ICN-K04 generally demonstrating values closer to those of the control group. Isolongifolene and ICN-K04 significantly ($P < 0.05$) enhanced mitochondrial enzyme activities compared with B (a) P-treated animals. Overall, these findings suggest that isolongifolene and its chitosan nanoparticle formulation, particularly ICN-K04, may offer protective effects against B (a) P-induced hematological alterations, oxidative stress, and mitochondrial dysfunction. ICN-K04 demonstrated promising potential as a protective formulation.

Keywords: ICN-K04, Isolongifolene, Benzo(a)pyrene B(a)P, Haematological parameters, Antioxidant enzymes, Mitochondrial enzymes, Sprague Dawley rats.

Original Research Article

Article History

Received: 27-08-2026

Accepted: 20-10-2026

Published: 03-11-2026

Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

Environmental pollution and chemical toxicity pose major public health challenges; widespread exposure to hazardous xenobiotics is associated with organ dysfunction and cellular damage. Among these environmental pollutants, Benzo (a) pyrene, a high-molecular-weight polycyclic aromatic hydrocarbon is one of the most potent and extensively studied carcinogenic and mutagenic agents (ATSDR, 1995; WHO, 1998). Benzo(a)pyrene primarily originates from the incomplete combustion of organic matter; it is also found in vehicle exhaust, industrial emissions, cigarette smoke, and charred or grilled foods (EFSA, 2008). Once absorbed by living organisms, B(a)P undergoes complex biotransformation, mediated primarily by cytochrome P450 enzymes specifically CYP1A1 and CYP1B1 via the aryl hydrocarbon receptor (AhR) signalling pathway (Goksoyr and Forlin, 1992; Nebert *et al*., 2004). This metabolic activation leads to the formation of reactive intermediates notably benzo(a)pyrene-7,8-dihydrodiol-9,10 epoxide (BPDE) which bind covalently to cellular macromolecules, such as DNA, thereby causing mutations and genomic instability (Baird *et al*., 2005; Manimaran *et al.,*2019).

Beyond direct genotoxicity, it is well established that exposure to Benzo (a) pyrene induces severe systemic oxidative stress through the excessive

production of intracellular reactive oxygen species (ROS) (Manimaran *et al*., 2026, Bozena Bukowska and Piotr Duchnowicz., 2022). This redox imbalance significantly disrupts physiological homeostasis, manifesting in ways that include hematological alterations, the inhibition of endogenous antioxidant defense mechanisms, and structural or functional damage to subcellular organelles (Manimaran *et al*., 2024). Due to their high metabolic activity and reliance on oxidative phosphorylation, mitochondria are a primary target for Benzo (a) pyrene induced toxicity. The impairment of mitochondrial enzymes disrupts cellular energy (ATP) production, enhances ROS formation, and triggers apoptotic signaling pathways. Consequently, the analysis of hematological parameters (such as red and white blood cell counts and hemoglobin concentration), *in vivo* antioxidant enzyme activity (notably superoxide dismutase, catalase, and reduced glutathione), and mitochondrial enzyme function provides critical insights into the extent of systemic toxicity and cellular vulnerability. The Benzo (a) pyrene acts as a potent carcinogen and toxic agent, causing tumor induction and severe weight reduction. Co-treatment with ICN-K04 and Isolongifolene demonstrates varying degrees of protection and anti tumor efficacy has been reported (Kalimuthu Tulasi Ramakrishnan *et al.,* 2026).

Alongside toxicological assessments, modern biomedical research is increasingly focused on identifying protective or therapeutic candidates such as novel pharmacological compounds or plant-derived active substances, like ICN-K04 aimed at mitigating cellular damage induced by xenobiotics. In these experimental approaches, substances are typically evaluated for their ability to scavenge free radicals, restore depleted antioxidant reserves, preserve the integrity of mitochondrial enzymes, and normalize hematological parameters altered by toxic agents, such as Benzo (a) pyrene.

Consequently, investigating the combined and potentially protective effects of ICN-K04 in the presence of Benzo (a) pyrene (with regard to hematological parameters, *in vivo* antioxidant enzymes and mitochondrial enzyme function) is of critical importance for understanding the underlying mechanisms of chemically induced toxicity and for exploring potential pharmacological countermeasures.

