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in-vivo Chemopreventive Study of designed Herbomineral Tablet in Tobacco Smoke induced Lung Cancer Animal Model

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Abstract: Cancer is a major killer throughout human history. Lung cancer has lion's share in cancer statistics. Consumption of cigarettes dramatically increased worldwide. Total elimination of sale and use of tobacco products or making the smokers to quit such a habit is practically not possible. The idea of present work is to develop a herbo-mineral formulation for chemoprevention of lung cancer in tobacco severe adductors. Conventional oral tablets (each tablet wt. 400mg), containing Green tea aqueous extract and Sodium selenite (200mg and 1mg per tablet respectively) were prepared by direct tableting method, using Micro Crystalline Cellulose as a directly compressible binder. All the prepared tablets were evaluated for weight variation, hardness, friability and disintegration. Chemopreventive activity of the prepared tablets was carried out in tobacco induced lung cancer animal model. Male albino mice were selected for the study. All the mice were fed with standard diet with or without Green tea, Sodium selenite tablets (GST) before, during & after the carcinogenesis made by tobacco smoke. First 4 months mice were exposed to filtered air, followed by tobacco smoke for 3 months (6h/day, 5 days/week). Finally, the animals were recovered for another 2 months in filtered air. Mice received GST, showed five times lower tumor incidences and multiplicity. The obtained results allow to concluding that, our prepared GST effective against the initiation and progression of lung cancer, induced by tobacco smoke in albino mice.

Key Words: Tobacco, Green Tea, Sodium Selenite, Chemoprevention, Lung Cancer, Mice.

INTRODUCTION

Cancer is a global challenge and it considered being the most dreadful of all diseases existing in the world as it is fatal. Cancer statistics lifted eyebrows across the world. Estimated cancer cases in India is eight lacks per year. One in every 15 Men & 12 Women develops cancer during their life time.[1]

Tobacco smoke induced lung cancer constitutes a major health problem and it constitutes major part of cancer statistics. Smoking of cigarettes became affordable with the invention of machines that could produce cigarettes in large quantities. Consumption of cigarettes increased dramatically during and after World War I. Clinicians began to suspect a link between cigarette smoking and the disease of lung cancer in the late 1920s.[2]

"Cancer doesn't begin with the appearance of a tumor; by the time a tumor has formed, the processes that lead to cancer have been developing for years, often for decades. The idea behind chemoprevention is to interrupt the process before it is too firmly entrenched".[3]

The term 'Chemoprevention is coined in the mid 1970s by Michael Sporn,[4] Later in 1981, the National Cancer Institute established a division of cancer prevention. Chemoprevention can be defined as the use of natural or synthetic compounds to prevent, suppress or delay the development of invasive carcinoma. Chemoprevention offers a promising

approach to primary cancer prevention for a variety of organ systems. Phytochemicals due to low toxicity, relative safety and minerals due to their high efficacy at low doses, are promising potential chemopreventive agents. These agents after emerging successful through a series of invitro and invivo assays enter clinical trials.[5]

Stopping the sale of tobacco or pulling the smokers from the habit is practically not possible. Chemopreventive approaches with phyto chemicals and minerals designed to prepare a formulation that prevents the formation of lung tumors in smokers have been the interest of this work. Among all herbals, Green Tea (*Camellia Sinensis*), shows affordable protection against most types of cancers like lung, liver, esophagus, forestomach, duodenum, pancreas, colon, and breast.[6] Green tea is native to China South and Southeast Asia, but it is today cultivated across the world in tropical and subtropical regions. It is an evergreen shrub or small tree belong to family *Theaceae*. Among the mineral kingdom, Sodium selenite is safe, have potential chemopreventive and anticancer effect.[7]

Possible target populations for use of chemopreventive agents might include subjects unable to quit because of severe addiction, smokers who have been smoking for less than 10 years and former smokers that have already quit and might benefit from a further reduction in risk. In addition, chemoprevention might be used to prevent the recurrence of second primary tumors in head and neck and lung cancer patients following successful treatment of the first tumor. Spouses and children of heavy smokers involuntarily exposed to environmental tobacco smoke at work are at increased risk and might be additional beneficiaries. [2]

OBJECTIVE

The objective of the study was to obtain oral solid dosage form, an uncoated conventional tablet, from dry aqueous extract of Green tea leaves and Sodium Selenite using suitable adjuvants and to investigate their chemopreventive effect in the tobacco smoke induced lung cancer animal model.

