ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In-vivo chemopreventive study of designed herbomineral tablet in ethanol induced liver cancer animal model

AV Badari Nath*, C Madhusudhana Chetty

Department of Pharmaceutics, Annamacharya College of Pharmacy, New boyanapalli, Rajampet, Kadapa District, Andhra Pradesh.

ABSTRACT

Cancer is a global challenge. Cancer statistics lifted eye brows across the world and liver cancer occupies its major share. Consumption of alcohol spectacularly increased worldwide. Total abolition of sale and exploit of alcohol products or making the adductors to leave such a habit is practically not promising. The idea of present work is to develop a herbo-mineral formulation for chemoprevention of liver cancer in alcohol rigorous adductors. Conventional oral tablets (each tablet wt. 400mg), containing Green tea aqueous extract and Sodium selenite (GST) (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 ethanol induced liver cancer animal model. Male albino mice were selected for the study. All the mice were fed with standard diet throughout the 17 weeks of study period. Initial 12 weeks animals were fed with designed GST, followed by induction of tumors by ethanol in liver for next 4 weeks. The mice were scarified; liver tissues are isolated and plaid for tumor incidence and multiplicity. GST received mice, showed 91.7% and 67% reduction in tumor incidences and tumor multiplicity respectively. The obtained results allow to concluding that, our prepared GST is effective against the initiation and progression of liver cancer, induced by ethanol in albino mice.

Keywords: Ethanol, Green Tea, Sodium Selenite, Chemoprevention, Liver Cancer, Mice

Corresponding author
Email: avbadrinatha@rediffmail.com

ISSN: 0975-8585

INTRODUCTION

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

Primary liver cancer remains one of the most lethal malignancies worldwide. The American Cancer Society has estimated that, in 2007, there were over 700 000 new cases of primary liver cancer worldwide. It is the fifth most common malignancy in men and the eighth in women. Liver cancer is among the most lethal cancers (five-year survival rates under 11%), which makes it the third most frequent cause of cancer death in men and the sixth in women [2].

Liver cancer consists of several histologically different primary hepatic malignancies, such as cholangiocarcinoma, hepatoblastoma and haemangiosarcoma, but hepatocellular carcinoma (HCC) is by far the most common type, accounting for 70%-85% of cases [2,3].

Cirrhosis (due to for instance hemochromatosis), chronic hepatitis B and C viral infections, chronic alcohol consumption, aflatoxin-B1 intake (from contaminated food) are the most important of the well-defined risk factors for HCC. Variations in the prevalence of these etiological factors mirror the geographical distribution of the incidence of HCC. The worldwide (age-adjusted) incidence per 100 000 persons is 14.9/5.5 (men/women), varying from 2.6/1.3 in Northern Europe to 35.4/12.6 in East Asia [3,4].

Animal models for human HCC can be helpful to our understanding of the (molecular) mechanisms underlying the pathogenesis of HCC. The laboratory mouse remains one of the best models to study cancer *in vivo* due to various features, such as the small size, the similarities to humans and the entirely sequenced genome and the similarities to humans [5].

“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” [6].

The term ‘Chemoprevention is coined in the mid 1970s by Michael Sporn [7], 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 [8].

Stopping the sale of alcohol or pulling the addictors from the habit is sensibly not achievable. Chemopreventive approaches with phyto chemicals and minerals designed to

prepare a formulation that prevents the formation of liver tumors in alcoholic consumers have been the attention 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 [9] 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 [10].

Possible target populations for use of chemopreventive agents might include subjects unable to quit drinking alcohol because of severe addiction, alcoholics who have been taking for less than 10 years and former drinkers 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 liver [11].

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 ethanol induced liver 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 Machinery 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 [12]. 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) [13].

Experimental protocol

Even though, there are a number of methods to induce the liver tumors in mice, an animal model which mimic or duplicate the human experience in experimental animals was selected for theis study. For instance, the risk of HCC in males is approximately 2-5 times greater than in females. This gender difference is seen in mice as well [14]. Hence, an male mice were selected for this study.