MATERIALS AND METHODS

Determination of haematological parameters

Whole blood was collected in EDTA coated tubes. Hematological parameters like Red Blood Cell (RBC), Packed Cell Volume (PCV), White Blood Cell (WBC), Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) were estimated using an automated Hematological cell Counter (CD 1700) CELL-DYN 1700 (Abbott Diagnostics, Abbott Park, IL, USA)

***In vivo* enzymatic antioxidants:**

Assay of Superoxide Dismutase (SOD)

Superoxide dismutase activity was measured by the method of Kakkar *et al*. (1984). Reaction mixture contained 0.1 mL of cytosolic fraction, 1.2 mL of sodium pyrophosphate buffer, 0.1 mL PMS, 0.3 mL of 300 µM NBT, 0.2 mL NADH. Reaction was started by addition of NADH. The reaction was then stopped by the addition of 0.1 mL TCA, stirred with 4.0 mL of n-butanol after incubation at 30°C for 90 seconds. Then the mixture was allowed to stand for 10 min, centrifuged (3000 ×g, 10 min) and butanol layer was separated. Colour intensity of the chromogen in the butanol layer was measured at 560 nm and SOD was expressed as units/mg protein.

Estimation of Catalase

Catalase activity was assayed as described by Luck (1963).0.05 M phosphate buffer (2.5 mL), 0.019M hydrogen peroxide (50 Nl) and 20 Nl of cytosolic fraction forms the reaction mixture. Change in absorbance was recorded at 240 nm. Enzyme activity was expressed as Units/mg of protein.

Estimation of Glutathione peroxidase

Glutathione peroxidase (GPx) activity was measured following Lawrence and Burk (1976). Phosphate buffer (1.44 mL), EDTA (0.1 mL), sodium azide (0.1 mL), glutathione reductase (GR) (0.05 mL), glutathione (0.1 mL), NADPH 0.1 mL, hydrogen peroxide (0.01 mL), and cytosolic fraction (0.1 mL) forms the reaction mixture. The disappearance of NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated as units of NADPH oxidized/mg of protein.

Estimation of Vitamin A

The level of Vitamin A was estimated as described the method of Bayfield and Cole (1980). Saponification agent was added equally to 1.0 mL of tissue extract and incubated for 20 min at 60°C. After attaining the room temperature, 20 mL of water and 10 mL of preheated (60°C) petroleum ether was added to the reaction mixture to extract Vitamin A. Excess moisture in the extract was removed by adding anhydrous sodium sulphate. 1.0 mL of extract was aspirated and allowed to dryness at 60°C and re-dissolved in 1.0 mL of chloroform. Similarly, different concentrations of standard vitamin A palmitate (0.10 µg) were dissolved in 1.0 mL of chloroform. Further, 2.0 mL of TCA reagent was added to all the tubes and the absorbance was read at 620 nm spectrophotometrically. The values are expressed as µg/mg of protein. The entire experiment was carried out in dark to avoid light interference.

Estimation of Vitamin C (Ascorbic acid)

The level of vitamin C was estimated by the method of Omaye *et al*. (1979). About 0.5 ml of tissue extract was added to 0.5 ml of H_2O and 1.0 ml of TCA to form the reaction mixture. The contents were mixed well and centrifuged at 2000 g for 10 minutes. To 1.0ml

of the supernatant, 0.2 ml of DNPH reagent was added and incubated at 37°C for 3 hours. After incubation, 1.5 ml of ice cold sulphuric acid was added and the solution was allowed to stand for 30 minutes at 37ºC. The color intensity was read at 520nm spectrophotometrically. A graph was plotted using standard ascorbic acid to determine the corresponding concentration of vitamin C present in the tissue extract. The values are expressed as µg/mg of protein.

Assay of Vitamin E (α-Tocopherol)

The content of vitamin E in all the experimental animals was assayed by the method of Palan *et al*. (1973). About 0.5 ml of tissue extract/ standard was added to 1.5 ml of ethanol (100%) and centrifuged at 2000 x g for 10 minutes. The supernatant was aspirated in to fresh tube and allowed to dry at 80ºC for 3 hours. To this tube, 0.2 ml of 2, 2 dipyridyl solution, 0.2 ml of ferric chloride and 4.0 ml of butanol was added and mixed vigorously. The color developed was measured at 520 nm against reagent blank. Graph was plotted using α-tocopherol standard to determine the concentration of α-tocopherol in the tissue extract and the values are expressed as µg/mg of protein.

