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ORIGINAL ARTICLE

Anticancer Effect of Isoflavone Prunetin on Benzo(a)pyrene Induced Lung Cancer Mice Model

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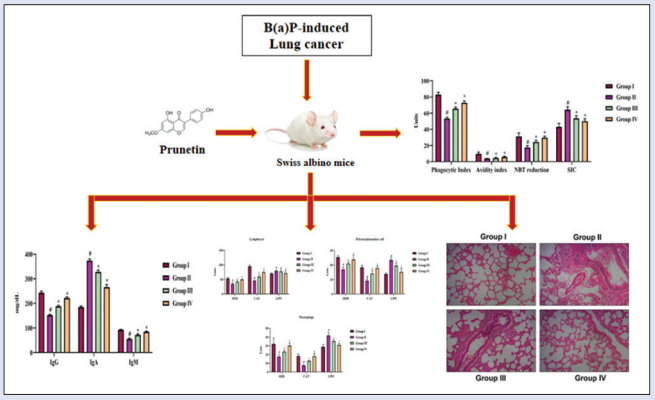
ABSTRACT

Background: Cancer, a deadly disease seems to be a major concern for the global medical field. Controlling the spread of disease is not only a problem for under developed countries but also the developed countries face a lot of concern in diagnosing and treating cancer. Among various cancer types lung cancer mortality incidence seems to be a global threat. Surgery and Chemoradiation therapies are mostly prescribe for lung cancer patients which renders post treatment side effects. Hence, treating cancer with alternative medicine tends to be a choice at present. **Materials and Methods:** In this study, we induced lung cancer in healthy Swiss albino mice by treating with polycyclic aromatic hydrocarbon carcinogen benzo(a)pyrene. The treatment was carried out for a period of 18 weeks; both pre supplementation and post supplementation of prunetin was administered to the mice. **Results:** The changes in hematological and immunological parameters induced by prunetin in lung cancer induced mice were assessed. Oxidative stress in white blood cells which plays a key role in lung cancer induction was analyzed. The therapeutic of efficacy of prunetin was evaluated by estimating the levels of metabolizing enzymes in cancer induced mice. The anticancer effect of prunetin was assessed by estimating the tumor marker CEA and anti-inflammatory markers. It was further confirmed with histopathological analysis of lung tissue. Our results had proven that prunetin significantly increased the white blood cell count, phagocytic, and activity index in lung cancer induced mice. It also decreased the levels of immunoglobulins and oxidative stress in white blood cells. Prunetin treatment decreased the levels of metabolizing enzymes, tumor marker CEA, and proinflammatory markers in cancer induced mice which proven the anticancer efficacy of prunetin. It was further confirmed with histopathological analysis of lung tissue. **Conclusion:** Over all our results authentically confirms prunetin is a potent anticancer drug to treat lung cancer.

Key words: Aryl hydrocarbon hydroxylase, Benzo (a) pyrene, carcinoembryonic antigen, immunoglobulin A, lymphocytes, prunetin

SUMMARY

- Prunetin significantly increased the white blood cell count, phagocytic and activity index in lung cancer induced mice
- Prunetin significantly scavenged the ROS, decreased immunoglobulin IgA and increased the IgG, IgM levels
- Prunetin decreased the levels of metabolizing enzymes, tumor marker CEA and pro-inflammatory markers in lung cancer induced mice



Abbreviations used: CEA-carcinoembryonic antigen; SOD-superoxide dismutase; CAT-catalase; LPO-lipid peroxidation; AHH- aryl hydrocarbon hydroxylase, LDH- lactate dehydrogenase, γ GTP- γ glutamyl transpeptidase, 5'NT-5' nucleotidase

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INTRODUCTION

Cancer is the medical term which threatens not only the global population's quality of life but also their socio-economic status. It tends to be the foremost cause of fatality in almost all the country around the globe.[1] In the wold wide, aged population's leading cause of death is reported to be cancer.[2] Every year about 19.3 million new cases of cancer and 10 million cancer related deaths were estimated worldwide.[3] The lung cancer ranked to be the second most occurring type of cancer in females and males. The mortality rate in lung cancer patients were comparatively higher than the patients with colorectal, prostate, liver, or stomach cancer.[4]

Smoking tobacco is the prevalent cause for lung cancer and about 80–90% of lung cancer patients are smokers.[5] Secondhand smokers are also at high risk in lung cancer incidence which affects the younger population. Tobacco use causes 80% to 90% of all lung cancers. Secondhand tobacco smoke exposure is also a significant risk factor with younger age at exposure associated with higher risk of lung cancer.[6,7] Smoking tobacco liberates various cancer inducing metabolites; one among them

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is benzo (a) pyrene (BaP), a major metabolite of tobacco smoke. BaP is a polycyclic aromatic hydrocarbon also released from automobile exhaust.[8] BaP metabolized into benzo (a) pyrene-7,8-diol-9,10-epoxide which forms adduct with DNA there by initiate cancer induction.[9,10]

The most common adverse effect caused due to chemotherapeutic treatment in lung cancer patients is leukopenia and neutropenia.[11] These clinical conditions were reported to cause increased mortality and morbidity in cancer patients.[12] Increased levels of immunoglobulins are reported in cancer patients. Immunoglobulins, which tend to carry out defense mechanism, act as tumor reactive antibodies which induces the cancer progression.[13] Hence, discovering a drug which renders nil side effect and possess targeted inhibitory action on the carcinogenic activity of BaP will be more effective in treating lung cancer patients.