A protocol made by Ina Bergheim et al. was slightly modified and implemented [15]. Healthy, male albino mice, 8 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. Mice were randomized into 4 groups as showed in Table No. 2. They were allowed to adopt the environment for 1 week. All groups provided standard diet (Hindustan Liver Ltd.,) and water *ad libtum* throughout study period (1week adaptation period + 16 weeks test period). Since the study related to chemoprevention, mice should receive GST long before the carcinogenesis. Hence first 12 weeks of test period, mice provided with only GST and allowed to develop a resistance to initiation of cancer. Later from 13^{th} week to 16^{th} week, liver tumors were induced by giving ethanol. 10mg of GST was given to Group C and D mice. The tablet has made into powder; 10mg of powder was mixed with enough quantity of water to make solution, and placing through oral route by gavage. Group B & D received ethanol as carcinogen, where as equivalent amount of saline was received by group A & C [15].

Surgical implantation of the intra gastric cannula and enteral feeding was performed. Either ethanol or saline was fed for 4 weeks. The initial rate of ethanol delivery (16g/kg/day) was increased in stepwise manner (1g/kg every 2 days) until the end of the 1^{st} week, followed by 1g/kg increment for every 4 days until the end of experiment [15]. 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. Our approval number is 1220/a/08/CPCSEA/ANCP/02.

Study of liver tumor incidence and multiplicity

At the end of test period, all the mice were killed by decapitation. The mice were dissected to isolate the liver. The body weight and liver weight were measured and hepatic tumors on the surface were examined by glucose-6-phosphatase (G6P) biochemical staining technique. The principle of this staining technique includes, normal tissues of liver will stain strongly, and Hepato Cellular Carcinoma (HCC), tissues staine very weakly.

Slides of cryostat sections were incubated in a buffer containing 0.05% G6P and 0.18% lead nitrate, pH 6.7, for 5-10 minutes at 37°C. The slides were then rinsed with distilled water and placed in 0.5% yellow ammonium sulfide for 2 minutes, rinsed again with distilled water and finally mounted. Cells with enzyme activity stained very dark [16]. (color of the precipitated lead sulfide).

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.

Liver tumor development in mice

The mice tolerated the ethanol intake well, and no ethanol consuming deaths were observed. Ethanol consuming mice showed a little slower weight gain during period of carcinogenesis. However, there is no significant difference in weight loss/gain between alcohol consuming mice and non-consuming mice.

The effects of GST on body weight, liver weight, liver tumor incidence and liver tumor multiplicity are showed in Table No.4. The body weights were not affected by GST (compared group A with C & group B with D). The difference between normal liver tissue and tumor incidence liver tissue is showed in Figure No.1. Group A & C does not showed even single tumor incidence and multiplicity. However, both were observed in Group B & D. Group B shows 100% tumor incidences. The prominent effect of GST was calculated (by the formula showed in Figure No. 2) as 91.7% reduction in tumor incidences and 67% reduction in tumor multiplicity.

The data were statistically analyzed by one way ANOVA, and in all the analysis, statistical significance is claimed at $P < 0.05$.

ISSN: 0975-8585

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 liver cancer in ethanol induced mice.

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	Diet	GST	Ethanol/Saline
A	Standard Diet	-	Saline (No Carcinogen)
B		-	Ethanol (Carcinogen)
C		+	Saline (No Carcinogen)
D		+	Ethanol (Carcinogen)

ISSN: 0975-8585

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 Details of Tumor Incidence and Multiplicity