Mitochondrial Isolation

At the end of the experimental period, the animals were killed by cervical decapitation. Lungs were immediately excised, weighed and then used for the isolation of mitochondria. The mitochondria were isolated by the method of Mustafa *et al*. (1977); Johnson and Lardy (1967). 10% homogenate was prepared in 0.25 M sucrose and centrifuged at 600g for 10 min. The supernatant fraction was decanted and then again centrifuged at 15000g for 10 min. The resultant mitochondrial pellet was then washed and resuspended in 0.25 M sucrose. The purity of the mitochondria were estimated by assaying the activity of succinate dehydrogenase (SDH) and used for further analyses. Total protein of the lung mitochondrion was estimated by the method of Lowry *et al*. (1951).

Isocitrate dehydrogenase activity

The activity of isocitrate dehydrogenase was estimated the protocol described earlier by King (1965). To 0.1 mL of Tris-HCl, 0.2 mL of trisodium isocitrate, 0.3 mL of manganese chloride, 0.2 mL of mitochondrial suspension and 0.2 mL of NADP+ were added. Instead of mitochondrial suspension and 0.2 mL of water served as control. After 60 min of incubation, 1 mL of DNPH followed by 0.5 mL of EDTA was added and kept at 37 °C for 20 min. Ten ml of NaOH was added and read at 390 nm. A standard containing α-ketoglutarate was run simultaneously. The isocitrate dehydrogenase activity was expressed as Nmoles of α-ketoglutarate liberated/min/mg of protein.

Estimation of α-Ketoglutarate dehydrogenase (E.C.1.2.4.2)

The activity of α-Ketoglutarate dehydrogenase was estimated the protocol by Reed and Mukherjee (1969). To 0.15 mL of phosphate buffer, 0.1 mL of each thiamine pyrophosphate, magnesium sulphate, α-Ketoglutarate and potassium ferricyanide were added. The total volume was made upto 1.2 mL with water followed by 0.2 mL of mitochondrial suspension and incubated at 30°C for 20 min. Then 1 mL of TCA was added, centrifuged (5000 ×g, 15 min, 4°C) and 0.2 mL of mitochondrial suspension was added to the control after the addition of TCA. To the supernatant, 0.1 mL of potassium potassium ferricyanide, 1mL of dupanol, then incubated for 20 min at 25°C and measured at 540 nm. A standard containing potassium ferrocyanide was run simultaneously.

Succinate dehydrogenase (E.C.1.3.5.1)

The activity of succinate dehydrogenase was estimated the protocol described earlier Slater and Bonner (1952). The reaction mixture containing 1 mL of phosphate buffer, 0.1 mL of EDTA, 0.1mL of BSA, 0.3 mL of sodium succinate, 0.2 mL of potassium ferricyanide and 0.1mL of potassium cyanide were added made upto2.8 mL with water. The reaction was started by the addition of 0.2 mL of mitochondrial suspension. The change in absorbance was recorded at 15 sec intervals for 5 min at 420 nm. The succinate dehydrogenase activity was expressed as Nmoles of succinate oxidized/min/mg of protein.

Estimation of Malate dehydrogenase (E.C.1.1.1.37)

The activity of Malate dehydrogenase was estimated by Mehler *et al*., (1948). To 0.3 mL of buffer, 0.1 mL of NADH and 0.1mL of oxaloacetate were added and the total volume was made upto 2.9 mL with water. About 0.1 mL of mitochondrial suspension was added to commence the reaction. The change in optical density was measured at 340 nm in an interval of 15 sec for 5 min in a spectrophotometer. The enzyme activity was expressed as n moles of NADH oxidized/min/mg protein.

Statistical analysis

All data were expressed as Mean ± Standard Error Mean (SEM) of number of experiments. One-way analysis of variance (ANOVA) using GraphPad PRISM (Version-5.01, USA) was used to determine the statistical significance and the individual comparison were obtained by "Dunnett" comparisons. A value of $P<0.05$was considered to indicate a significant difference between the groups. Values not sharing common superscript are significantly different with each other at $P< 0.05$.

RESULTS

Hematological parameters

Full blood count has been shown to predict the severity and mortality risk in cancer. The effect of ICN-K04 on hematological parameters such RBC, WBC, platelets were analyzed which was as given in Table 1. The results show that Hb(g/dl) and WBC (X10^3/µl) cell

count were significantly decreased in Group 2 when compared to Group 1.

ICN-K04 status in polymorphs, lymphocytes, eosinophil, monocytes and PCV in the various experimental groups (Table 6). Group 2, (i.e.) the animals treated with B(a)P, showed a significant *(P<0.05*) decrease in the cell counts when compared with the Group 1 (control) animals. ICN-K04 caused a significant increase in the counts of polymorphs, lymphocytes, eosinophil, monocytes and PCV cells compared with those for Group 2. As well as when compared to Group 2, the cell counts were increased significantly in all the treatment groups.

