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Antiproliferative effect of *Ocimum sanctum* against benzo[A]pyrene induced lung tumors in rats

Jyotsna Sethi[1], Chandrajeet Kumar[1] and Arun Kumar[2]*

[1]Department of Biotechnology, YBN University, Ranchi, Jharkhand, India
[2]Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India

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ABSTRACT

The disease of lung has increased many folds in the recent times. Unfortunately, the lung cancer disease cases have also been reported many times. Although smoking tobacco products are thought to be the causative agents. But the environmental pollution has elevated the disease risk. Among the common carcinogens, Benzo [A]pyrene is one of the known carcinogens which is directly responsible for the cause of the disease. It is a member of polycyclic aromatic hydrocarbon group and is usually generated during the partial burning of organic matter. Benzo (a) pyrene has been shown to inflict damage on the lungs as well as liver. Thus, the present study has been aimed to study the anticancer activity of *Ocimum sanctum* leaf extract on Benzo [A]pyrene induced lung cancer in rats. Male Charles Foster rats, 6 weeks old weighing around (150-180 g) were used for the study and were induced Benzo[A] pyrene (25 mg/Kg dissolved in Olive oil) orally in two intervals (1st day and 14th day) and were left for 3 months. After 3 months, there were development of lung tumors, which were histopathologically confirmed. Thereafter, *Ocimum sanctum* leaf extract at the dose of 200mg/Kg body weight was administered to the rats for 5 weeks. After the treatment there was significant reduction in the lung tumor size in the studied rats. All the parameters were studied and their data were analyzed. The haematological parameters, the biochemical parameters and the histopathologically parameters were also correlated for the efficacy of the drug.

Through the entire study, it can be concluded that, leaf extract of *Ocimum sanctum* possesses anti-proliferative effect against Benzo [A]pyrene induced lung cancer. This drug after various trials can be recommended as therapeutic drug for lung cancer disease in the future.

Keywords: Benzo[A]pyrene induced lung model; Tumor volume; Biochemical parameters study; Histopathological study; Leaf extract of *Ocimum sanctum*; Anti-proliferative activity; Novel drug discovery

Address for correspondence:

Dr. Arun Kumar,
Mahavir Cancer Sansthan and Research Centre,
Patna, Bihar, India
E-mail: 12018636@zju.edu.cn

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INTRODUCTION

In the Indian state of Bihar, the majority of the population lives in rural areas where farming is the primary economic activity. Numerous rural households rely on cooking over open flames, often by burning wood or cow dung cakes, which produces toxic fumes and poses health risks. In most cases, PAHs are released into the environment as a result of the combustion of organic materials [1] The PAHs are a broad class of chemicals that have a common structural feature: aromatic rings comprised entirely of carbon and hydrogen. The long duration exposure to the PAHs causes irritation in the trachea and lungs, cough for long period, bronchitis, asthma etc [36].

Moreover, this long-term exposure too many different PAHs have been shown to be hazardous, altering genes and increasing the risk of illnesses such lung cancer [2-6]. Asthma, bronchitis, chronic cough, and shortness of breath are just some of the symptoms of modern air pollution, which has also been linked to an increased risk of lung cancer in people of all ages who are exposed to it regularly. Polycyclic Aromatic Hydrocarbons (PAHs), including the highly carcinogenic benzo(a)pyrene, are produced during the incomplete combustion of a wide variety of organic materials. Through cytochrome P450 metabolism, it is converted from 7,8-dihydrodiol-9 to epoxide, which is then thought to cause DNA damage and carcinogenesis [7].

In the *Ayurveda,* the Indian medicine system, there are significant numbers of medicinal plants which have potent effect against the lung disease. The most significant effect of medicinal plant has been documented is *Ocimum sanctum* (Tulsi) leaves. *Ocimum sanctum* leaves has been shown to have a wide range of health benefits, including those of an antimicrobial, mosquito repellent, antioxidant, anti-cataract, anti-inflammatory, chemopreventive, radioprotective, hepato-protective, neuro-protective, cardiovascular, anti-carcinogenic, analgesic, anti-pyretic, anti-diabetic, anti-hypertensive anti-hypercholesterolemia, anti-allergic, immunomodulatory, central nervous system depressant, memory enhancement, anti-asthmatic, anti-tussive, diaphoretic, anti-thyroid, anti-fertility, anti-ulcer, anti-emetic, anti-spasmodic, anti-arthritic, adaptogenic, anti-stress, anti-cataract, anti-leukodermal and anti-coagulant activities[8-10].