Supplementation of phytoestrogens have been proven to render protective therapeutic effect in various epidemiological studies. Isoflavanoid phytoestrogen are reported to inhibit cell differentiation, angiogenesis in cancer condition and also regulates the platelet aggregation, wound healing process. Prunetin is one such O-methylated isoflavone present in plants such as Pisum sativum, Trifolium pretense.[14] It possess various pharmacological properties anti-obesity, anti-inflammatory, inhibits liver aldehyde dehydrogenase, and also regulates the proteolytic activity in articular chondrocytes.[15-17] The anticancer potency of prunetin was not well established. Hence, in the present study, we assessed the anticancer effect of prunetin on BaP-induced lung cancer mice model through averting oxidative stress and inflammatory responses.

MATERIALS AND METHODS

Chemicals

HPLC grade Benzo[a] pyrene and TLC grade Prunetin used for the treatment of mice were procured from Sigma Aldrich, USA. Chemicals used for experiments such as nitro blue tetrazolium, sucrose and reagents such as hank's solution, leishman's stain, haematoxylin and eosin stain, and all the other chemicals utilized for the present study was purchased were of high quality grade (Himedia, USA).

Experimental animals

Young healthy male Swiss albino mice weighing about 25–30mg were acclimatized in a laboratory condition maintained at temperature of 22–24°C with a relative humidity of 50–60% for a period of 10 days. The mice were housed in hygienic propylene cage bedded with paddy husk. The bedding was changed daily, and the cage was changed thrice a week. Standard laboratory pellet diet and clean water was given ad libidum for the mice. The mice were taken care with utmost concern.

Experimental design

After the acclimatization period, the mice were grouped into 4 each group consisting of 6 mice. Group I are control group where the mice were treated with corn oil for 18 weeks through oral gavage. Group II are lung cancer induced mice treated with 50mg/kg bwt of B[a] P through oral gavage for 4 week from the 2nd week to 6th week. The B[a] P was administered twice a week. Group III mice are prunetin post supplemented mice which were treated with B[a] P as per group II treatment protocol and then supplement with 30mg/kg bwt prunetin from 12th week to 18th week. Group IV are prunetin pre supplemented mice which were treated with 30mg/kg bwt prunetin for whole treatment period of 18 weeks and also treated with B[a] P as per group II treatment protocol. Both B[a] P and purnetin were dissolved in corn oil and administered through oral gavage. The mice were monitored throughout the treatment period and were finally euthanized by cervical dislocation. Blood samples and tissue samples were collected for further analysis.

Quantification of hematological parameters

The blood samples of lung cancer induced and prunetin treated mice were collected in EDTA coated tubes to separate the plasma samples. The buffy coat was removed gently by rinsing the packed cells with saline solution. Using repetitive pipetting technique, the hemolysis was performed and the red blood cells were collected in propylene tubes. The red blood cells were then centrifuged at 20,000 g at 4°C for 20 min, and the supernatant which is the haemolysate was collected for further investigation. Leishman staining was performed in blood smears to access the differential count of white blood cells.

Evaluation of phagocytic and activity index

The phagocytic and activity index were performed using the method of Wilkinson, 1977,[18] to evaluate the immunological and humoral changes in lung cancer induced and prunetin treated mice. About 200µl of normal serum and leukocyte suspension was mixed with Hank's solution and incubated for 15 min at 37°C. The suspension was then centrifuged for 10 min at 2000 rpm and the sediment was collected. A smear was prepared with the collected sediment and stained using Leishman's stain. The stained smears were viewed under light microscope to view the phagocytic cell and to calculate the phagocytic and activity index.

Evaluation of respiratory burst

The respiratory burst in the neutrophils of lung cancer induced mice and prunetin treated lung cancer induced mice were assessed using the nitro blue tetrazolium reduction test.[19] About 500µl of uncoagulated blood sample was incubated at 37°C for 30 min and the neutrophils were collected after incubation. The collected neutrophils were mixed with 100µl of nitroblue tetrazolium solution, 0.35% of sucrose solution and serum. The suspension was smeared on the glass slide to form a thin layer using a clean cover slip. The layer was incubated at 37°C for 30 min and then the cover slip was removed and the layer was air dried. The slide was further fixed with methanol for 180 sec and then rinsed with distilled water. The rinsed slide was dried and then stained with safranin for a period of 10 min. The safranin-stained slide was viewed and examined for formazin positive cells under light microscope.

Evaluation of inflammation

The inflammatory response in lung cancer induced mice and prunetin treated lung cancer induced mice were assessed using the soluble immune complex test.[20] Serum sample was collected from coagulated blood centrifuged at 1500 rpm for 25 min at 4°C. The serum was further diluted with buffer solution in ratio of 1:3. The diluted samples were separated into two portions to the first portion 2 ml of buffer solution which acts as control. The second portion was mixed with 2 ml of PEG solution and the samples were incubated at 37°C for 30 min. After incubation period the OD value of samples were measured at 450 nm using the ELISA microplate reader. The PEG index was calculated using the formula

PEG index = (OD of PEG solution – OD of PBS solution) ×1000

Quantification of serum immunoglobulins

The level of immunoglobulins in serum of lung cancer induced mice and prunetin treated lung cancer induced mice were assessed using commercially available ELSIA kit (Abcam, USA). The levels of IgG, IgA, and IgM were quantified using the protocol of manufacturer provided in the kit. The experiment was performed in triplicates ant the absorbance was measured at 450 nm using ELISA microplate reader.

