Advances in Bioresearch
Adv. Biores., Vol 15 (3) May 2024: 177-183
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.3.177183

Advances in Bioresearch

ORIGINAL ARTICLE

Screening of Chemoprevention on *Madhuca longifolia* ethanolic seeds extract against Benzo(a)pyrene Carcinogenesis induced Lung Cancer in Swiss Albino Male Mice Model

Dibyajyoti Saha[1*], Satish Kumar Sarankar[2]
[1,2] Faculty of Pharmacy, Mansarovar Global University, Billkisganj, Sehore, Madhya Pradesh- 466001, India
* Corresponding Author's E-Mail: [1]djsmgupharmacy@gmail.com

ABSTRACT
Exhaustive research showed that Madhuca longifolia have anti-cancer, anti-ulcer and ethnopharmacological value. The use of substances that can change, decrease or delay the progression of cancer at various stages is known as cancer chemoprevention. Chemical carcinogenesis benzo(a)pyrene was selected for this research. The objective of the research was to explore the chemopreventive activity of Madhuca longifolia ethanolic seeds extract against benzo(a)pyrene carcinogenesis induced lung cancer in mice model. Five groups were designed for the study and Swiss Albino male mice were intraperitoneally injected with vincristine 50mg/kg b.w. at a standardized dose. Mahua extract was given orally at an optimum dose of 15 mg/kg b.w. and 30 mg/kg b.w. as per experimental protocol. The parameters LPO, GST, SOD, CAT, GSH have been evaluated. The results obtained showed significant elevation of antioxidant and oxidative enzymatic parameters in lung. Thus, the present data strongly indicated that the plant seeds have potential to act as anti-lung cancer promoting agent in animal model. However, further studies need to be done to understand the mechanism of action.
Keywords: *Benzo(a)pyrene, Carcinogenesis, Chemoprevention, Lung, Madhuca longifolia*

Received 29.11.2023 Revised 31.12.2023 Accepted 23.03.2024

How to cite this article:
Dibyajyoti S, Satish K S. Screening of Chemoprevention on *Madhuca longifolia* ethanolic seeds extract against Benzo(a)pyrene Carcinogenesis induced Lung Cancer in Swiss Albino Male Mice Model. Adv. Biores., Vol 15 (3) May 2024: 177-183.

INTRODUCTION

DNA damage caused by oxidants and free radicals is one of the most common reasons of cancer's genesis. Lung cancer is the term used to describe tumors that start in the bronchi or lung parenchyma [3]. Utilizing organic and inorganic compounds to stop or slow down the promotion stage of carcinogenesis, in which the initial cells expand to become a tumor, is known as cancer chemotherapy [4]. Plants include a variety of compounds referred to as phytochemicals. It is also showed that chronic oxidative and nitrosative stress can harm cells and play a role in different phases of carcinogenesis [10, 14]. Tumor cells divide and multiply uncontrollably, have the capacity to continue growing unabatedly and independently, and are resistant to apoptosis. Additionally, reactive oxygen species promoted their growth [16]. Free radicals are produced by a range of endogenous and external mechanisms in the body [20]. Free radical overproduction has been linked to the emergence of several chronic conditions, including cancer. Compounds that are alien to animal existence were looked at in the research of xenobiotics [12]. New pathways of research into the mechanisms underlying and influencing the development of cancer have been opened up. These include the significance of DNA damage and mutation in the development of cancer, the part played by metabolic activation in the development of cancer, and more. The first item on the list is DNA repair [15]. Xenobiotics are substances that are present in an organism but are not normally produced by it or anticipated to be there. They can be compounds from natural or manufactured sources [19]. Oxidative stress (OS) also characterized by an increase in ROS production and/or a decrease in antioxidants that scavenge ROS [2, 5]. GST proteins are crucial anti-oxidant enzymes that regulate

stress-related signaling cascades. Glutathione, an antioxidant, serves as a detoxifier and free radical scavenger in cells. It is the molecule that is most frequently observed to have higher levels following oxidative stress and is crucial for a number of processes, including cell division, proliferation, and differentiation [6]. The body uses the essential antioxidant superoxide dismutase (SOD) to defend itself against oxidative stress. Cancer can be effectively treated using the enzyme. Cancer is significantly impacted by catalase as well [9].