Therefore, the present study aims to develop Benzo[a] pyrene induced lung cancer model in Charles Foster rats and assess the efficacy of leaf extract of *Ocimum sanctum* against the lung tumors in the animal model.

MATERIALS AND METHODS

Chemicals and reagents

Benzo[a]pyrene (C20H12) manufactured by Sigma-Aldrich, USA, Lot# SLBV8459, P code: 1002545809 was purchased from the scientific chemical store of Patna, Bihar, India and provided by the Research Department of Mahavir Cancer Sansthan and Research Centre, Patna, India. The other solvents and chemicals used were all 99% analytical grade.

Medicinal plant

Ocimum sanctum, commonly referred to as Tulsi, was utilized in the study as a therapeutic herb. The leaves of *Ocimum sanctum* were obtained from a local garden in Patna and identified and authenticated by a botanist, Prof. Ashok Kumar Ghosh. The leaves were separated, cleaned well, and dried in an incubator at 37^{oC}. The powder was then ground into a fine powder and soaked in absolute ethanol for 48 hours before being extracted with absolute ethanol using a Rota vapour apparatus. After the determination of the LD50 value, the dosage of the ethanolic leaf extract of *Ocimum sanctum* was titrated to 200mg/kg body weight each day for 5 weeks.

Ethical approval

Before using animals, ethical permission was acquired from the institute's Institutional Ethics Committee (IAEC) through CPCSEA (GoI), with CPCSEA Registration number. 1129/bc/07/CPCSEA. The study was authorized by IAEC number. 2021/1B-06/10/21 on October 6, 2021.

Animals

The animal facility at the Mahavir Cancer Sansthan and Research Centre in Patna, India, provided male Charles Foster rats for this study. Two rats were kept in each of the standard polypropylene cages. They were randomly assigned to treatment and control groups. Rats had a constant room temperature of 22± 2 °C, a light/dark cycle of 12 hours, and ad libitum access to food and drink.

Lung tumor model development

To induce lung tumors, rats were administered Benzo[a] pyrene dissolved in olive oil at a concentration of 25 mg/Kg on days 1 and 14 and then left alone for three months. After 3 months, fine needle aspiration cytology study confirmed the presence of lung tumors in the animals, and lung biopsies were performed on a small number of mice provided definitive proof of lung cancer.

Experimental design

Thirty male Charles Foster rats, 6 weeks old weighing (150-180 g) were divided into groups of n=6 animals in each.
Group I- Control (n=6)
Group II- Benzo[a]pyrene treated (n=12)
Group III- *Ocimum sanctum* treated (n=6) - Upon Benzo[a] pyrene induced treated with *Ocimum sanctum* ethanolic leaf extract (200mg/kg body weight per day) for 5 weeks (Group II rats).

Anesthetic drug ketamine was used to induce anesthesia in rats, and then the animals were euthanized after the drug had taken effect. The rats used in the experiments had their orbits punctured to collect blood samples. Serum was drawn for biochemical analysis and lipid peroxidation calculation. Various organ tissues, including lung tissue, were fixed in 10% formalin for histological examination.

Biochemical assay

Serum was analyzed using a (UV - Vis) spectrophotometer (UV-10, Thermo Scientific, USA) to conduct biochemical tests. The Liver function test (LFT) as serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were measured according to the method [42] alkaline phosphatase (ALP) by using method of [29] (1954), total bilirubin activity by method [26] (1938). The kidney function test (KFT) was analyzed through urea by the method of [11] (1960); [12] (1859), creatinine by the method of [13] (1945) and uric acid by the method of [11] (1980).

Lipid peroxidation (LPO)

The double heating method [14] (1990) based on the spectrophotometric measurement of color reproduction during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA) was used to assess thiobarbituric acid reactive substances (TBARS), which are used as markers of LPO. In this experiment, 0.5 milliliters of serum was combined with 2.5 milliliters of Trichloroacetic acid (TCA) solution and cooked in a water bath at 90 degrees Celsius for 15 minutes. After letting the mixture settle to ambient temperature, centrifugation at 3000 rpm for 10 minutes was performed. After heating the supernatant for 15 minutes at 90°C in a water bath, 2 ml of it was combined with 1 ml of 0.675% TBA solution in a test tube. At room temperature, the solution was allowed to cool down. A UV-Visible spectrophotometer (Thermo Scientific UV-10 USA) was used to determine absorbance at 532nm.