Quantification of oxidative stress in leukocytes

The lung lavage was collected from lung cancer induced mice and prunetin treated lung cancer induced mice using saline solution. The leukocyte count in lavage fluid was counted using counting chamber. The levels of SOD, CAT, and LPO in lymphocytes, polymorphonuclear cells and macrophages were measured according to the protocol of Hu *et al.*[21]

Estimation of metabolizing enzymes

The drug efficacy of prunetin on lung cancer induced mice was assessed by estimating the levels of metabolizing enzymes aryl hydrocarbon hydroxylase, lactate dehydrogenase, γ glutamyl transpeptidase, and 5˙ nucleotidase. The lung homogenates of control and experimental mice were prepared with phosphate buffered saline. The homogenate was subjected to centrifugation at 7500 rpm for 20 min at 4°C. The supernatant was collected subjected to protein estimation using Bradford reagent. The levels of aryl hydrocarbon hydroxylase were estimated according to the protocol of Mildred (1981),[22] lactate dehydrogenase protocol of King (1965),[23] γ glutamyl transpeptidase protocol of Orlowski *et al.* (1965),[24] and 5˙ nucleotidase protocol of Hardonk (1968).[25]

Estimation of tumor markers

The tumor marker carcinoembryonic antigen was measured in the serum of lung cancer induced mice and prunetin treated lung cancer induced mice. The CEA levels were estimated using the commercially available ELISA kit procured from BioVision, MA, USA. The test was performed according to the protocol of manufacturers and the absorbance was measured at 450 nm.

Estimation of pro-inflammatory cytokines

The levels of pro-inflammatory cytokines TNFα, IL-6, and IL-β were quantified from the lung tissue homogenate of lung cancer induced mice and prunetin treated lung cancer induced mice. The homogenate was subjected to centrifugation at 7500 rpm for 10 min at 4°C and the supernatant collected was utilized for further analysis. The test was performed using commercially available ELISA kit procured from Abcam, USA. The test was performed according to the protocol of manufacturers, and the absorbance was measured at 450 nm.

Histopathological analysis

The lung tissue collected from the lung cancer induced mice and prunetin treated lung cancer induced mice were fixed with 10% formalin. The fixed lung tissues were subjected to tissue processing protocol with successive xylene and ethanol treatment. The processed tissue was then prepared as blocks with paraffin wax. The tissue blocks were then subjected to sectioning of 5 micron thickness using microtome. The sectioned tissue was deparaffinized by placing on to hot water bath and then fixed on to an albumin coated glass slide. The sections were then stained with hematoxylin and eosin stains. The stained lung tissue smears were viewed under light microscope for histopathological changes.

Statistical analysis

All the experiments were performed in triplicates and the data were analyzed using statistical software SPSS 17.0. The results were subjected to One Way ANOVA analysis followed by the *post hoc* test Student's Newman Keul test. The statistical significance was considered to be $P < 0.05$.

RESULTS

Protective role of prunetin on leukocytes in lung cancer induced mice

Figure 1 depicts the results of leukocyte, lymphocyte, and neutrophils in the lung cancer induced and prunetin treated lung cancer induced mice. The level of leukocytes was drastically decreased in the lung cancer induced mice whereas both prunetin pre and post treatment in lung cancer induced mice increased the levels of leukocytes. Significant decrease in lymphocytes and neutrophils were observed in lung cancer induced mice. The treatment with prunetin significantly increased the count of both lymphocytes and neutrophils in the lung cancer induced mice.

Immunomodulatory effect of prunetin in lung cancer induced mice

Figure 2 illustrates the phagocytic and activity index of prunetin in lung cancer induced mice. The phagocytic and activity index were significantly decreased in the lung cancer induced mice whereas prunetin treatment significantly increased both the phagocytic and activity index in lung cancer induced mice. The respiratory burst in the neutrophils of lung cancer induced and prunetin treated lung cancer induced mice were assess with NBT reduction test and the results were depicted in Figure 2. Prunetin treatment significantly increased the NBT reduction in lung cancer induced mice compared to untreated cancer induced mice. Prunetin treatment decreased the levels of soluble immunoglobulin complex in lung cancer induced mice which is an indicator of inhibition of inflammatory process.

Protective role of prunetin on Immunoglobulins in lung cancer induced mice

Figure 3 represents the levels of immunoglobulins IgG, IgA, and IgM in lung cancer induced untreated and prunetin treated lung cancer induced mice. The levels of IgG and IgM were significantly decreased

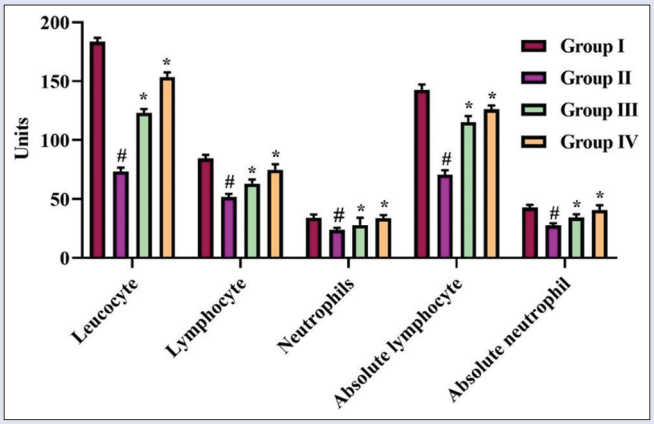

Figure 1: Effect of prunetin on the leukocytes level in the lung cancer-induced mice. The levels of leukocyte, lymphocyte, and neutrophils. The experiment was performed in triplicates. Data were analyzed with One Way ANOVA and Student's newman Keul's post host test and values are illustrated as mean ± SD of triplicates. '#' is significant at $P < 0.01$ from control and '*' is significant at $P < 0.05$ from B (a) P-induced group. Group I: Control mice; Group II: B (a) P-induced lung cancer mice; Group III: B (a) P-induced + 30mg/kg of prunetin post-treated from 12th week; Group IV: B (a) P-induced +30mg/kg of prunetin treated for 18 weeks

