

**Original Research Paper****PHARMACOLOGICAL EVALUATION OF INFLUENCE OF ORTHOSIPHON THYMIFLORUS ON BENZOPYRENE INDUCED MOUSE FORESTOMACH TUMEROGENESIS****Zainab Fatima*, A.Venkateshwar Reddy**

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ABSTRACT

The modulating effect of plant extract on benzo(a)pyrene (BP)-induced forestomach tumours was investigated in male Swiss albino mice, receiving oral administration of BP at a dose of 1 mg twice weekly for 4 weeks. Plant extract inhibited both BP-induced forestomach tumour incidence and multiplicity. The present data may indicate the potential of Plant extract as a powerful chemo preventive agent against BP-induced forestomach tumours in mice. The possible modes of action of *Orthosiphon thymiflorus* may be through its antioxidant and anti-inflammatory activities, coupled with enhancement of detoxification activity.

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**1. INTRODUCTION**

It is common knowledge that cancer mostly leads to fatal results, regardless of age and sex. At the same time, many cancer-related types of research and studies indicate that there have been certain improvements in the cancer treatment for more than two decades. Still, even though there was an apparent progress in the studies, there are a few aspects that are unknown and not clarified.^[1]

Traditional medicine uses natural products based on plants (herbs) as it is known such products have positive healing effects on the human health system. To carry out such treatment an entire plant, including leaves, fruit, stem and roots, was used.^[1]

Many current researches in the field of cancer therapeutics is dedicated to innovate and develop medicine and/or vaccine for combating the growth of tumour cells. As a matter of fact, a lot of scientific studies provide sufficient evidence that natural products, such as fruit, vegetables, teas, spices, and traditional medicinal herbs, have strong anti-inflammatory, antioxidant or anticancer properties. In addition, a range of epidemiological and animal studies show distinctively that healthy diet full of fruit, vegetables, cereal grains, and spices, reduces the risk of cancer and cancer-related symptoms.^[2]

Ongoing evaluations demonstrate that gastric disease is the fourth most incessant sort of malignant growth and the second most common reason for disease worldwide. Benzopyrene [BP], a powerful genius cancer-causing agent utilized in starting stomach malignancy, is the prototypical and best described individual from PAHs group of synthetic carcinogens. It has been appeared to instigate tumors of skin, lung, mammary organs and forestomach tissues of different test animals.

The Main Objectives of the present Study is to evaluate Influence of *Orthosiphon thymiflorus* on benzopyrene [BP] induced mouse forestomach tumorigenesis.^[2]

2.MATERIAL AND METHODS

The leaves of *Orthosiphon thymiflorus* was collected from Kurumalai, Thuthukudi District in the month of December 2018. The leaves were authenticated by Prof. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy and Research centre, Chennai 600045.

Extraction of the Plant Material

The plant powder was subjected to extraction with organic solvents. The plant was subjected to successive extraction with n-hexane followed by chloroform, ethyl acetate and alcohol using cold percolation technique.

The plant powder was loosely packed in an aspirator bottle and was immersed in sufficient quantity of n-hexane and left to stand for 3 days. The solvent was then filtered and distilled to yield a gummy mass which was then evaporated to dryness. To the same plant powder chloroform was added, allowed to stand filtered. This was followed with ethyl acetate and alcohol. The plant was soaked in each solvent for 3 days. The filtrates were then filtered and distilled to obtain the respective extracts.^[4]

All the four extracts (n-hexane, chloroform, ethyl acetate, alcohol) of the whole plant of *Orthosiphon thymiflorus* were subjected to phytochemical analysis.^[4]

3. EXPERIMENTAL SETUP

Animals

Male Swiss albino mice (7-8 weeks old) were used for the present study. They were maintained in the air-conditioned animal facility with a 12-h light /dark cycle, and provided (unless otherwise stated) with standard food pellets and tap water ad libitum. Throughout the feeding protocol of test diet or standard feed, animals were observed for food and water consumption or for signs of any toxicity. The experimental protocol was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and was maintained with strict adherence to the guidelines of institutional Animal Ethic Committee (IAEC).^[6]

Benzopyrene (carcinogen) was dissolved in peanut oil and the concentration adjusted to 1mg P/O.1 ml of peanut oil. Benzopyrene was administered to the animals through oral gavage route.^[5]

Experimental Design

For each modulator independent experiments were planned to assess the cancer chemopreventive potentials. The experiment was carried out by the method described by Azuine and Wattenberg et al (1980). The animals were assorted in the following groups

Group-I: Animals were kept on a normal diet for two weeks. After that each mouse received 1 mg twice weekly for four weeks. This group of animals served a positive control group.

Group-II : Animals were kept on 2.5% diet of *Orthosiphon thymiflorus* starting two weeks before, during and two weeks after the carcinogen treatment (1 mg twice weekly for four weeks) as given to Group-I animals.

Group-III: Animals was kept on 5% diet of *Orthosiphon thymiflorus* starting two weeks before, during and after the carcinogen treatment (1 mg BP twice weekly for four weeks) as given to Group-II animals.

Group-IV: Animals was kept on 5% diet of *Orthosiphon thymiflorus* throughout the experimental duration and served as negative control group.^[6]

Histopathological Preparation

At the end of experiments, the forestomachs from the normal and carcinogen treated animals were removed, and fixed in 10% formalin. Following a standard technique, the tissue was embedded in paraffin wax and sections 4-5 micron thick was stained in Ehrlich Hematoxylin and Eosin for histopathological study. The neoplastic nature of the tumor was confirmed with the help of Histopathologists.^[7]

Data analysis: All the values are represented as Mean $\pm$ SEM from 8 animals per group. Statistical differences of the mean were analyzed using one-way analysis of variance (ANOVA) followed by turkey's multiple comparison test using graph pad prism 5.0. P value < 0.05 was considered as statistically significant (*, ** indicates p < 0.05 and 0.01, respectively vs. control). The results are represented as Mean + SEM from 6 animals per group.