Chemoprevention has novel and promising approach to suppress or inhibit the formation of cancer or tumor using natural, synthetic and biological substances. The poly aromatic hydrocarbon class prototypical lung carcinogen benzo (a) pyrene is known to cause large numbers of free radicals, which leads to oxidative stress [11]. In this experiment, chemical carcinogen was used by inducing lung cancer namely benzo(a)pyrene occurred chronic inflammation, create oxidative stress and damaged DNA. The present study was undertaken to evaluate the chemopreventive effect of *Madhuca longifolia* seeds (ethanolic extract) against lung cancer in Swiss albino male mice model.

The plant have nutraceutical, ethno medicinal & ethnopharmacological action such as anti- inflammatory, antipyretic, antihyperglycemic, antifertility, antiulcer, antimicrobial, antioxidant, cardio protective etc. This plant-based foods also effective against the various diseases i.e., ulcer, snakebite, scabies, rheumatism etc. As best of knowledge, no cancer chemoprevention activity of this plant seeds previously reported. The aim of research was to find out the chemo preventive efficacy of *Madhuca longifolia* seeds to prevent lung cancer in the animal model.

MATERIAL AND METHODS

Chemicals

Benzo[a]pyrene (96%), ethylene diamine tetra acetic acid (EDTA), pyrogallol, thiobarbituric acid (TBA), bovine serum albumin (BSA), vincristine, 1-chloro-2, 4-dinitrobenzene (CDNB), sodium dodecyl sulphate (SDS), reduced glutathione (GSH), Hydrogen peroxide 30% etc.

Animals

Swiss albino healthy male mice weighing 20-24 gm, were taken for this study and maintained at twelve hours light/dark cycle, humidity (55% to 65%), temperature (21^0C to 25^0C). Six mice per cage were housed in wire-mesh cages. Standard food pellets diet (rat/mice feed) and drinking water was given ad libitum.

Preparation of Drug

Ethanolic extract of *Madhuca longifolia* seeds (15mg/30 mg) was dissolved in 200 µl phosphate buffer saline. Each day of the experiment, just before the treatment, it was prepared.

Preparation of Carcinogenesis

Benzo[a]pyrene (96%), at the dose of 50mg/kg body wt. dissolved in corn oil, was given orally weekly twice for four consecutive weeks to induce lung cancer by the 16th week. Each day of the experiment, just before the treatment, it was prepared.

Experimental Design

Vehicle control group (Group-I): This group received corn oil orally (200 µl /mouse) for 16 weeks.

Carcinogen control group (Group-II): Animals received benzo[a]pyrene (96%), at the dose of 50mg/kg b.wt. dissolved in corn oil, was given orally weekly twice for four consecutive weeks to induce lung cancer by the 16th week served as carcinogen control.

Positive control group (Group III): The treatment for the animals in this group was the same as for group II and also administered intraperitoneally with 50 mg/kg standard drug of Vincristine from the day started 7 days before the exposure of the B(a)P.

Lower dose test group (Group-IV): The treatment for the animals in this group was the same as for group II and also received the Mahua Extract (ME) at a dose of 15 mg/kg body weight/day orally from the day started 7 days before the exposure of the B(a)P.

Higher dose test group (Group-V): The treatment for the animals in this group was the same as for group II and also received the Mahua Extract (ME) at a dose of 30 mg/kg body weight/day orally from the day started 7 days before the exposure of the B(a)P.

Mice of group I, II, III, IV, V were sacrificed based on IAEC guidelines after 16 weeks of 1st application of B(a)P.

Estimation of Antioxidant and oxidative enzymatic parameters

Assay of Lipid per oxidation (LPO): Thiobarbituric acid was employed to quantify the lipid peroxidation in microsomes, and the results were computed using the nanomoles of TBARS produced per milligram of protein [8].