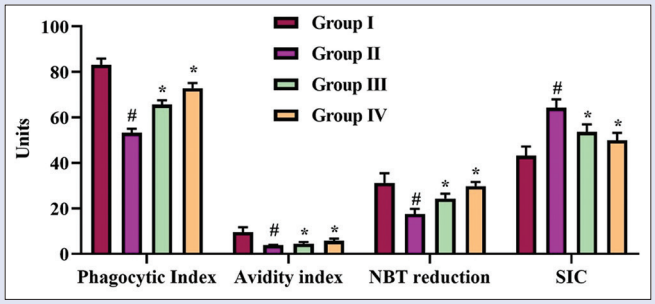

Figure 2: Effect of prunetin on the phagocytic and avidity, index, NBT reduction, and SIC in the lung cancer-induced mice. The experiment was performed in triplicates. Data were analyzed with One Way ANOVA and Student's newman Keul's post host test and values are illustrated as mean ± SD of triplicates. '#' is significant at $P < 0.01$ from control and '*' is significant at $P < 0.05$ from B (a) P-induced group. Group I: Control mice; Group II: B (a) P-induced lung cancer mice; Group III: B (a) P-induced + 30mg/kg of prunetin post-treated from 12th week; Group IV: B (a) P-induced + 30mg/kg of prunetin treated for 18 weeks

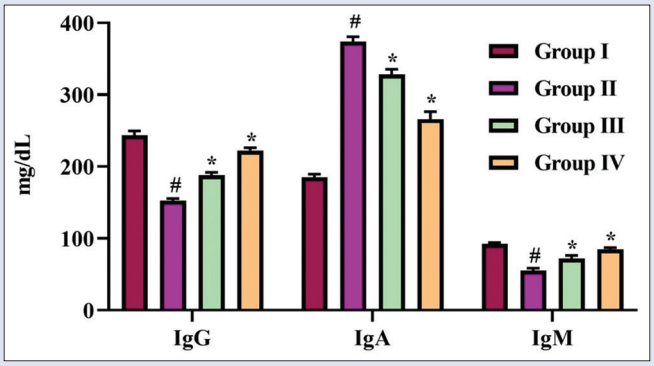

Figure 3: Effect of prunetin on the Immunoglobulins in the lung cancer-induced mice. The levels of serum immunoglobulins IgG, IgA and IgM of control and experimental mice. The levels were estimated using commercially available ELISA kit. The experiment were performed in triplicates. Data were analyzed with One Way ANOVA and Student's newman Keul's post host test and values are illustrated as mean ± SD of triplicates. '#' is significant at $P < 0.01$ from control and '*' is significant at $P < 0.05$ from B (a) P-induced group. Group I: Control mice; Group II: B (a) P-induced lung cancer mice; Group III: B (a) P-induced + 30mg/kg of prunetin post-treated from 12th week; Group IV: B (a) P-induced + 30mg/kg of prunetin treated for 18 weeks

in lung cancer induced untreated mice compared to the control group whereas prunetin treatment significantly increased the levels of both IgG and IgM in lung cancer induced mice. The increase in IgG and IgM levels were observed in both pre and post prunetin supplemented mice. IgA levels were significantly increased in lung cancer induced untreated mice compared to the control group. Prunetin treatment significantly decreased the levels of IgA in lung cancer induced mice compared to the lung cancer induced untreated mice.

Antioxidant role of prunetin on leukocytes in lung cancer induced mice

Oxidative stress in leukocytes plays a key role in cancer cell progression and invasion hence we estimated the levels of antioxidants superoxide dismutase, catalase and the oxidative stress marker lipid peroxidation in leukocytes and the results were illustrated in Figure 4. The levels of SOD and CAT were significantly decreased in lymphocytes, polymorphonuclear cells, and macrophages in lung cancer induced untreated mice. Whereas the prunetin treatment significantly increased the levels of both SOD and CAT in lung cancer induced mice compared to the untreated mice. Compared to polymorphonuclear cells and macrophages, the lymphocytes depicted low level of lipid peroxidation in lung cancer induced mice. Prunetin treated lung cancer induced mice shown significant decreased levels of lipid peroxidation in all the leukocytes compared to the lung cancer induced untreated mice.

Drug efficacy of prunetin in lung cancer induced mice

Figure 5 shows the level of metabolizing enzyme aryl hydrocarbon hydroxylase (AHH), lactate dehydrogenase (LDH), γ glutamyl transpeptidase (γ GTP) and 5' nucleotidase (5'NT) which are the potential targets for a drug design. The levels of metabolizing enzymes AHH, LDH, γ GTP, and 5'NT were significantly increased in lung cancer induced untreated mice compared to the control mice. Prunetin treatment significantly decreased the levels of all the four enzymes in lung cancer induced mice. Compare to post treatment prunetin supplemented mice, the pretreatment prunetin supplemented mice showed significant decrease in the levels of metabolizing enzymes.

Anticancer role of prunetin in lung cancer induce mice

Figure 6a depicts the results of tumor marker carcinoembryonic antigen in the lung cancer induced untreated and prunetin mice. The levels of CEA were significantly increased in lung cancer induced untreated mice compared to the control mice. Prunetin pretreatment in lung cancer induced mice shown significant decrease in the levels of CEA compared to lung cancer induced mice. The levels of inflammatory markers TNF-α, IL-6, and IL-1β were measured in lung cancer induced untreated and prunetin treated mice and the results were illustrated in Figure 5b. Compared to IL-6 the levels of TNF-α and IL-1β were significantly increased in lung cancer induced untreated mice. TNF-α, IL-6, and IL-1β were significantly decreased in prunetin pre supplemented lung cancer induced mice compared to the lung cancer induced untreated and prunetin pretreated mice.

Ameliorative role of prunetin on lung histology in lung cancer induced mice

Figure 7 illustrates the lung tissue histology of control, lung cancer induce, and prunetin post and pretreated lung cancer induced mice. Control mice lung histology shows normal alveoli with thin walls and the interalveolar septa appeared to be thin with increased air space whereas the lung cancer induced mice shows thickened alveolar walls with decreased air space. Prunetin supplementation has restore the alveolar wall thickness and increased the air space in lung tissue.