MCV, MCH, MCHC levels were decreased considerably *(P<0.05*) in B(a)P treated Group 2 animals, along with an increase *(P*<0.05) in the MCV, MCH, MCHC level in rest of the groups when compared with the values for the Group 2 (Benzo (a) pyrene) animals and the significant values shown in Table 1.

Table.1. Effect of ICN-K04 on the hematological count in control and experimental animal

Haematological Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
HB	17.4±0.11	9.33±0.17 [a]	16.8±0.11 [b]	10.13±0.14	16.2±0.23 [c]	16.96±0.17 [ns]
PCV	55.98±0.26	41.12±0.28 [a]	55.79±0.30 [b]	39.4±0.23	49.24±0.39 [c]	54±98±0.10 [ns]
RBC	9.69±0.05	6.83±0.01 [a]	9.48±0.05 [b]	6.95±0.02	8.69±0.01 [c]	9.43±0.01 [ns]
WBC	14.13±0.01	7.85±0.01 [a]	14.61±0.09 [b]	7.52±0.04	11.84±0.01 [c]	13.86±0.08
MCV	55.26±0.18	50.42±0.14 [a]	53.46±0.21 [b]	49.41±0.09	52.44±0.02 [c]	54.93±0.16
MCH	19.81±0.04	17.0±0.04 [a]	19.12±0.05 [b]	16.65±0.10	19.23±0.09 [c]	19.92±0.05
MCHC	33.73±0.14	30.12±0.18 [a]	32.82±0.08 [b]	30.18±0.14	33.03±0.10 [c]	33.14±0.38 [ns]
Platelets	386.14±0.47	294.24±0.53 [a]	365.48±0.88 [b]	300.36±0.01	359.28±0.11 [c]	361.35±0.17 [ns]
Polymorphs	0.69±0.08	0.32±0.01 [a]	0.65±0.01 [b]	0.38±0.01	0.59±0.02 [c]	0.67±0.05 [ns]
Lymphocytes	89.26±0.04	37.40±0.20 [a]	83.37±0.29 [b]	46.09±0.57	77.43±0.22 [c]	83.49±0.10 [ns]
Eosinophil	0.87±0.03	0.42±0.03 [a]	0.80±0.08 [b]	0.47±0.01	0.78±0.08 [c]	0.83±0.05 [ns]
Monocytes	17.4±0.11	13.33±0.17 [a]	16.8±0.11 [b]	14.13±0.14	16.20±0.23 [c]	16.96±0.17 [ns]

Note: Each value is expressed as the Mean± SEM for 8 rats in each group. The values are statistically significant at *P<0.05* by ANOVA test. a B(a)p vs. control. b,c differs from B(a)p. ns-non significant vs. B(a)p+5-FU. Hemoglobin (HB):g/dl; Packed cell volume (PCV) %; red blood cells (RBC) X10ˆ6/µ white blood cell (WBC) X10ˆ3/µl ; Mean corpuscular volume (MCV) fl; Mean corpuscular hemoglobin (MCH) pg; Mean corpuscular hemoglobin concentration (MCHC) g/dl; platelets X10ˆ3/µl; Polymorphs, lymphocytes, eosinophil, monocytes %. The platelet count is significantly decreased in benzo(a)pyrene induced groups when compared to control. There was a slight change in the platelet count of all experimental groups as compared to control, platelet counts are significantly increased when compared to benzo(a)pyrene induced groups as well in isolongifolene and ICN-K04 treated animals' platelets are non-significantly (*P<0.05*) increased when compared to 5-flurouracil treated animals (Table 1).

Effect of *in vivo* antioxidant enzymes

Effect of ICN-K04 and Benzo (a) pyrene on tissue antioxidant enzyme levels (SOD, CAT and GPx were shown in Table 2. The traits of these antioxidant enzyme levels were significantly (*P<0.05*) decreased in benzo (a) pyrene (Group 2) and chitosan nanoparticles (Group 4) induced rat when compared to control (Group 1). Administration of 5-FU (Group 3), isolongifolene (Group 5) and ICN-K04 (Group 6) after benzo (a) pyrene treatment showed significant (*P<0.05*) increase in enzymatic antioxidants status as compared to benzo(a)pyrene. No significant commutes were observed in 5-FU and ICN-K04 treated SD rat. Similar results were obtained in non-enzymatic antioxidant levels (GSH, Vitamin A, Vitamin C and Vitamin E). However, the activities of both enzymatic and non-enzymatic antioxidants are not statistically different with 5-FU or ICN-K04 treated rat.