Assay of Glutathione - S - Transferase (GST): Utilizing 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate, glutathione-S-transferase (GST) activity was assessed by observing a rise in absorbance at 340 nm. Nanomoles of the CDNB-GSH conjugate were believed to constitute the enzyme's specific activity per milligram of protein [7].
Assay of Superoxide Dismutase (SOD): The cytosolic superoxide dismutase (SOD) activity was measured and expressed as units per milligram of protein. Pyrogallol autooxidation inhibition is quantified as a proxy for SOD activity. An "enzyme activity unit" is the amount of enzyme required for 50% inhibition. [18].
Assay of Catalase (CAT): A unit of catalase (CAT) activity is the quantity of enzyme needed to release half the peroxide oxygen from H_2O_2 in 100 seconds at 25°C. [1].
Assay of Reduced Gluthathione (GSH): The amount of GSH was determined as nanomoles per milligram of protein in the cytosol [17].

Statistical Analysis

MEAN ± SEM were used to present each experimental data. Following a one-way ANOVA comparison and analysis of all the groups, Dunnett's multiple comparison tests were carried out. [13].

RESULTS AND DISCUSSION

Estimation of Antioxidant & oxidative enzymatic parameters

Lipid per oxidation (LPO)

The significant increase was shown of lipid per oxidation in carcinogen control group II (157.2 ± 2.82%) compared to group I. The positive control group III (130.3 ± 2.12%) showed inhibition of lipid peroxidation. However, simultaneous treatment of group IV (106.1 ± 1.08%) showed better result than group III. The highest inhibition of lipid peroxidation showed by higher dose test group V (65.9 ± 2.27%) than group III & IV after 16 weeks treatment (Figure 1).

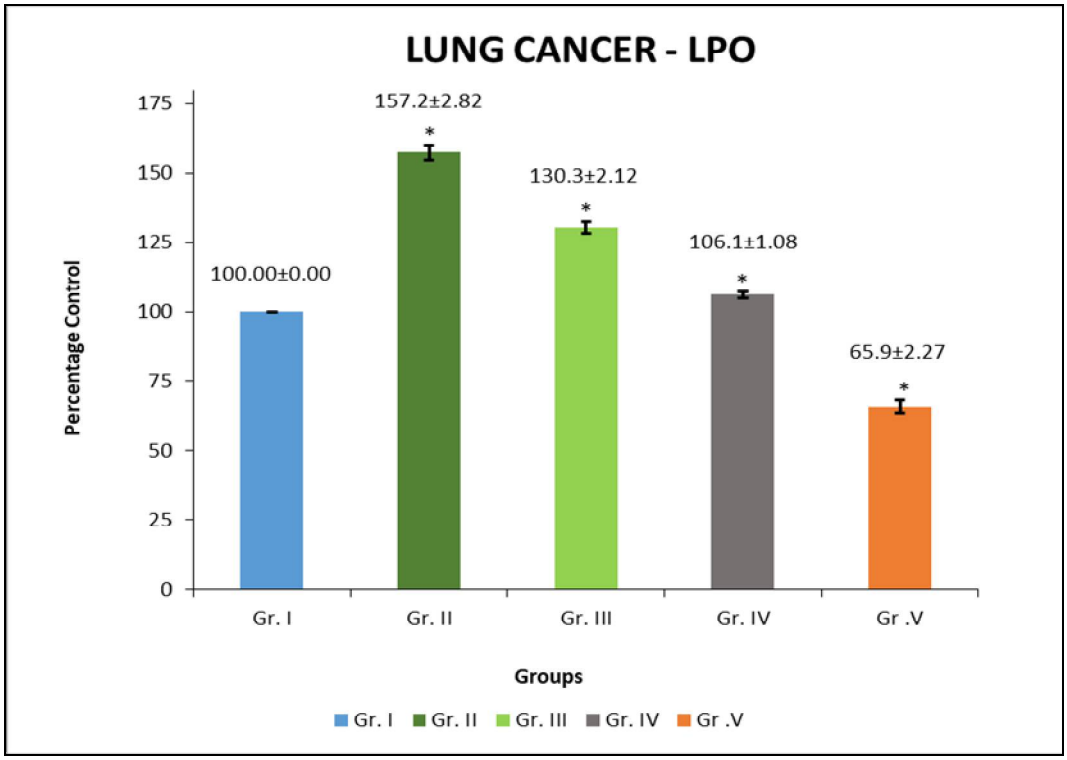

Figure 1: Lung Cancer-LPO.