Superoxide dismutase (SOD) activity

The Epinephrine Method -SOD activity in the supernatant was determined using the method described by [38] (1972). The technique relies on measuring the rate of epinephrine auto-oxidation inhibition by SOD contained in the examined samples in 50 mM sodium carbonate buffer pH 10.2, within the linear range of auto-oxidative curve and U/mg protein units were used to express the SOD activity.

Catalase (CAT) activity

The method developed by [19] 1991 was used to assess catalase (CAT) activity. The process included incubating serum samples in a substrate containing 65 mol/ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4 for 60s at 37 °C. One CAT unit was converted into one micromole of H_2O_2 every minute under these circumstances. Finally, the enzymatic process was halted using 32.4 mM $(NH_4)2MoO_4$ (Ammonium

molybdate), and the yellow complex of molybdate and hydrogen peroxide was identified at 405 nm, in contrast to a blank containing all the components but the enzyme.

Histopathologically study

For 24 hours, lung tissues were fixed in 10% formalin. The tissues were then dehydrated in a variety of ethanol concentrations before being embedded in paraffin wax. For histological analysis, a 4.5µm section was cut and stained with haematoxylin and eosin for histopathological study under light microscope [15,16] (2014).

Statistical analysis

The data from each group of rats (n = 6) is shown as a mean standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons (p0.05 was regarded statistically significant) were used to examine the total variation reflected in a set of data. We used the statistical software GraphPad Prism (GraphPad 5 Software, Inc, San Diego, USA) to do the statistics.

RESULTS

Morbidity and mortality

Death rates were found to be somewhat elevated in the Benzo[a]pyrene-treated group. At the conclusion of the treatment, however, some moderate restlessness was seen. Every single group had severe shortness of breath, which subsided after receiving *Ocimum sanctum*.

Effect of *Ocimum sanctum* on liver functional test (LFT)

There was a statistically significant (p<0.0001) increase in SGPT, SGOT, ALP, and total bilirubin levels in the Benzo[a]pyrene-treated group compared to the control group. However, blood levels of SGPT, SGOT, ALP, and total bilirubin all decreased following treatment with the hydroxyethanolic leaf extract of *Ocimum sanctum* (p<0.0001). *Ocimum sanctum* leaf extract seems to provide protection against the hepatotoxicity caused by Benzo[a] pyrene, according to the current research **(Tab. 1).**

Effect of *Ocimum sanctum* on kidney functional test (KFT)

There was a substantial (p< 0.0001) increase in the levels of urea, uric acid, creatinine, and albumin in the group that was treated with Benzo[a]pyrene in contrast to the group that served as the control. However, after treatment with the hydroxyethanolic leaf extract of *Ocimum sanctum*, there was a substantial (p <0.0001) reduction in the levels of albumin, urea, and uric acid found in the blood. The present study demonstrates that *Ocimum sanctum* has a protective effect against the nephrotoxicity caused by Benzo[a]pyrene **(Tab. 2).**

Effect of *Ocimum sanctum* on lipid peroxidation (LPO)

In compared to the rats in the control group, the rats that were given Benzo[a]pyrene had blood levels of LPO that were statistically considerably higher (p<0.0001). However, a significant reduction (p< 0.0001) was seen following the administration of the hydroxyethanolic leaf extract of *Ocimum sanctum*. This was in comparison to the control group, which did not see any significant alterations. This indicates that the leaf extract of *Ocimum sanctum* has the potential to serve as an antioxidant(**Fig. 1).**