Table.2. Effect of ICN-K04 on *in vivo* antioxidant enzymes.

Parameters	SOD	CAT	GPx	GSH	Vit-A	Vit C	Vit E
Group 1	7.48±0.14	30.31±0.11	9.36±0.02	12.87±0.02	6.22±0.03	11.47±0.13	8.51±0.12
Group 2	4.24±0.08 [a]	15.14±0.07 [a]	4.11±0.02 [a]	6.49±0.12 [a]	2.56±0.11 [a]	6.22±0.09 [a]	2.46±0.13 [a]
Group 3	6.55±0.10 [b]	28.60±0.14 [b]	8.51±0.07 [b]	9.69±0.06 [b]	5.59±0.16 [b]	10.48±0.08 [b]	7.29±0.07 [b]
Group 4	5.46±0.11	22.62±0.13	5.19±0.05	8.49±0.04	4.26±0.06	8.23±0.06	5.63±0.15
Group 5	5.62±0.12 [c]	23.25±0.20 [c]	5.19±0.08 [c]	8.51±0.07 [c]	4.39±0.08 [c]	8.48±0.12 [c]	5.67±0.16 [c]
Group 6	6.40±0.05 [ns]	28.27±0.19 [ns]	8.35±0.02 [ns]	9.92±0.22 [ns]	5.44±0.1 [ns]	10.25±0.06 [ns]	7.23±0.05 [ns]

Note: Each value is expressed as the Mean ± SEM for 8 rats in each group. The values are statistically significant at p<0.05 by ANOVA test. a B(a)P vs. control, b,c differs from B(a)p. ns non-significant vs. B(a)p+5-FU. Enzyme activities are expressed as SOD: NBT Reduction/min/mg of protein; CAT-µmole of H_2O_2 hydrolyzed/min/mg of protein; Gpx- µg of glutathione oxidized/min/mg of protein; GSH-µg of GSH oxidized / mg of protein. Vit-A, C, E- µg/mg of protein.

Effect of Mitochondrial Enzymes

Table 3 displays the effect of ICN-K04 along with B(a)P on the activities of mitochondrial enzymes such as alpha-KGDH, IDH, SDH and MDH in the lung of control and experimental animals. The activities of the enzymes were found to be significantly (*P<0.05*) decreased in B(a)P treated group (Group 2) animals when compared with the control animals (Group 1). 5-FU (Group 3) administration caused a significant increase in the activities of the mitochondrial enzymes when compared with Group 2 and Group 4. Isolongifolene (Group 5) and ICN-K04 (Group 6) treated animals showed a significant (P<*0.05*) increase in these enzyme activities when compared with B(a)P treatedgroup. However, there found to be no significant difference in the activities of the mitochondrial enzymes between the control animals and the animals treated with ICN-K04.

Table. 3. Effect of ICN-K04 on Mitochondrial enzymes

Mitochondrial enzymes	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
ICDH	0.97±0.05	0.55±0.03 [a]	0.76±0.03 [b]	0.52±0.02	0.72±0.02 [c]	0.84±0.08 [ns]
αKGDH	0.95±0.09	0.54±0.01 [a]	0.74±0.01 [b]	0.43±0.01	0.75±0.01 [c]	0.89±0.0 [ns]
SDH	8.14±0.03	5.60±0.10 [a]	7.24±0.09 [b]	4.98±0.02	6.88±0.15 [c]	7.34±0.06 [ns]
MDH	7.10±0.05	3.75±0.09 [a]	6.69±0.07 [b]	4.24±0.14	6.32±0.18 [c]	7.03±0.08 [ns]

Note: Each value is expressed as the Mean±SEM for 8 rats in each group. The values are statistically significant at *P<0.05* by ANOVA test. a B(a)P vs. control. b,c differs from B(a)p. ns non-significant vs. B(a)P+5-FU. Mitochondrial Enzyme activities are expressed as molar fractions/min/mg of proteins.

DISCUSSION

The principal tissue in human body is the blood and whenever the toxic effect of chemicals is found it shows abnormal modification in its parameters that leads to diseased condition. In a number of human pathologic conditions or after exposure to xenobiotic displaying oxidative stress changes in RBCs level have been detected. Erythrocytes are permanently in contact with potentially damaging levels of oxygen, but their metabolic activity is capable of reversing this injury under normal conditions, which are equipped by many defence systems representing their antioxidant capacity (Kurata *et al*., 1993). Further, oxidative stress in red blood cells is an indicator of overall oxidative stress besides RBC-related disorders. Thus, the present study investigates the potential protective and curative effect of isolongifolene in erythrocyte oxidative damage in B(a)P induced lung carcinogenesis.