DISCUSSION

Approximately in the year 2020, about 10 million deaths globally were reported due to cancer. Breast, lung, colon, and prostate cancer are the most common type of cancer detected in the population worldwide. One-third cancer mortality occurs due to tobacco usage, high body mass index, alcohol consumption, and absence of physical activity.[26] Among all the cancer types, lung cancer is the most deadly type of cancer which immensely consumes the global health care funds. The mortality rate of lung cancer is twice higher than breast cancer in women and thrice

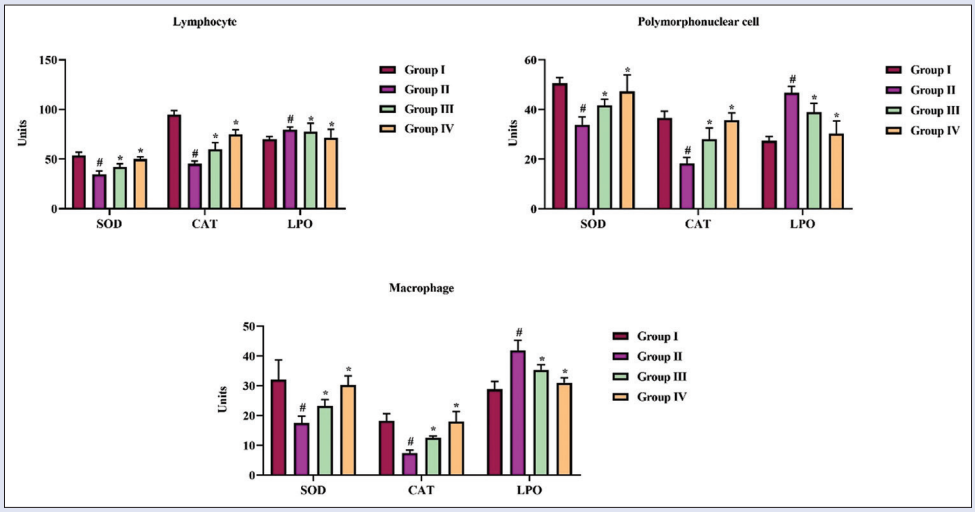

Figure 4: Effect of prunetin on the LPO and antioxidants in the leukocytes of lung cancer-induced mice. The levels of Superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO) in a) Lymphocytes, b) Polymorphonuclear cells and c) Macrophages of control and experimental mice. The experiment were performed in triplicates. Data were analyzed with One Way ANOVA and Student's newman Keul's post host test and values are illustrated as mean ± SD of triplicates. '#' is significant at $P < 0.01$ from control and '*' is significant at $P < 0.05$ from B (a) P-induced group. Group I: Control mice; Group II: B (a) P-induced lung cancer mice; Group III: B (a) P-induced + 30mg/kg of prunetin post-treated from 12th week; Group IV: B (a) P-induced + 30mg/kg of prunetin treated for 18 weeks

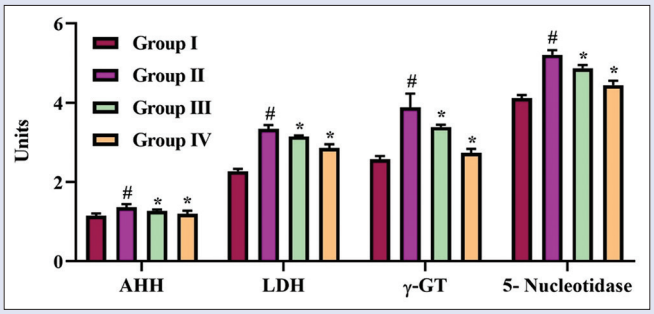

Figure 5: Effect of prunetin on the metabolizing enzyme activity in the lung cancer-induced mice. The levels of metabolizing enzymes aryl hydrocarbon hydroxylase, lactate dehydrogenase, γ glutamyl transpeptidase and 5′ nucleotidase in lung homogenates of control and experimental mice. The experiment were performed in triplicates. Data were analyzed with One Way ANOVA and Student's newman Keul's post host test and values are illustrated as mean ± SD of triplicates. '#' is significant at $P < 0.01$ from control and '*' is significant at $P < 0.05$ from B (a) P-induced group. Group I: Control mice; Group II: B (a) P-induced lung cancer mice; Group III: B (a) P-induced + 30mg/kg of prunetin post-treated from 12th week; Group IV: B (a) P-induced + 30mg/kg of prunetin treated for 18 weeks

higher than prostate cancer. It accounts about 32% of cancer mortality in males and 20% in females.[27] Cancer occurs due to DNA damage and epigenetic changes which in turn disrupts the cell functions such as proliferation, apoptosis, and repair mechanism of DNA.[28] Smoking is the prior cause of lung cancer; 80% of lung cancer patients are reported to be cigarette smokers. The incidence rate of lung cancer is considerably high in passive smokers.[29] The smoke of cigarette consists of numerous carcinogens one of the most active carcinogen is benzo (a) pyrene.[30,31]

In this study, we induced lung cancer in mice by administering benzo (a) pyrene because it affects the initiation, invasion, and progression of a cancer cell.[32,33] BaP is a both genotoxic and a non-genotoxic carcinogen which alters the targeted cells genetic materials and also disrupts receptor activation, cell-cell communication, etc.[34,35] Induction of lung cancer with BaP is well accepted in animal models and the dosage administered is equivalent to the human smoker life time exposure to BaP.[36]

Leukopenia is a clinical condition which occurs due to the massive reduction of white blood cells in circulation. Leukopenia occurs during various conditions such as infection, consumption of drugs, cancer, hypersplenism, etc.[37] BaP, carcinogen induces immunosuppression in experimental animal models and also causes inflammation.[38] Previous studies have reported BaP significantly decreased the levels of leukocytes.[21] Currently available chemotherapeutic drugs to treat cancer also reported to cause leukopenia especially neutropenia upon long term usage. Therefore, in this study, we analyzed the immunomodulatory effect of prunetin treatment on lung cancer induced mice. Prunetin significantly increased the levels of neutrophils and lymphocytes which proves the immunomodulatory effect of prunetin on cancer induced mice.