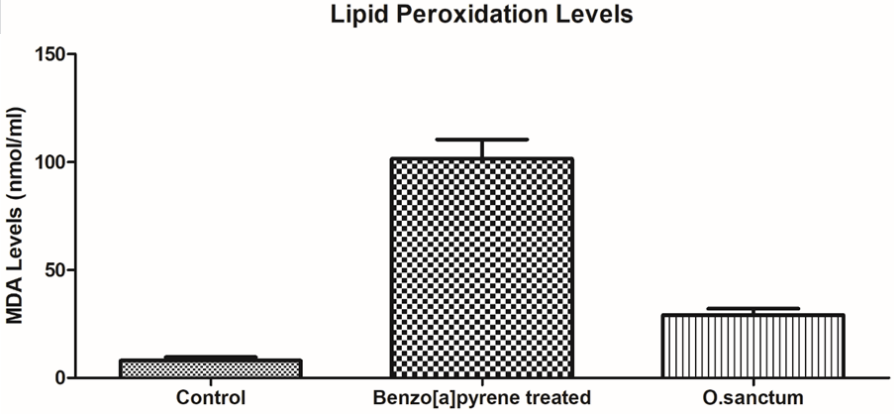
Tab.1. Showing liver function test data.

Parameters	Control	Benzo [a] pyrene Treated	*Ocimum sanctum* Treated for 5 Weeks
SGPT (U/mL)	31.21 ± 1.4	179.23± 5.34*	77.3 ± 2.45**
SGOT (U/mL)	30.23 ± 1.6	235.12± 6.45**	56.34 ± 3.34*
ALP (KA units)	10.43 ± 0.95	28.14± 3.11*	20.5 ± 2.14*
Bilirubin (mg/dL)	0.91 ± 0.22	2.89± 0.6*	1.3 ± 0.43**

Tab.2. Showing kidney function test data.

Parameters	Control	Benzo [a] pyrene Treated	*Ocimum sanctum* Treated for 5 Weeks
Urea (mg/dL)	29.23± 1.97	89.12 ± 2.45**	53.45 ± 2.45**
Uric acid (mg/dL)	4.54 ± 1.02	23.14± 2.10**	7.34 ± 1.35**
Creatinine (mg/dL)	0.88 ± 0.66	4.93± 1.11*	1.13 ± 0.95*

Fig. 1. Showing levels of lipid peroxidation activity in different treatment groups.



Effect of *Ocimum sanctum* on superoxide dismutase (SOD)

In compared to the rats in the control group, the rats that were given Benzo[a]pyrene had a substantial drop in their SOD activity (p< 0.0001). However, a significant rise (p< 0.0001) was seen following the administration of the hydroxyethanolic leaf extract of *Ocimum sanctum*. This was in comparison to the control group, which did not see any significant alterations. This indicates that the leaf extract of *Ocimum sanctum* has the potential to act as an antioxidant (**Fig. 2).**

Effect of *Ocimum sanctum* on catalase (CAT) activity

In compared to the rats in the control group, the rats that were given Benzo[a]pyrene had a CAT activity that was considerably lower (p< 0.0001). However, a significant rise (p< 0.0001) was seen following the administration of the hydroxyethanolic leaf extract of *Ocimum sanctum*. This was in comparison to the control group, which did not see any significant alterations. This indicates that the leaf extract of *Ocimum sanctum* has the potential to act as an antioxidant(**Fig .3).**

Histopathological study

The histological investigation reveals that the tissue of the lung contains alveolar sacs that have a normal morphology. It would suggest that the parietal and visceral layers are not interfering with the lung's regular functioning (**Fig .4A).** In the rats that were treated with Benzo[a]pyrene, there was evidence of lung cells that had papillary tubulo-alveolar carcinoma (**Fig .4B).** There was a considerable reversal in the condition of the lung cells following the treatment with the leaf extract of *Ocimum sanctum*. Despite this, the disease is still present, although in a very minor form (**Fig. 4C).**

DISCUSSION

The most prevalent environmental carcinogen that individuals are routinely exposed to is Benzo (a) pyrene. This chemical is known to cause cancer in humans. Papillary tubulo-alveolar carcinoma was the kind of lung tumor that developed in the current research as a result of the treatment with Benzo[a]pyrene in the tissue. This disease model emerged as the most prevalent one created in the present investigation. In most cases, exposure to benzo[a] pyrene promotes the activation and rise in expression of the histone H3 lysine 9 methyl-transferase. Additionally, it inhibits the action of the tumor-suppressor gene SOCS3,

Fig. 2. Showing levels of superoxide dismutase activity in different treatment groups.