In this study, lung cancer bearing animals showed reduction in hemoglobin percentage and RBC count, which is an indication of anemia. It may be assumed that the free radicals resulting from B(a)P metabolism caused liver injury and a proportion of these free radicals were liberated from the liver into the blood and may affect the membranes of circulating red cells. The depression in RBC count and Hb content recorded in the present work could be attributed to disturbed hematopoiesis, destruction of erythrocytes, and reduction in the rate of their formation or their enhanced removal from circulation.

The reduction in the values of blood parameters (RBC and Hb) may be attributed to the hyperactivity of bone marrow, which leads to production of red blood cells with impaired integrity that are easily destroyed in the circulation. (Tung *et al*., 1975). Depletion of oxygen to all organs (hypoxia) and tumor hypoxia are considered as potential therapeutic problem. Continued hypoxia condition may result in cellular changes leading to a more aggressive tumor phenotype as reflected by accelerated malignant progression, increased potential for local invasiveness and tumor spreading (Hockel *et al*., 2001).

B(a)P administration reduced the body weight and increased lung weight and tumors, whereas ICN-K04 (Isolongifolene Chitosan Nanoparticles) treatment effectively counteracted these changes (Kalimuthu Tulasi Ramakrishnan *et al.,* 2026). Isolongifolene might have protected the tissue from hypoxia and reduced the extent of tumorigenesis and this is indicated by the restoration of Hb contents and RBC count in animals that received isolongifolene after B(a)P. White blood cell populations, lymphocytes and neutrophils play a crucial role in the systemic inflammatory response, often observed in cancer patients. The foremost indication of the effect of carcinogenesis in the tissue is understood by the rapid increase in WBC count and alterations in differential count (lymophocytes, monocytes and neutrophils). In the current study, the lung cancer bearing animals showed elevated WBC count and neutrophil count with reduced lymphocyte and monocyte count, which was in line with the previous findings (Rahma *et al*., 2012).

The observed results also coincide with the results of Saeed *et al*., (2013) who attributed the increase in the WBC count to antioxidant activity of vitamin E and isolongifolene. Similarly, isolongifolene could increase the WBC count, due to its role in free radical scavenging. Lymphocytes play the key role in all immune reactions and are always directed against the specific foreign antigens (toxins). Lymphocytes were significantly decreased in number in response to stressful condition. Additionally, lymphocytes migrate to the site of inflammations which may be resulted due to toxic effect of B (a)P. We observed a significant difference in

circulating WBC after isolongifolene treatment and no significant difference was observed between control and isolongifolene alone treated animals.

Free radicals and associated oxidative stress induced by Benzo (a) Pyrene are implicated in eliciting pathological changes in lung toxicity. Since ancient times *Terminalia arjuna* bark (Arjuna chaal), an indigenous plant used in ayurvedic and unani Medicine, primarily as a cardiotonic as well as in other diseases also.

Oxidative stress occurs in all the stages of the progress of cancer with the genesis of other diseases. Antioxidant defence enzymes consists of superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (GPx) and reduced glutathione (GSH), Vit A, Vit C and Vit E these enzymes and vitamins defends and shields the cellular macromolecules against oxidative damage by detoxifying carcinogens both by destroying their reactive centers or by conjugating with endogenous ligands facilitating their excretion (Nafees *et al*., 2012). The present study, has demonstrated Isolongifolene in combination with chitosan exhibits prominent inhibitory effects against several aspects of Benzo (a) Pyrene induced lung toxicity. In the cancer scenario, the cells becomes benefit of the capability of cellular proliferation control as a result of this there is a loss of lipid peroxidation in preneoplastic cellular membranes (Nafees *et al*., 2013). Isolongifolene ameliorated Benzo (a) Pyrene induced inhibition of the activities of antioxidant enzymes, viz., glutathione peroxidase and phase-II metabolising enzymes such as glutathione-S-transferase.

B (a) P is a contaminant that occurs ubiquitously in the environment together with other polycyclic aromatic hydrocarbons as a product of incomplete combustion or pyrolysis of organic materials containing carbon and hydrogen. Main sources of B (a) P and polycyclic aromatic hydrocarbons in the environment are residential heating, industrial plants, vehicle exhausts and cigarette smoke (Rahimi *et al*., 2005). It was found by other researchers (Florence, 1995 and Murata *et al*., 1998) that polycyclic aromatic hydrocarbons such as B (a)P, play a major role in lung carcinogenesis. Enzymatic antioxidants such as SOD, CAT and GPx synergistically scavenge reactive oxygen species. Several reports have cited decreased activities of SOD and CAT in various carcinogenic conditions (Nijveldt *et al*., 2001).