Neutrophils plays a crucial role in phagocytic function, the disrupted phagocytic function of neutrophils leads to chronic inflammation or lung tissue damage.[39] Therefore, we assessed the role of prunetin on phagocytic and activity index in lung cancer induced mice. Prunetin significantly increased the levels of both phagocytic and activity index of neutrophils. The phagocytic ability of neutrophils was further confirmed with NBT reduction test; prunetin increased the levels of NBT reduction confirming the phagocytic potency of neutrophils.

Tumor induced immunoglobulin differs in the function of B lymphocytes produced immunoglobulin. They induce the tumor cell proliferation, metastasis, and immune escape.[40] Tumor derived immunoglobulin are mostly detected in epithelial cancer cells such as breast, colon, cervical, lung, liver, prostate cancer, etc.[13] It also detected the secretion of cultured cancer cells.[41] In our study, the levels of soluble immunocomplex was significantly increased in BaP treatment whereas the treatment with prunetin decreased the level of immunocomplex. Prunetin treatment significantly increased the levels of IgG and IgM in lung cancer induced mice whereas the levels of IgA level were decreased. The decrease in IgG and IgM in lung cancer induced mice may be due to the humoral immunosuppression which was observed in cancer patients.[42]

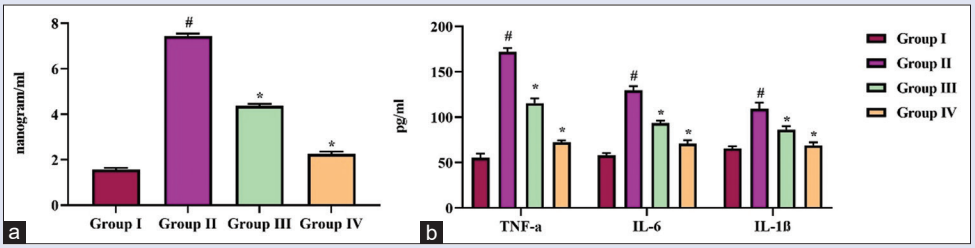

Figure 6: Effect of prunetin on the CEA and pro-inflammatory cytokines level in the lung cancer-induced mice. a) Carcinoembryonic antigen, b) Inflammatory markers. The levels of CEA and inflammatory markers were measured using commercially available ELISA kit and the absorbance was measured at 450 nm. Data were analyzed with One Way ANOVA and Student's newman Keul's post host test and values are illustrated as mean ± SD of triplicates. '#' is significant at $P < 0.01$ from control and '*' is significant at $P < 0.05$ from B (a) P-induced group. Group I: Control mice; Group II: B (a) P-induced lung cancer mice; Group III: B (a) P-induced + 30mg/kg of prunetin post-treated from 12th week; Group IV: B (a) P-induced + 30mg/kg of prunetin treated for 18 weeks

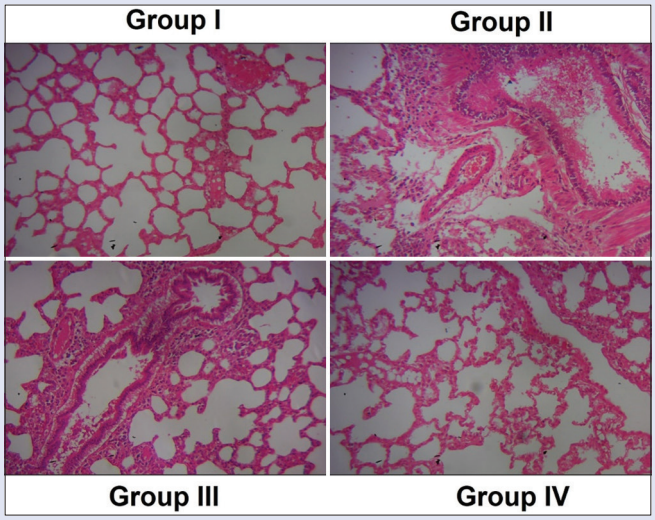

Figure 7: Ameliorative role of prunetin on lung histology in lung cancer-induced mice. Histopahtological assessment in lung tissue of mouse with hematoxylin and eosin staining. Group I: Control mouse with normal lung histology, Group II: B[a] P treated lung cancer induced mice with thickened aleveolar wall, Group III: B[a] P treated lung cancer induced mice post treated with prunetin with normal alveoli and increased air space, Group IV: B[a] P treated lung cancer induced mice pretreated with prunetin with normal alveoli and increased air space. 40X magnification. Group I: Control mice; Group II: B (a) P-induced lung cancer mice; Group III: B (a) P-induced + 30mg/kg of prunetin post-treated from 12th week; Group IV: B (a) P-induced + 30mg/kg of prunetin treated for 18 weeks

Oxidative stress is a key factor in cancer initiation and metastasi; the increased levels of reactive oxygen species increases the metabolic activity of cells and thereby disrupts the mitochondrial function and facilitates the uncontrolled proliferation of cancer cells.[43] The altered cell signaling and damages to DNA was reported in various cancer such as breast, lung, colon, liver etc.[44] Therefore, we evaluated the role of prunetin on scavenging oxidative stress in leukocytes of lung cancer induced mice. Prunetin treatment significantly increased the levels of antioxidant enzymes SOD and CAT in leukocytes thereby subsequently decreased the levels of lipid peroxidation.