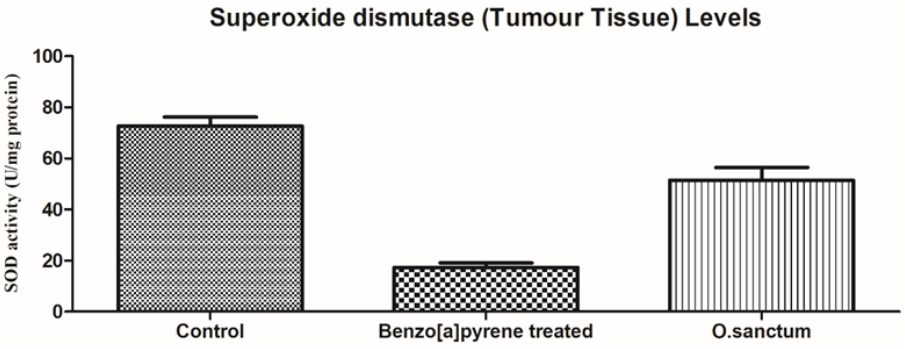


Fig. 3. Showing levels of catalase activity in different treatment groups.

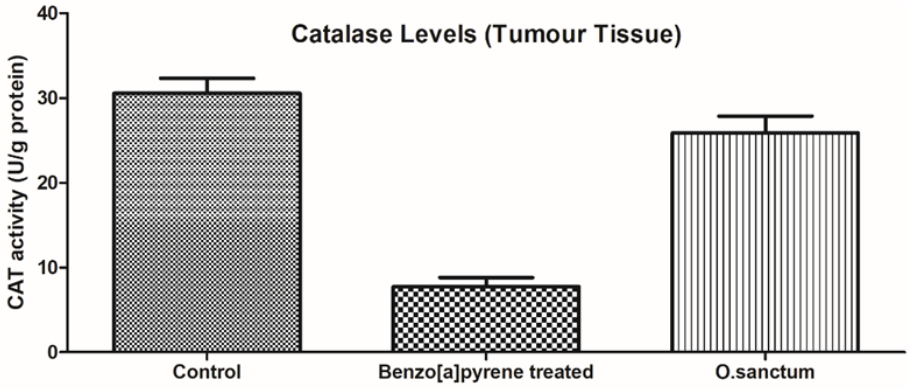
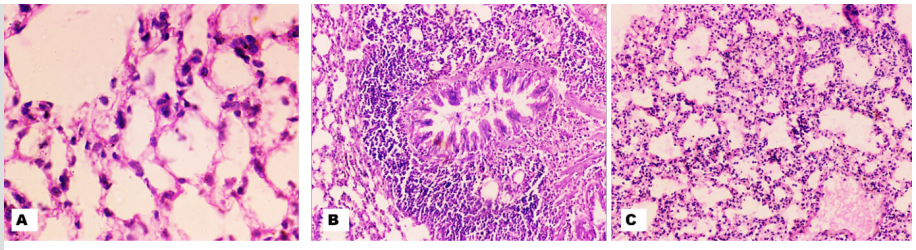


Fig. 4. Showing a microphotograph of lung tissue sections of **(A)** control lung section exhibiting normal architecture of alveolar sacs x 500 **(B)** lung tissue section showing abnormal architecture of alveolar sacs x 500 **(C)** *Ocimum sanctum* leaf extract treatment group showed considerable normalization in the lung tissue with least residual disease x500 in comparison to Benzo (a) pyrene treated group which showed papillary tubulo-alveolar carcinoma x500.



which in turn causes a disruption in the activities of the caspase series and additionally activates the Akt and Erkl/2 pathways, which ultimately results in the development of tumors in the lungs. Researchers from an array of institutions all came up with variations of a similar model [17-24] (2014); [25] (2018); [26] 2008; [27] (2018); [28-30] (2016).