Glutathione - S - Transferase (GST)

The significant decrease was shown of glutathione-S-Transferase (GST) in carcinogen control group II (45.28 ± 1.58%) compared to the vehicle control group I. The positive control group III (55.29 ± 1.98%) showed significant increase of GST activity. However, simultaneous treatment of group IV (76.52 ± 2.09%) showed better result than group III. The highest increase of GST showed by higher dose test group V (118.31 ± 4.03%) than group III & IV after 16 weeks treatment (Figure 2).

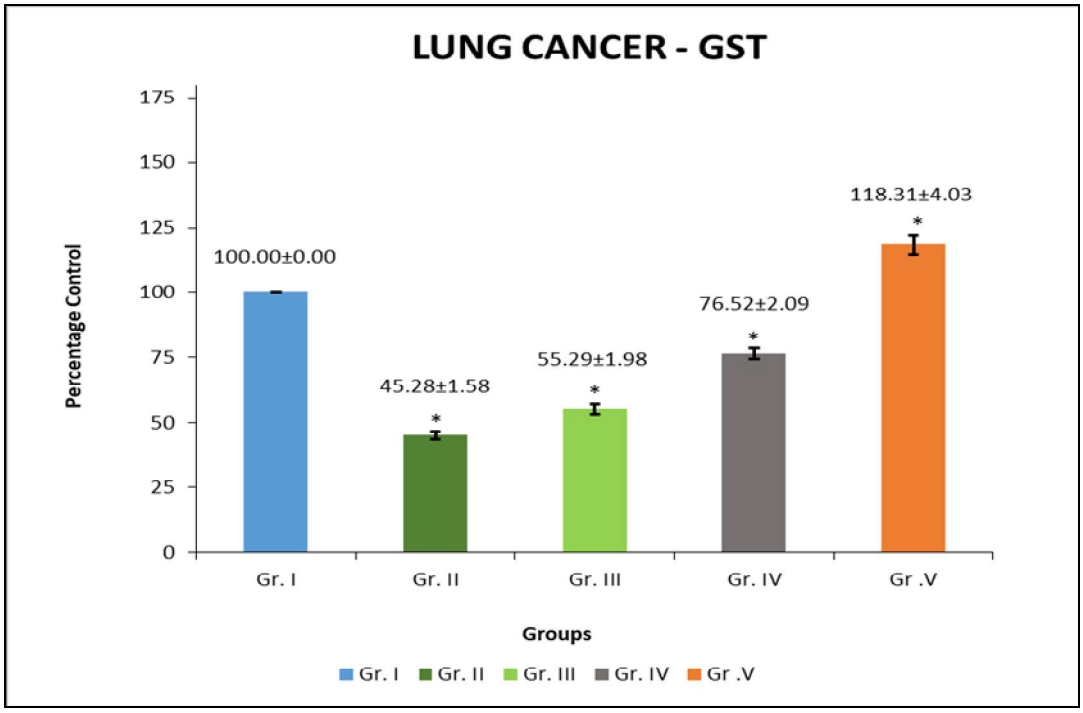

Figure 2: Lung Cancer-GST.

Reduced Gluthathione (GSH)

The significant decrease was shown of reduced Gluthathione (GSH) in carcinogen control group II (58.52 ± 1.78%) compared to group I. The positive control group III (77.21 ± 3.27%) showed significant increase of GSH activity. However, simultaneous treatment of group IV (100.05 ± 5.01%) showed better result than group III. The highest increase of GSH showed by higher dose test group V (145.42 ± 2.08%) than group III & IV after 16 weeks treatment (Figure 3).