GPx is a well-known first line of defence against oxidative stress, it catalyzes the transformation of Hydrogen peroxide to harmless by products, thereby curtailing the quantity of cellular destruction and several studies have reported the decreased activities of GPx in various cancerous conditions (Gagnon and Holdway, 2000). Antioxidant enzymes constitute a mutually supportive team of defence against ROS. These enzymes have been found to be decreased in B (a)P induced lung cancer bearing experimental animals (Manimaran *et al*., 2022).

In the present study it clearly emphasizes that the combination of isolongifolene with chitosan has exhibited increased or more or less equivalent levels of all antioxidant enzymes and vitamins A, C, E as well as vitamins when compared to the control. Simultaneously when compared to the group administered with only B (a)P has showed very significantly marked increased levels of all antioxidant enzymes and vitamins A, C, E.

Conflicts of interest

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

REFERENCES

1. Agency for Toxic Substances and Disease Registry (ATSDR). (1995). Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs). U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.
2. World Health Organization (WHO). (1998). Selected Non-Heterocyclic Polycyclic Aromatic Hydrocarbons. Environmental Health Criteria 202. WHO, Geneva.
3. EFSA Panel on Contaminants in the Food Chain (CONTAM). (2008). Scientific opinion of the panel on contaminants in the food chain on a request from the European commission on Polycyclic aromatic hydrocarbons in food. EFSA Journal, 6(8), 724. 1-114
4. Goksoyr A, Forlin L. (1992). The cytochrome P450 system in fish, aquatic toxicology and environmental monitoring. Aquatic Toxicology, 22(4), 287–311.
5. Nebert DW, Dalton TP, Okey AB, Gonzalez FJ. (2004). Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. Journal of Biological Chemistry, 279(23), 23847–23850.
6. Baird WM, Hooven LA, Mahadevan B. (2005). Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environ Mol Mutagen. 45(2-3):106–114
7. Manimaran D, Ghanendra S, Sridhar M, Kaliaperumal J, Muniyaraj S, Mohankumar T, Vijayakumar R, Manigandan K, Balakrishnan R, Elangovan N (2019) Molecular insights of newly identified potential peptide inhibitors of hypoxia inducible factor 1α causing breast cancer. J Mol Struct 1177:558–563
8. Manimaran, D., Kaliaperumal, J., Elangovan, N., & Palanisamy, V. (2026). Peptide pACC1-Nano Complex Abrogates ACC1-Mediated Lipogenesis in Breast Cancer Cells Via Modulating p53/NF-κB Signaling Cascades. Journal of Bioengineering and Applied Biosciences, 2(1), 1-11.
9. Bozena Bukowska, Piotr Duchnowicz., (2022). Molecular mechanisms of action of selected substances involved in reduction of Benzo(a)Pyrene induced oxidative stress. Molecules, 27(4), 1379.
10. Manimaran, D., Elangovan, N., & Palanisamy, V. (2024). Anti-Tumorgenic Impact of Nano-Formulated Peptide HIF-Alpha Therapy by DMBA Induced