4.RESULTS AND DISCUSSION

Phenol and coumarin were found to be present in chloroform, ethyl acetate and alcohol extracts. Coumarin is known for its pharmacological properties such as antiinflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, and neuroprotective properties. Flavonoids, alkaloids and sugars were present in ethyl acetate and alcohol extracts.

TABLE 1: Effect of Plant extract on Benzo(a)pyrene Induced Forestomach Tumorigenesis in Swiss Albino Mice

Groups	Body Weight (gms)		Total tumour incidence (%)	Tumor Burden	Inhibition of tumour burden (%)
	Initial	Final			
Group- I	27.4 $\pm$ 3.2	32.0 $\pm$ 3.5	100	7.182 $\pm$ 1.3	0.00
Group- II	29.9 $\pm$ 3.1	34.0 $\pm$ 3.5	81.0	1.182 $\pm$ 0.5	83.5
Group- III	30.6 $\pm$ 1.0	35.0 $\pm$ 1.9	63.6	0.818 $\pm$ 0.7	88.6
Group- IV	31.6 $\pm$ 2.0	36.7 $\pm$ 2.3	0.00	0.00	0.00

Values are expressed as the mean $\pm$ S.D; Statistical significance' p' value are calculated by one way ANOVA followed by Dunnett's test.

Tumor Burden

The mean number of papilloma in mouse (tumor burden) in the control group of animals was 7.182 $\pm$ 1.3 In contrast, animals treated with 2.5% and 5% diet of *Orthosiphon thymiflorus*, tumor burden reduced to 1.182 $\pm$ 0.7 (p<0.01) respectively. The percentage inhibition of tumor multiplication was 83.54 and 88.61 by the low and high doses of *Orthosiphon thymiflorus* treatment respectively.^[9]

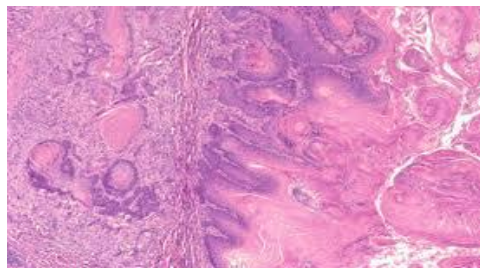
Tumor Incidences

All of the control animals developed forestomach papilloma's by treatment. Relative to control tumor incidences were 81.8% and 63.6% in case of 2.5% and 5% dose of *Orthosiphon thymiflorus* respectively. Total tumor incidence decreased was 18.1 % with the low dose and 36.3% with the high dose of *Orthosiphon thymiflorus*. 5% diet of *Orthosiphon*

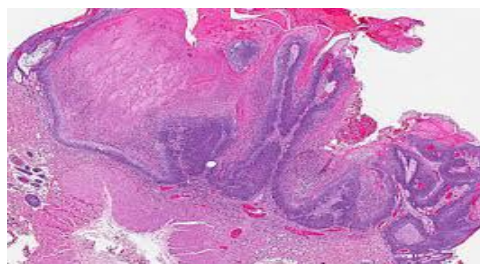
thymiflorus treatment (negative control group) did not result in any tumor.^[9]

Histopathological of stomach

Group –I :



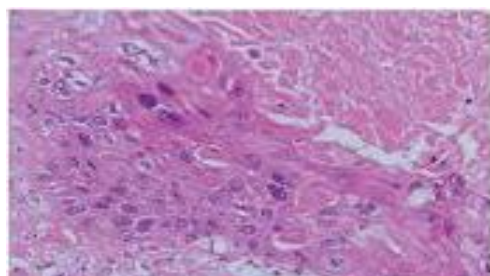
Group –II :



Group–III:



Group –IV :



Based on the research findings, it was proved that with application of these extracts at low concentrations during the 24-hour exposure time it became possible to suppress cell viability, so, stopping these malignant cells and cell lines from growth and spreading. So, it opened the great new opportunity to treat cancer and other associated diseases. With reference to the paper findings, it is indicated that particularly the *Orthosiphon thymiflorus* extract has shown the high inhibitory impact on stomach cancer.

In addition, we are inclined to believe that further research should be carried out based on these paper findings in order to acquire the sufficient evidence that, first of all, the extracts of these medicinal plants will not have the adverse impact on healthy cells and the general human condition, and, secondly, it will effectively suppress and create the inhibitory effect on cancer.^[10]

5. CONCLUSION

The present research was set out to explore and analyse the effect medicinal plants, namely *Orthosiphon thymiflorus* in the form of extracts. In this aspect it was assumed that the properties medicinal plants obtain might help in reducing the activity of damaged or bad cells, particularly cancer cells. In fact, it was concluded that *Orthosiphon thymiflorus* extracts have anti-cancer effects on cells and their growth and development. At the same time, these extracts were noticed to show the high rates of inhibition when exposed to the chosen samples of a few different cancer cell lines. In general, many researches and studies have been conducted to find much more favourable and caring treatment means of cancer and tumour cells, than chemotherapy and other immune-suppressing therapies. It is considered that these paper findings might provide the sufficient evidence that the medicinal plants studied have the great potential to suppress cell viability, especially those cells that present reasons for cancerous diseases. In such a way, it may become possible to find proper solutions to treatment of cancer and other associated diseases.

6. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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