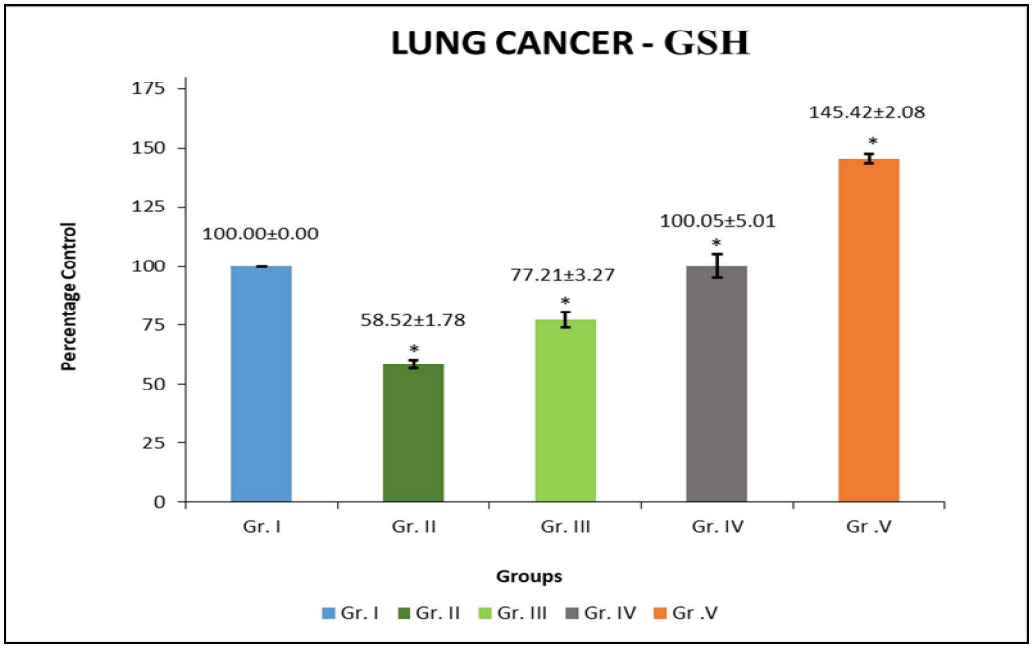

Figure 3: Lung Cancer-GSH.

Superoxide Dismutase (SOD)

The significant decrease was shown of superoxide dismutase (SOD) in carcinogen control group II (42.53 ± 0.98%) compared to group I. The positive control group III (68.91 ± 2.37%) showed significant increase

of SOD activity. However, simultaneous treatment of group IV (94.18 ± 2.15%) showed better result than group III. The highest increase of SOD showed by higher dose test group V (120.05 ± 1.09%) than group III & IV after 16 weeks treatment (Figure 4).