Mammary Carcinoma in Rodent Type. Jordan Journal of Pharmaceutical Sciences, 17(4), 783.
11. Kalimuthu Tulasi Ramakrishnan, Kowsalya Rengasamy, Dharmar Manimaran. (2026). Phytoprotective Effects of Isolongifolene Nanoparticles (ICN-K04) against Benzo (a) pyrene - Induced Lung Cancer in Sprague Dawley Rats. IOASD Journal of Medical and Pharmaceutical Sciences, 3(4), 269-274.
12. Kakkar P, Das B, Viswanathan PN. (1984). A modified Spectrophotometric assay of superoxide dismutase. Indian Journal of Biochemistry and Biophysics. 21: 130-132.
13. Luck H. (1965). Catalase. In: Bergmeyer, H.U., Ed., Method of Enzymatic Analysis, Academic Press, New York and London, 885-894.
14. Lawrence RA, Burk RF. (1976). Glutathione Peroxidase Activity in Selenium-Deficient Rat Liver. Biochemical and Biophysical Research Communication. 71: 952-958.
15. Bayfield RK, Cole ER. (1980). Colorimetric estimation of vitamin A with Trichloroacetic acid. Methods in Enzymology. 67: 189-195.
16. Omaye ST, Turnbull JD, Sauberlich HE. (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods in Enzymology. 62: 3-11.
17. Palan PR, Mikhail BS, Basin J, Romney SL. (1973). Plasma Levels of antioxidant betacarotene and tocopherol in uterine cervix dysplasia and cancer. Nutrition and Cancer. 15: 13-20.
18. Mustafa M, Hacker AD, Husain MZ, Ospital JJ, Lee SD. (1977). Biochemical Effects of Environmental Pollutants in Animal Lungs. In Biochemical effects if Environment pollutants (S.D.Lee, Ed.) Ann Arbor Science, Michigan. 59-96.
19. Johnson D, Lardy H. (1967). Isolation of Liver or Kidney mitochondria. Methods in Enzymology. 10: 94-96.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 193: 265-275.
21. King J. (1965). The dehydrogenases or oxidoreductases — Lactate dehydrogenase. In: King J., editor. Practical Clinical Enzymology. London: Van Nostrand Company Ltd; pp. 83–93.
22. Reed LJ, Mukherjee BB. (1969). α-Ketoglutarate dehydrogenase complex from Escherichia coli. Methods in Enzymology. 13: 55-61.
23. Slater EC, Bonner WD. (1952). Effect of fluoride on the Succinate oxidase system. Biochemical Journal. 52(2): 185-196.
24. Mehler AH, Kornberg A, Grisolia S, Ochoa S. (1948). The enzymatic mechanisms of oxidation reduction between malate or isocitrate and pyruvate. Journal of Biological Chemistry. 174: 961-77.
25. Kurata M, Suzuki M, Agar NS. (1993). Antioxidant systems and erythrocyte life-span in mammals. Comparative Biochemistry and Physiology B. 106(3): 477- 487.
26. Tung His Tang, Cook FW, Wyatt RD, Hamilton PB. (1975). The anemia caused by aflatoxin. Poultry Science. 54(6): 1962-1969.
27. Hockel M, Vanupel P, J. (2001). Tumour hypoxia: definitions and current clinical, biologic and molecular aspects. Journal of National Cancer Institute. 93: 266-76.
28. Harzallah HJ, Grayaa R, Kharoubi W, Maaloul A, Hammami M, Mahjoub T. (2012). Thymoquinone the Nigella sativa Bioactive Oxidative Stress Caused by 1,2 Dimethylhydrazine in Erythrocyte during colon post initiation carcinogenesis. Oxidative Medicine and Cellular Longevity. 2012 : 854065.
29. Saeed M, Abbas ZM, Ali S. (2013). Preparation and Characterization of Sodium Alginate Nanoparticles Containing ICD-85 (Venom Derived Peptides). International Journal of Innovation and Applied Studies. 4(3): 534-542.
30. Nafees S, Ahmad ST, Arjumand W, Rashid S, Ali N, Sultana S. (2012). Modulatory effects of gentisic acid against genotoxicity and hepatotoxicity induced by cyclophosphamide in Swiss albino mice. Journal of Pharmacy Pharmacology. 64(2): 259-267.
31. Nafees S, Ahmad ST, Arjumand W, Ali N, Rashid S , Sultana S. (2013). Hibiscus rosasinensis alleviates Thioacetamide induced Acute Hepatotoxicity in Wistar Rats. International Journal of Drug Development and Research. 5(1): 143-153.
32. Rahimi RL, Shekoufeh and Mohammad A. (2005). A review on the role of antioxidants in the management of diabetes and its complications. Biomedicine and Pharmacotherapy. 59: 365-373.
33. Florence TM. (1995). The role of free radicals in diseases. Australian and New Zealand Journal of Ophthalmology. 23(1): 3-7.
34. Murata MI, Mika L, Sumiko and Shosuke K. (1998). Metal-mediated DNA damage induced by diabetogenic alloxan in the presence of NADH. Free Radical Biology and Medicine. 25: 586-595.
35. Nijveldt R, van Nood, van Hoorn, van Leeuwen. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. Americal Journal of Clinical Nutrition. 4: 418-425.
36. Gagnon M, Holdway D. (2000). EROD induction and biliary metabolite excretion following exposure to the water accommodated fraction of crude oil and to chemically dispersed crude oil. Archives of Environmental Contamination Toxicology. 38: 70-77.
37. Manimaran, D., Elangovan, N., Mani, P., Subramanian, K., Ali, D., Alarifi, S., ... & Kalirajan, A. (2022). Isolongifolene-loaded chitosan nanoparticles synthesis and characterization for cancer treatment. Scientific Reports, 12(1), 19250.