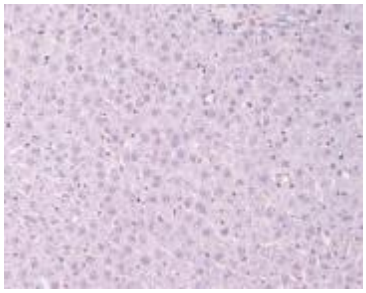
Group	Body Wt	Liver Wt	Tumor Incidence [a]	Tumor Multiplicity [b]
A	27.62±2.09	1.32±0.22	0/12 (0%)	0.00 ± 0 (0)
B	30.52±1.14	1.83±0.12	12/12 (100%)	4.27±1.07 (12)
C	28.42±1.09	1.30±0.02	0/12 (0%)	0.00 ± 0 (0)
D	31.02±1.22	1.79±0.33	1/12 (8.3%)	1.02±0.02 (1)

[a] Number of tumor bearing animals per total number of animals in group (Percentage)

[b] Mean ± SD (Number of Animals)

Figure No.1 Showing the different between and normal liver tissue tumor incidence liver tissue.

Normal Liver Tissue



ISSN: 0975-8585

Tumor incidence liver tissue

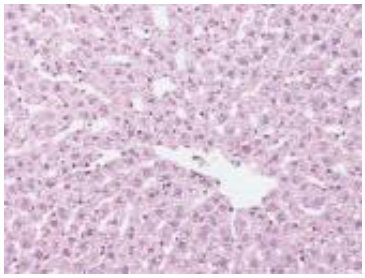


Figure No. 2 Showing the formula to calculate the liver tumor multiplicity and incidence

$$\text{Prominent effect of GST in liver tumor multiplicity} = 100 - \left(\frac{\text{Tumor Multiplicity in mice receives carcinogen and CST}}{\text{Tumor Multiplicity in mice receives carcinogen alone but not CST}} X100 \right)$$

$$\text{Prominent effect of GST in liver tumor incidence} = 100 - \left(\frac{\text{Tumor incidence in mice receives carcinogen and CST}}{\text{Tumor incidence in mice receives carcinogen alone but not CST}} X100 \right)$$

REFERENCES

[1] Raghavendra Babu.V D, Cancer connections, Heritage Amruth, 2007, 5-8.
[2] American Cancer Society. Global Cancer Facts & Figures 2007. Available from: URL: http://www cancer org.
[3] Farazi P A, DePinho R A. Nat Rev Cancer 2006; 6: 674-687.
[4] Llovet J M, Burroughs A, Bruix J. Lancet 2003; 362: 1907-1917
[5] Frese K K, Tuveson D A. Nat Rev Cancer 2007; 7: 645-658
[6] Dianne C Witter. Oncolog 2007: 52-60.
[7] Young Joon Surh. www.nature.com/reviews/cancer; 2003, 3, 768-780.

[8] Rachana Patel, Rachana Garg, Suvarna Erande, Girish B. Maru. J Cli Biochem Nutr 2007; 40:82-91.
[9] Hasan Mukhtar and Nihal Ahmad. Toxicolog Sci 1999; 52: 111-117.
[10] Alexis black; The mineral selenium proves itself as powerful anti cancer medicine; Natural news.com; Retrieved on 01/04/2006.
[11] Hanspeter Witschi, Imelda Expiritu, Mang Yu and Neil H. Willits. Carcinogenesis 1998; 10: 1789-1794.
[12] Zbigniew Marczynski, K. Henryka Bodek. Polish Chitosan society, Monograph XII, 2007.
[13] Margret Chandira, Jayakar B. International J Pharm Sci Rev Res 2010; 3:20-28.
[14] Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. Science 2007; 317: 121-124
[15] Ina Bergheim, Luping Guo, Molly Anne Davis, Jason C Lambert, Juliane I Beier, Ilinca Duveau, James P Luyendyk, Robert A Roth, Gavin E Arteel. Gastroenterol 2006; 130: 2099-2112.
[16] Chuang S E, Kuo M L, Hsu C H, Chen C R, Lin J K, Lai G M, Hsich C Y, Cheng A L. Carcinogenesis 2000; 21: 331-335.