Metabolizing enzyme plays a crucial part in designing a potent anticancer drug; hence, we analyzed the effect of prunetin of metabolizing enzyme γ-Glutamyl transpeptidase, lactate dehydrogenase, aryl hydrocarbon hydroxylase, and 5' nucleotidase. γ-Glutamyl transpeptidase involved in glutathione antioxidant metabolism,[45] and LDH converts pyruvate to lactate at glycolysis process.[46] Both γ GTT and LDH are potential target for anticancer drug development and has been focused by various researchers. In our study, prunetin significantly decreased the levels of both γ GTT and LDH in lung cancer induced mice. Aryl hydrocarbon hydroxylase which is determined to genetic susceptibility to lung cancer.[47] was significantly decreased with prunetin treatment in lung cancer induced. Prunetin also decreased the levels of 5' nucleotidase which involved in multidrug resistance.[48,49]

Inflammation triggers the conversion of normal cell to malignant cell, proliferation malignant cells, and metastases of cancer cells. It enriches the tumor neovascularization, inhibits apoptosis and inhibits the immune response.[50] In the present study, prunetin treatment significantly decreased the levels of pro inflammatory cytokines TNF-α, IL-6, and IL-1β in lung cancer induced mice and also decreased the level of carcinogenic embryonic antigen the prominent marker for tumor induction. This proves the anti-inflammatory and anticancer property of prunetin against BaP induced lung cancer mice.

CONCLUSION

In conclusion, prunetin effectively increased the phagocytic and avidity index in lung cancer-induced mice. Prunetin significantly scavenged the ROS, decreased immunoglobulin IgA, and increased the IgG and IgM levels. It also decreased the levels of metabolizing enzymes, tumor marker CEA, and pro-inflammatory markers in lung cancer-induced mice. Our histopathological analysis authentically confirms the therapeutic effect of prunetin on lung tissue of cancer-induced mice. Overall, our results confirm that prunetin is a potent anticancer drug which possess immunomodulatory, anti-inflammatory, and antioxidant properties. Hence, further studies in the future could lead to the development of prunetin as a promising anticancer candidate to treat the lung cancer.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49.
2. World Health Organization (WHO). Global health estimates 2020: Deaths by cause, age, sex, by country and by region. Dec 11. WHO; 2000-2019. p. 2020.