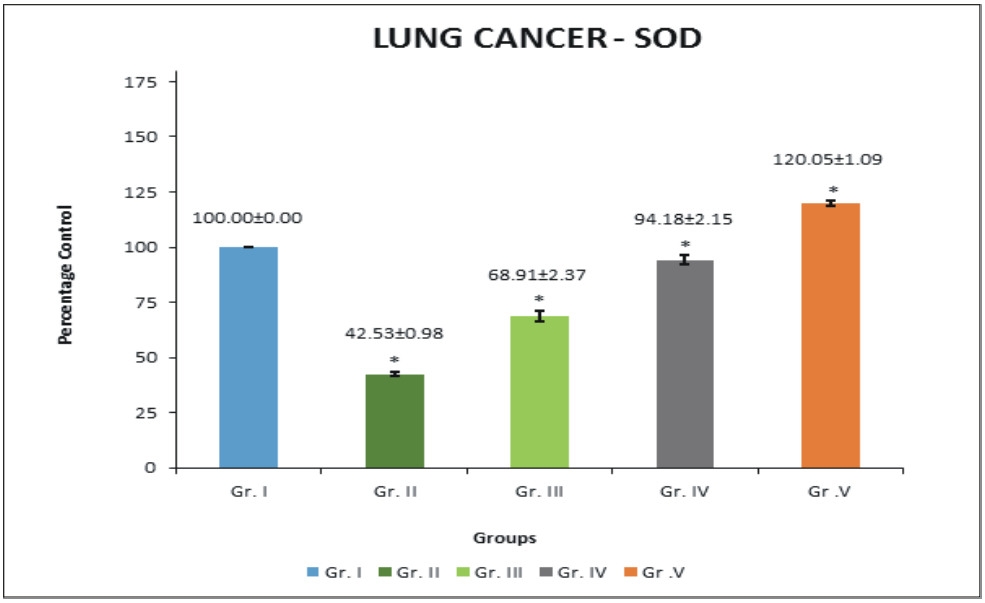

Figure 4: Lung Cancer-SOD.

Catalase (CAT)

The significant decrease was shown of catalase (CAT) in carcinogen control group II (30.24 ± 2.93%) compared to group I. The positive control group III (50.73 ± 2.01%) showed significant increase of CAT activity. However, simultaneous treatment of group IV (75.08 ± 1.61%) showed better result than group III. The highest increase of CAT showed by higher dose test group V (103.59 ± 2.25%) than group III & IV after 16 weeks treatment (Figure 5).

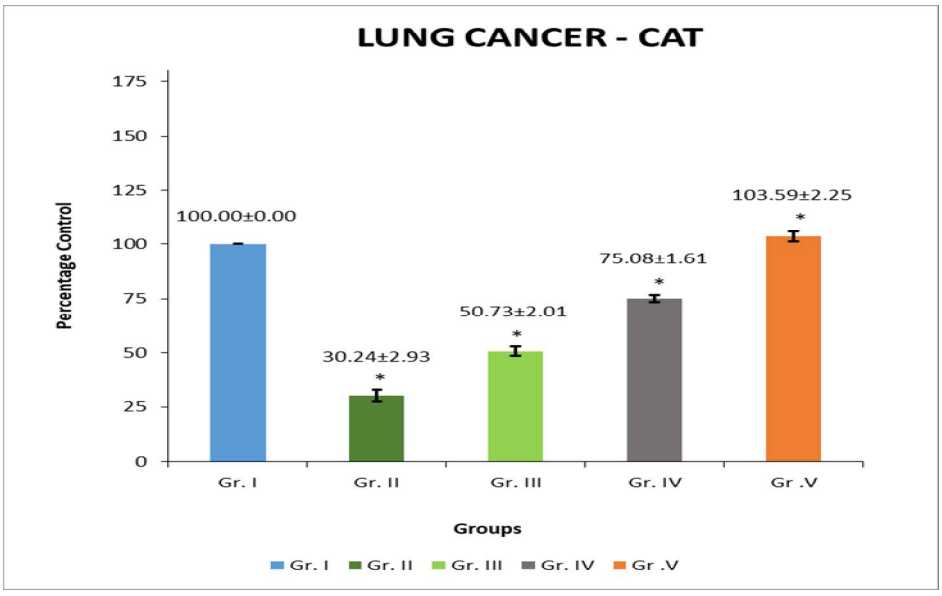

Figure 5: Lung Cancer-CAT.

The significant increase was shown of lipid per oxidation in carcinogen control group II compared to group I. The positive control group III showed inhibition of lipid peroxidation. However, simultaneous treatment of group IV showed better result than group III. The highest inhibition of lipid peroxidation showed by higher dose test group V than group III & IV after 12 to 16 weeks treatment. The significant decrease was shown of glutathione-S-Transferase (GST) in carcinogen control group II compared to group I. The positive control group III showed significant increase of GST activity. However, simultaneous