MATERIALS AND METHODOLOGY

Sodium Selenite was obtained as a gift sample from Seeco Biotech Pvt. Ltd., Guntur, Andhra Pradesh. Microcrystalline cellulose, lactose, talc powder and magnesium sterate were purchased from Rajesh Chemicals Pvt. Ltd. Mumbai. All the chemicals and reagents used in the study were analytical grade. Healthy albino male mice were purchased from Natural Remedies Pvt. Ltd., Bangalore, Karnataka. Standard diet for the mice was procured from Food Products Division, Hindustan Liver Ltd.,

PREPARATION OF GREEN TEA AQUEOUS EXTRACT

Green tea leaves were collected in the season from ooty, Tamilnadu, and were authenticated by Dr. C. Madhava Chetty, Department of Botany, S.V. University, Tirupathi, Andhra Pradesh. Leaves were cleaned and shade dried completely. 100g of leaves were boiled with 1 liter of distilled water for 10 min at 70 C. The heated solution was filtered and marc was freeze dried. Resulted green, dry mass was used to prepare the tablet.

PREPARATION AND EVALUATION OF GREEN TEA AND SODIUM SELENITE TABLETS (GST)

Direct tableting is simpler, cost effective and is preferable even from the point of view of good manufacturing practice than wet granulation or dry compacting. Micro Crystalline Cellulose (MCC) was selected due to its direct compressible binding, disintegrating and improving liquidity properties. The tablets were manufactured in a nine station rotary punching machine (Chamunda Pharma Machinary Pvt. Ltd., Ahmadabad. Model PP-1, Machine No. 101/81) equipped with cancave12 mm punches. The list of tablet contents was showed in Table No. 1. The components of tablet mass were weighed, mixed thoroughly and tableted.[8] The prepared tablets were subjected to weight variation, hardness (Monsanto type, Singhala Scientific Industry), friability (Friabilator, Singhala Scientific Industry) and disintegration time (Disintegration Tester USP, Electro Lab, Model ED 21).[9]

EXPERIMENTAL PROTOCOL

Even though, there is a number of methods to induce the lung tumors in mice, An animal model which mimic or duplicate the human experience in experimental animals was selected for theis study. A protocol made by Hanspeter Witschi et.al. was slightly modified and implemented.[10] Healthy, male albino mice, 5 weeks old, were purchased from Natural Remedies Pvt. Ltd. Bangalore. The mice were housed in polypropylene cages with tight fitting wire screen lids on conventional bedding material. After an acclimatization period, the mice were divided into three groups. Diet and exposure system of each group is showed in table No.2. All groups provided standard diet (Hindustan Liver Ltd.,) and water *ad libtum* throughout study, where as Group-III receives GST additionally. The tablet has grinded into powder; 10mg of powder was mixed with enough quantity of water to make solution, and placing through oral route by gavage. Group-I animals exposed only to filtered air for total test period. Group-II & III were exposed to filtered air (first 4 months), tobacco smoke (next 3

months) and followed by recovery in filtered air (2 months). Since the formulation is used to prevent the cancer, animals have to feed long before the carcinogenesis. Study conditions include temperature at 20-21°C, humidity at 40-70%, 12h light-dark cycle system. The mice were monitored daily and weighed weekly. All experimental protocols had been reviewed and approved by the Committee For the Purpose of Control & Supervision of Experiments on Animals (CPCSEA), New Delhi. Approval No. 1220/a/08/CPCSEA/ANCP/02.

Table No. 1. Composition of Each Tablet

S. No.	Ingredient	Use	Quantity per Tablet (mg)
1	Green Tea Aqueous Extract	Chemopreventive agent	200
2	Sodium Selenite	Chemopreventive agent	001
3	Lactose	Filler	085
4	Micro Crystalline Cellulose	Direct compressible binding, disintegrating and improving liquidity properties	012
5	Talc Powder	Glidant	001
6	Magnesium Stearate	Lubricant	001
Total Tablet Weight			400

Table No. 2 Group, Diet and Exposure details

Group No. (Each group contains 12 mice)	Diet	Exposure
I	Standard Diet	Only filtered air throughout study
II	Standard Diet	First 4 months to filtered air, next 3 months to tobacco smoke, Last 2 months in filtered air. (6h/day, 5d/week)
III	Standard Diet + 10 mg GST/mice/day	

Figure No.1 Showing the complete details of tobacco smoke generated apparatus.

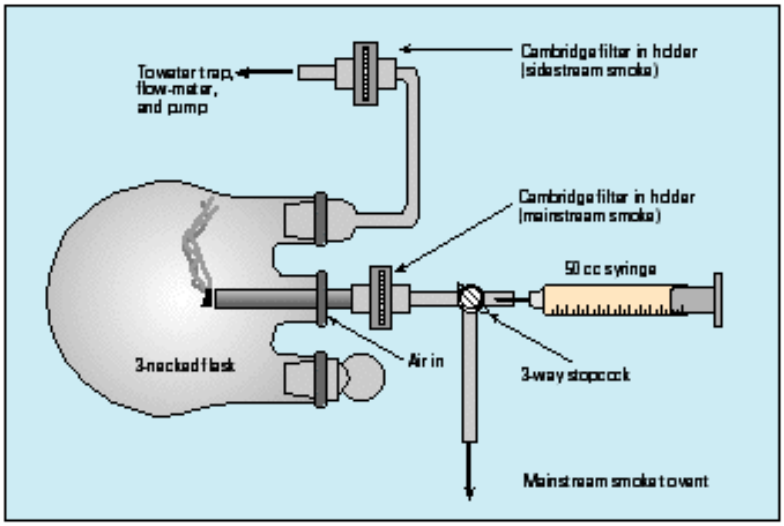

Figure No. 2 Showing the Laboratory photograph of burning cigarette in the chamber.