3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
4. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, *et al.* Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 2019;144:1941-53.
5. Latimer KM, Mott TF. Lung cancer: Diagnosis, treatment principles, and screening. Am Fam Physician 2015;91:250-6.
6. Jemal A, Thun MJ, Ries LA, Howe HL, Weir HK, Center MM, *et al.* Annual report to the nation on the status of cancer, 1975-2005, featuring trends in lung cancer, tobacco use, and tobacco control. J Natl Cancer Inst 2008;100:1672-94.
7. Alberg AJ, Brock MV, Ford JG, Samet JM, Spivack SD. Epidemiology of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143(Suppl):e1S-29S.
8. Alexandrov K, Rojas M, Satarug S. The critical DNA damage by benzo (a) pyrene in lung tissues of smokers and approaches to preventing its formation. Toxicol Lett 2010;198:63-8.
9. Memmott RM, Mercado JR, Maier CR, Kawabata S, Fox SD, Dennis PA. Metformin prevents tobacco carcinogen--induced lung tumorigenesis. Cancer Prev Res (Phila) 2010;3:1066-76.
10. Zuo J, Brewer DS, Arlt VM, Cooper CS, Phillips DH. Benzo pyrene-induced DNA adducts and gene expression profiles in target and non-target organs for carcinogenesis in mice. BMC Genomics 2014;15:880.
11. Zhou JG, Tian X, Cheng L, Zhou Q, Liu Y, Zhang Y, *et al.* The risk of neutropenia and leukopenia in advanced non-small cell lung cancer patients treated with erlotinib: A Prisma-compliant systematic review and meta-analysis. Med (Baltim) 2015;94:e1719.
12. Liu W, Zhang CC, Li K. Prognostic value of chemotherapy-induced leukopenia in small-cell lung cancer. Cancer Biol Med 2013;10:92-8.
13. Cui M, Huang J, Zhang S, Liu Q, Liao Q, Qiu X. Immunoglobulin expression in cancer cells and its critical roles in tumorigenesis. Front Immunol 2021;12:613530. doi: 10.3389/fimmu.2021.613530.
14. Sandhu KV, Demiray YE, Yanagawa Y, Stork O. Dietary phytoestrogens modulate aggression and activity in social behavior circuits of male mice. Horm Behav 2020;119:104637. doi: 10.1016/j.yhbeh. 2019.104637.
15. Ahn TG, Yang G, Lee HM, Kim MD, Choi HY, Park KS, *et al.* Molecular mechanisms underlying the anti-obesity potential of prunetin, an O-methylated isoflavone. Biochem Pharmacol 2013;85:1525-33.
16. Nam DC, Kim BK, Lee HJ, Shin HD, Lee CJ, Hwang SC. Effects of prunetin on the proteolytic activity, secretion and gene expression of MMP-3 *in vitro* and production of MMP-3 *in vivo*. Korean J Physiol Pharmacol 2016;20:221-8.
17. Hu H, Li H. Prunetin inhibits lipopolysaccharide-induced inflammatory cytokine production and MUC5AC expression by inactivating the TLR4/MyD88 pathway in human nasal epithelial cells. Biomed Pharmacother 2018;106:1469-77.
18. Janusz MJ, Austen KF, Czop JK. Phagocytosis of heat-killed blastospores of Candida albicans by human monocyte beta-glucan receptors. Immunology 1988;65:181-5.
19. Gifford RH, Malawista SE. A simple rapid micro method for detecting chronic granulomatous disease of childhood. J Lab Clin Med 1970;75:511-9.
20. Seth P, Srinivas RV. Circulating immune complexes in cervical cancer-simple method for detection and characterization. Indian J Med Res 1981;73:926-9.
21. Hu X, Geetha RV, Surapaneni KM, Veeraraghavan VP, Chinnathambi A, Alahmadi TA, *et al.* Lung cancer induced by benzo (A) pyrene: Chemoprotective effect of sinapic acid in swiss albino mice. Saudi J Biol Sci 2021;28:7125-33.
22. Buening MK, Chang MT, Huang MT, Fortner JG, Wood AW, Conney AH. Activation and inhibition of benzo (a) pyrene and aflatoxin B1 metabolism in human liver microsomes by naturally occurring flavonoids. Cancer Res 1981;41:67-72.
23. King C. The transferases-alanine and aspartate transaminases. In: Van D, editor. PracticalClinical Enzymology. 1965. p. 121-38.
24. Orlowski M, Meister A. Isolation of γ-glutamyl transpeptidase from hog kidney. J Biol Chem 1965;240:338-47.
25. Hardonk HG, Boer HGB. 5′-Nucleotidase 3. Determinations of 5′-nucleotidase isoenzymes in tissues of rat and mouse. Histochemie 1968;12:29-41.
26. Cabasag CJ, Fagan PJ, Ferlay J, Vignat J, Laversanne M, Liu L, *et al.* Ovarian cancer today and tomorrow: A global assessment by world region and Human Development Index using GLOBOCAN 2020. International Journal of Cancer. 2022 Mar 23.
27. Goodarzi E, Sohrabivafa M, Adineh HA, Moayed L, Khazaei Z. Geographical distribution global incidence and mortality of lung cancer and its relationship with the Human Development Index (HDI); An ecology study in 2018. WCRJ 2019;6:e1354.
28. Brown KM, Keats JJ, Chapter SA. Holland –Frei CancerMedicine. 8th ed, vol 8. People's Medical Publishing House; 2010. p. 978-1-60795-014-1.
29. Biesalski HK, Bueno de Mesquita B, Chesson A, Chytil F, Grimble R, Hermus RJ, *et al.* European consensus statement on lung cancer: Risk factors and prevention. CA Cancer J Clin 1998;48:167-76; discussion 164.
30. Hecht SS. Lung carcinogenesis by tobacco smoke. Int J Cancer 2012;131:2724-32.
31. Kumar V, Abbas AK, Aster JC. Chapter 5. Robbins Basic Pathology. 9th ed, vol 199:978-1-4377-1781-5. Elsevier Saunders. 2013. p. 2013.
32. Luch A. Nature and nurture – lessons from chemical carcinogenesis. Nat Rev Cancer 2005;5:113-25.
33. Klaunig JE, Kamendulis LM. Chemical carcinogenesis. In: Casarett and Doull's Toxicology: the Basic Science of Poisons. 7th ed, vol 603. New York: McGraw-Hill; 2007. p. 329-80.
34. Williams GM. Mechanisms of chemical carcinogenesis and application to human cancer risk assessment. Toxicology 2001;166:3-10.
35. Hernández LG, van Steeg H, Luijten M, van Benthem J. Mechanisms of non-genotoxic carcinogens and importance of a weight of evidence approach. Mutat Res 2009;682:94-109.
36. Tuveson DA, Jacks T. Modeling human lung cancer in mice: Similarities and shortcomings. Oncogene 1999;18:5318-24.
37. Ing VW. The etiology and management of leukopenia. Can Fam Physician 1984;30:1835-9.
38. Herr M, Bostedt H, Failing K. IgG and IgM levels in dairy cows during the periparturient period. Theriogenology 2011;75:377-85.
39. Shanmugam L, Ravinder SS, Johnson P, Padmavathi R, Rajagopalan B, Kindo AJ. Assessment of phagocytic activity of neutrophils in chronic obstructive pulmonary disease. Lung India 2015;32:437-40.
40. Zhao J, Peng H, Gao J, Nong A, Hua H, Yang S, *et al.* Current insights into the expression and functions of tumor-derived immunoglobulins. Cell Death Discov 2021;7:148.
41. Zheng H, Li M, Ren W, Zeng L, Liu HD, Hu D, *et al.* Expression and secretion of immunoglobulin alpha heavy chain with diverse VDJ recombinations by human epithelial cancer cells. Mol Immunol 2007;44:2221-7.
42. Ravichandiran V, Nazeer H, Nirmala S. Natural flavonoids and lung cancer. Pharm Globale 2011;2:1-9.
43. Fiaschi T, Chiarugi P. Oxidative stress, tumor microenvironment, and metabolic reprogramming: A diabolic liaison. Int J Cell Biol 2012;2012:762825. doi: 10.1155/2012/762825.
44. Saha SK, Lee SB, Won J, Choi HY, Kim K, Yang GM, *et al.* Correlation between oxidative stress, nutrition, and cancer initiation. Int J Mol Sci 2017;18:1544.
45. Hu X, Legler PM, Khavrutskii I, Scorpio A, Compton JR, Robertson KL, *et al.* Probing the donor and acceptor substrate specificity of the γ-glutamyl transpeptidase. Biochemistry 2012;51:1199-212.
46. Fiume L, Manerba M, Vettraino M, Di Stefano G. Inhibition of lactate dehydrogenase activity as an approach to cancer therapy. Future Med Chem 2014;6:429-45.
47. Kiyohara C, Hirohata T. [A role of aryl hydrocarbon hydroxylase inducibility in susceptibility to lung carcinogenesis]. Nihon Eiseigaku Zasshi 1994;48:1027-36. [Japanese].
48. Mikhailov A, Sokolovskaya A, Yegutkin GG, Amdahl H, West A, Yagita H, *et al.* CD73 participates in cellular multiresistance program and protects against TRAIL-induced apoptosis. J Immunol 2008;181:464-75.
49. Baqi Y, Lee SY, Iqbal J, Ripphausen P, Lehr A, Scheiff AB, *et al.* Development of potent and selective inhibitors of ecto-5′-nucleotidase based on an anthraquinone scaffold. J Med Chem 2010;53:2076-86.
50. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011;144:646-74.