treatment of group IV showed better result than group III. The highest increase of GST showed by higher dose test group V than group III & IV after 16 weeks treatment. The significant decrease was shown of reduced Gluthathione (GSH) in carcinogen control group II compared to group I. The positive control group III showed significant increase of GSH activity. However, simultaneous treatment of group IV showed better result than group III. The highest increase of GSH showed by higher dose test group V than group III & IV after 16 weeks treatment. The significant decrease was shown of superoxide dismutase (SOD) in carcinogen control group II compared to the vehicle control group I. The positive control group III showed increasing of SOD activity. However, simultaneous treatment of group IV showed better result than group III. The highest increase of SOD showed by higher dose test group V than group III & IV after 16 weeks treatment. The significant decrease was shown of catalase (CAT) in carcinogen control group II compared to the group I. The positive control group III showed significant increase of CAT activity. However, simultaneous treatment of group IV showed better result than group III. The highest increase of CAT showed by higher dose test group V than group III & IV after 16 weeks treatment.

Chemoprevention should be able to treat lung cancer. The B(a)P, contribute to the onset and development of carcinogenesis by harming several biomolecules and causing a variety of cellular alterations. It has been established that produce reactive oxygen species and free radical intermediates were responsible for transformation of carcinogen. Several mutations may then be caused by the interaction of MDA-DNA adducts with DNA, which may result in carcinogenesis.

CONCLUSION

The present research explored whether the plant ethanolic seed extract provides significant protection from free radical scavenging, initiating inflammation, etc. in a Swiss albino mouse model of B(a)P-induced lung carcinogenesis through antioxidants. The findings of the current study established that an ethanolic extract of Mahua seeds has powerful anticancer activity in vivo and can effectively inhibit the proliferation of lung cancer cells against B(a)P. Hence, plant seed ethanolic extract can be used to treat or prevent lung cancer.

REFERENCES

1. Ansar M., Ivanciuc T., Garofalo R.P., Casola A. (2020). Increased Lung Catalase Activity Confers Protection against Experimental RSV Infection, Sci Rep., (10): 3653:1-10. https://doi.org/10.1038/s41598-020-60443-2.
2. Apaya MK, Chang M, Shyur L. (2016). Phytomedicine polypharmacology: cancer therapy through modulating the tumor microenvironment and oxylipin dynamics. Pharmacology & Therapeutics. (162): 58–68. https:// dx.doi.org/10.1016/j.pharmthera.2016.03.001
3. Ashok SR, Shivananda MK, Manikandan A, Chandrasekaran R. (2019). Discovery and synthesis of 2-amino-1-methyl-1H-imidazole-4(5H)-ones as GPCR ligands; an approach to develop breast cancer drugs via GPCR associated PAR1 and PI3Kinase inhibition mechanism. Bioorg. Chem. (86): 641–651. https:// dx.doi.org/10.1016/ j.bioorg. 2019.02.048
4. Bindu B, Vijayalakshmi S, Manikandan A. (2019). Discovery, synthesis and molecular substantiation of N-(benzo[d]thiazol-2-yl)-2-hydroxyquinoline-4-carboxamides as anticancer agents. Bioorg. Chem. (91): 103171. https:// dx.doi.org/10.1016/j.bioorg.2019.103171
5. Chaudhary T, Chahar A, Sharma JK, Kaur K, Dang A. (2015). Phytomedicine in the treatment of cancer: a health technology assessment. J Clin Diagn Res. (9): 12, XC04–XC09. https://dx.doi.org/10.7860/JCDR/2015/15701.6913
6. Cheng Y, Yang C, Shyur L. (2016). Phytomedicine-modulating oxidative stress and the tumor microenvironment for cancer therapy. Pharmacological Research. (114):128–143. https://dx.doi.org/10.1016/j.phrs.2016.10.022
7. Dai X., Dharmage S.C., Lodge C.J. (2022). Interactions between glutathione S-transferase genes and household air pollution on asthma and lung function, Front Mol Biosci. (29): 9:1-13. https://dx.doi.org/10.3389 /fmolb.2022.955193.
8. Ito F., Sono Y., Ito T. (2019). Measurement and Clinical Significance of Lipid Peroxidation as a Biomarker of Oxidative Stress: Oxidative Stress in Diabetes, Atherosclerosis, and Chronic Inflammation, Antioxidants (Basel). (8): 3:1-28. https://dx.doi.org/10.3390/antiox8030072.
9. Jivad N, Rabiei Z. (2014). A review study on medicinal plants used in the treatment of learning and memory impairments. Asian Pac J Trop Biomed. (4): 780–789. https://dx.doi.org/10.12980/APJTB.4.2014APJTB-2014-0412
10. Kadirappa A, Manikandan A, Sailaja M, Napoleon AA. (2018). Synthesis of substituted quinolinyl ether-based inhibitors of PI3K as potential anticancer agents. J. Heterocyclic Chem. (55): 7:1669–1677. https://dx.doi.org/10.1002/jhet.3201
11. Kasala E.R., Bodduluru L.N., Barua C.C., Madhana R.M., Dahiya V., Budhani M.K., Mallugari R.R., Maramreddy S.R., Gogoi R. (2016). Chemopreventive effect of chrysin, a dietary flavone against benzo(a)pyrene induced lung carcinogenesis in Swiss albino mice. Pharmacol Rep., (68): 2: 310-318. https://doi.org/ 10.1016/j.pharep.2015.08.014.