EXPOSURE SYSTEM

The detailed tobacco smoke exposure system was showed in Figure No.1. Briefly, mice were exposed to a mixture of 89% sidestream and 11% mainstream smoke generated from burning Gold Flake King reference cigarettes (purchased from Indian Tobacco Company, ITC). Sidestream smoke is smoke which goes into the air directly from a burning cigarette, where as mainstream smoke is smoke which directly inhaled by the smoker from the cigarette. Sidestream smoke contributes to Environmental Tobacco Smoke (ETS), and more harmful than mainstream smoke. Sidestream smoking has been classified as a Class A carcinogen by the U.S. Environmental Protection Agency. Chamber atmospheres were monitored for standard conditions. During the time of carcinogenesis, Group-II & III animal cages were placed in stainless steel inhalation chambers and exposed to 6 hours per day, 5 days per week for a period of 3 months.[10]

TISSUE PREPARATION

At the end of study, all animals were killed by pentobarbital overdose. For analysis of tumor incidence and multiplicity, the lungs were expanded to inspiratory volume and fixed for at least 24 hours. The number of tumor nodules on the lung surface was counted through projection microscope.[2]

RESULTS AND DISCUSSION

EVALUATION OF TABLETS

The tablet parameters observed are given in Table No. 3. The tablets were compressed at the specified weight 400mg. The maximum weight variation obtained was ± 1.02%, which falls within the acceptable weight variation range of ± 5%. Hence all the tablets passed the weight variation test. Hardness for tablets was in the range of 4.0 to 4.2 kg/cm^2, which falls above the limit of not less than 3.0 kg/cm^2. None of the tablets showed friability value more than 0.87% which is less than ideal limit 1%. The tablets were passed disintegration time also by showing 13 minutes 35 seconds, which is less than ideal limit 15 minutes.

LUNG TUMOR DEVELOPMENT IN MICE

The mice tolerated the tobacco smoke exposure well, and no exposure related deaths were observed. Tobacco smoke exposed mice showed a little slower weight gain during smoke exposure period. However, there is no significant difference in weight loss/gain between tobacco smoke exposed mice and air exposed mice. We examined the possibility of increasing lung tumor incidences and multiplicities in all the mice. The number of nodules visible on the lung surface was counted and the results expressed as tumor incidence (i.e., percentage of mice with one or several lung tumors) and tumor multiplicity (the average number of tumors per lung, including non tumor bearing animals). All numerical data were calculated as mean ± Standard Deviation (SD). Details of Total Suspended Particles, (TSP), Comparisons of tumor incidence and multiplicity between tobacco smoke exposed mice and air exposed group are made in Table No. 4. It was observed that Group-III mice showed 5 times less susceptible to carcinogen induced lung tumor formation than Group-II mice, where as Group-I mice does not shows any incidence of lung tumors. The data were statistically analyzed by one way ANOVA, and in all the analysis, statistical significance is claimed at $P < 0.05$.

Table No. 3. Evaluation of Tablets

Parameter	Obtained Results	Standard limits (I.P)
Weight Variation	400mg ± 1.02%	Within ± 5%
Hardness	4.0 to 4.2 kg/cm^2	Not less than 3.0 kg/cm^2
Friability	0.87%	Not less than 1%
Disintegration time	13 Min, 35 seconds	Not more than 15 Min.

Table No. 4. Evaluation of Chemopreventive Activity

Group No.	TSP (mg/m^3)[a]	Lung tumor Incidence[b]	Lung tumor multiplicity[c]
I	130 X 3 = 390	0/12 (0%)	0.00 ± 0
II		12/12 (100%)	35.3 ± 1.4 (12)
III		03/12 (25%)	06.0 ± 0.1 (02)

[a] Number in parentheses, Total Suspended Particles (TSP)
TSP = Concentration of Tobacco Smoke X duration of smoke exposure in months
[b] Number of tumor bearing animals per total number of animals in group (Percentage)
[c] Mean ± SD (Number of Animals)

CONCLUSION

The applied adjuvant substances in applied proportions appeared to be useful in the process of direct tableting of dried aqueous extract of Green tea and mineral Sodium selenite. The prepared tablets meet the pharmacopoeial requirements and are more comfortable in use. The obtained results point to the possibility of prevention of lung cancer in tobacco smoke induced mice.

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