In the current investigation, a lung tumor model that was produced by Benzo[a]pyrene was formed. This model was confirmed histopathologically, as there was a large formation of papillary tubulo-alveolar carcinoma in the lung. However, after treatment with an extract of the leaf of *Ocimum sanctum*, there was an enormous change in the lung cells, as normal alveolar sacs were shown to be present after examination. This finding lends credence to the hypothesis that the leaf extract of *Ocimum sanctum* has anti-carcinogenic properties. It has been discovered that the active component eugenol (1-hydroxy-2-methoxy-4-allylbenzene), which is contained in the leaf extract of the *Ocimum sanctum* plant, is primarily responsible for the medicinal potentials of the Tulsi plant. *Ocimum sanctum* leaf extract has also been shown to have an anticancer impact in a number of animal models. *Ocimum sanctum* and its phytochemicals eugenol, rosmarinic acid, apigenin, myretenal, luteolin, -sitosterol, and carnosic acid have shown in preclinical studies to increase antioxidant activity, alter gene expression, induce apoptosis, and inhibit angiogenesis and metastasis, thereby preventing chemically induced skin, liver, oral, and lung cancers. Orintin and vicenin, two flavanoids found in *Ocimum sanctum*, have been demonstrated to protect normal tissues against radiation's cytotoxic effects on tumors while also protecting mice from the illness and death caused by -radiation. Eugenol, rosmarinic acid, apigenin, and carnosic acid are some of the other key phytochemicals proven to protect DNA from radiation [31]2005; [32-34] 2005; [35-39] 2023; [40] 2023; [41] 2013; [42-43] 2013; [44] 2023; [45] 2020; [46-47] 2009; [48-49] 2020 & 2019; [50] 2023).

Furthermore, in the present study there has been significant decrease ($p<0.05$) in the antioxidant effects in Benzo[a] pyrene treated group in comparison to the control group. There was significant decrease ($p<0.05$) in the superoxide dismutase levels and catalase levels, while significant increase ($p<0.05$) in the lipid peroxidation levels. But after the treatment with the leaf extract of *Ocimum sanctum,* there was significant ($p<0.05$) normalization in the levels of superoxide dismutase, catalases and lipid peroxidation levels. The leaf extract of *Ocimum sanctum* contains Eugenol, Orintin and vicenin, two flavanoids that have been demonstrated to protect normal tissues against radiation's cytotoxic effects on tumors while also protecting mice from the illness and death caused by -radiation. Various authors have correlated their study with the effect of leaf extract of *Ocimum sanctum* [51] 2020; [52] 2012; [53-54] 2005; [55] 2006; [56-58] 2006; [59] 2004.

In the current study, the vital organs such as the liver and kidneys biochemical parameters were evaluated to observe the effect of drug side effects. The results showed a significant (p0.05) increase in the levels of SGPT, SGOT, ALP bilirubin, urea and uric acid and creatinine levels; however, after the administration of the leaf extract of *Ocimum sanctum*, there was a significant ($p<0.05$) normalization in the levels in the liver and although there was a substantial ($p<0.05$) drop in the levels as compared to the Benzo[a] pyrene treated group, the levels were still significantly higher than the usual limits. Because the liver and kidneys are metabolic organs, the anti-inflammatory activity in those organs may be delayed as a result of the significant damage induced by Benzo[a]pyrene. It is possible that this is the reason why the problem has arisen. However, the considerable decline in liver and kidney functions may be attributed to the active component eugenol and quercetol, which has been shown to have a protective effect on both organs (hepato-renal protective effect). The most important impact that the leaf extract of *Ocimum sanctum* has a regeneration that is modest. *Ocimum sanctum* has been shown to have a protective effect on both the liver and the kidneys by a number of different studies.

CONCLUSION

It is possible to draw the following conclusion from the complete research study that Benzo[a]pyrene is responsible for the development of lung tumors in Charles Foster rats; however, the leaf extract of *Ocimum sanctum* was able to significantly reduce the severity of the lung cancer disease. In addition to this, there was a notable restoration to normalcy in the levels of free radicals, including lipid peroxidation, superoxide dismutase activity, and catalase activity. However, in the biochemical parameters of liver function tests and kidney function tests that were analyzed, there was a considerable normalization in the levels of ALP, urea, and uric acid, but there was only a minor reduction in the levels of SGPT, SGOT, bilirubin, and creatinine levels. This was seen in both the liver and the kidneys. In the end, the researchers came to the conclusion that the leaf extract of *Ocimum sanctum* had anticancer, antioxidant, and a modest amount of hepato-renal protective action. In addition, the length of time that the leaf extract of *Ocimum sanctum* was taken might have provided better protection against the liver and renal organs. As a result, this medication has therapeutic anticancer properties, particularly with regard to lung cancer.

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COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

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