12. Lichterman BL. (2004). Aspirin: the story of a wonder drug. British Med J. 2004; 329 (7479):1408. PMCID: PMC535471
13. Morgan D. (2018). Bayesian applications in pharmaceutical statistics, Pharmaceutical Statistics, 17 (4): 298–300. https://dx.doi.org/10.1002/pst.1876.
14. Muralidharan VP, Manikandan A, Arumugam S, Iyer SK. (2018). Molecular substantiation and drug efficacy of relatively high molecular weight S- BINOLs; appraised as breast cancer medication and PI3 kinase inhibitors. J. Heterocyclic Chem. 55 (6):1339–1345. https://dx.doi.org/10.1002/jhet.3166
15. Petrovska BB. (2012). Historical review of medicinal plants' usage. Pharmacognosy Reviews. 6 (11):1–5. https://dx.doi.org/10.4103/0973-7847.95849
16. Rajesh KM, Manikandan A, Violet DV. (2018). N-substituted hydroxynaphthalene imino-oxindole derivatives as a new class of pi3-kinase inhibitor and breast cancer drug: molecular validation and SAR studies. Chem Biol Drug Des. 91 (1):277–284. https://dx.doi.org/10.1111/cbdd.13079
17. Robbins M.E., Cho H., Höhle M., Hansen J.M., Luchsinger J.R., Locy M.L., Velten M., Kleeberger S.R., Rogers L.K., Tipple T.E. (2021). Glutathione reductase deficiency alters lung development and hyperoxic responses in neonatal mice, Redox Biol., (38): 101797; 1–12. https://dx.doi.org/10.1016/j.redox.2020.101797.
18. Sul C., Lewis C., Dee N., Burns N., Oshima K., Schmidt E., Vohwinkel C., Nozik E. (2023). Release of extracellular superoxide dismutase into alveolar fluid protects against acute lung injury and inflammation in Staphylococcus aureus pneumonia, Am J Physiol Lung Cell Mol Physiol. 324 (4): L445-L455. https://dx.doi.org/10.1152 /ajplung.00217.2022.
19. Tapsell LC, Hemphill I, Cobiac L et al. (2006). Health benefits of herbs and spices: the past, the present, the future. Med. J. Aust. (185): S1-S24. https://dx.doi.org/10.5694/j.1326-5377.2006.tb00548.x
20. Thangarasu P, Thamaraiselvi S, Manikandan A. (2018). Unveiling novel 2- cyclopropyl-3-ethynyl-4-(4-fluorophenyl) quinolines as GPCR ligands via PI3- kinase/PAR-1 antagonism and platelet aggregation valuations; development of a new class of anticancer drugs with thrombolytic effects. Bioorg. Chem. (81): 468–480. https://dx.doi.org/ 10.1016/j.bioorg.2018.09.011