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Ameliorating effect of *Ganoderma lucidum* extract against solid Ehrlich Tumor in Swiss Albino Mice

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Abstract : This Paper deals with the reduction of tumor volume by a tiny fungus *Ganoderma lucidum*. Methanolic extract of *G.lucidum* was evaluated against solid Ehrlich tumor in male Swiss albino mice. Ix107 EAC cells were injected intramuscularly in right thigh of Swiss male mice .Two treatment group one treated with *G. lucidum* extract & other with 5 FU along with a Control group were studied. Longest and shortest diameters of tumor were measured with help of vernier calipers. The percent Tumor growth inhibition was calculated on day 13 by comparing the average values of treated groups with that of control group and was found that *Ganoderma lucidum* extract inhibited tumor growth upto 39.46%

Key words: *Ganoderma lucidum,* Antitumor activity, Ehrlich tumor, Swiss Albino Mice.

INTRODUCTION

Cancer continues to represent the largest cause of mortality in India and claims over 1 – 1.5 million lives every year with around 7-9 lakhs new cases being detected each year. Cancer contributes to 3.4% of all deaths reported from India.

Ganoderma lucidum commonly known as Reishi is considered as a Panacea in the treatment of a large number of diseases. It was indexed in the Shen Nong's Materia Medica (206BC-8AD) as a longevity promoting and tonic herb of the non-toxic superior class, and has been used in traditional Chinese medicine (TCM) for more than 2000 years to prevent various human diseases such as hepatitis, gastritis, bronchitis, tumor growth etc.[1,2]

An extremely promising strategy for cancer prevention today is chemoprevention which is defined as the use of synthetic or natural agents alone or in combination to block the development of cancer in human.

Ganoderma lucidum has attracted significant attention in recent years due to its large number of pharmacological properties. The fruiting bodies of these mushrooms contain a variety of chemical substances. However it has been reported that the physiological effects and distinguishing properties of *Ganoderma* are strain dependant. Hence, it was thought worthwhile to investigate the efficacy of *G. lucidum* cultivated artificially in our lab on solid Ehrlich tumor.[3,4,5]

Chemotherapy induced leucopenia leads to significant morbidity and mortality, a major limiting factor in clinical chemotherapy without efficacious remedies. As a tumor grows progressively, the immune system of tumor bearing host is subsequently impaired. (Bear 1986, Hoover *et al* 1990) our previous studies have shown that 70% Methanolic extract of *G.lucidum* significantly ameliorate the number of lymphocyte and reduce the cancer burden of Swiss albino to mice harboring Ehlich *Ascitis Carcinoma*

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along with the enhancement of RBC count and hemoglobin percentage as well.[8,10,13]

Ganoderma lucidum is very rare in nature. The amount of wild mushroom is not sufficient for commercial exploitation, hence, in this present investigation artificial cultivation method was adopted and its potential efficiency was measured upon a group of Swiss albino mice.[7,8,14]

MATERIAL AND METHODS

All chemical and solvents used were of analytical grade and were obtained from Genei (Bangalore) and Merck (Mumbai). The mushroom *Ganoderma lucidum* culture was procured from NRCM's, ICAR, Chambaghat , Solon (HP) mushroom Gene Bank, true- to- the type genuine DNA – finger printed.

ANIMAL

Swiss albino mice were obtained from CDRI, Lucknow, UP, India. All experiments were conducted strictly according to the guidelines prescribed by Ethical committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi.(regis.no.1317/c/09/CPCSEA) **.** Swiss Mice used in this experiment were all male 18-23 gms in weight.

CULTIVATION OF MUSHROOM

Pure culture tube procured from NRCM, ICAR *Chambaghat* Solan (HP) was used as a starter for cultivation of mushroom in lab. Sub – culture was done on PDA (Potato Dextrose) and the crop cultivation was carried out using paddy grains amended with wheat bran as substrate. The harvested fruit bodies were used for the Methanolic extraction. Prior to extraction they were air dried and powdered. The powder was dipped in 70% Methanolic water for 7 days at room temperature and were shaken. Methanolic water was filtered and again the process was repeated for the extraction of the residues with 70% Methanolic water for 7 days. The filtrate was combined with the organic solvent at reduced pressure at 40^0C and dissolved in water at least 2 times and the water content was evaporated to get the dry crystals from *Ganoderma lucidum* powder. Before sending these crystals to IIIM, CSIR, Jammu to test the anticancer activity a short term toxicity studies were done at Patna on Swiss albino mice.

Purification of crude extract of *G.lucidum*

Powder of *Ganoderma lucidum* was dissolved in distilled water and was partitioned with ethyl acetate in 1:1 volume for the removal of insoluble debris. The fraction was dried after partitioning. Standared solution of *G.lucidum* were prepared in several media. Using silica gel column chromatography, ingradients were separated.

Antitumor activity

Ehrlich ascites carcinoma (EAC) cells were collected from the peritoneal cavity of the Swiss mice harbouring 8-10 days old ascitic tumor. $1x10^7$ EAC cells were injected intramuscularly in right thigh of 24 Swiss male mice selected for the experiment on day 0. The next day, animals were randomized and divided into three groups. Two treatment groups contained 7 animals each and one control group contained 10 animals. One of the treatment groups was treated with *Ganoderma lucidum* extract (125 mg/kg i/p) from day 1-9.

Another treatment group was treated with 5-fluorouracil (22 mg/kg, i.p) from day 1-9 and it served as positive control. The control group was similarly administered normal saline (0.2 ml, i.p.) from day 1-9. On day 9 & 13, tumor bearing thigh of each animal was shaved and longest and shortest diameters of the tumor were measured with the help of vernier caliper. Tumor weight of each animal was calculated using the following formula.

$$\text{Tumor weight (mg)} = \frac{\text{Length (mm) x [width(mm)]}^2}{2}$$

The percent tumor growth inhibition was calculated on day 13 by comparing the average values of treated groups with that of control group. Tumor growth in saline treated control animals was taken to be 100%.

RESULTS AND DISCUSSION

5 FU used in this experiment as a Positive control is a proven antitumor drug. Effect of *G. lucidum* was assessed by observation of changes with respect to body weight and weight of tumor. The average body weight change of each group is given in Table–I. Percent tumor growth inhibition by tumor is approximately 40% considering the normal control as 100%. Hence it is clear from the data that G*anoderma lucidum* extract used in this investigation acts as a tumor suppressor.

The reliable criteria for judging the value of any anticancer drug is the reduction in average body weight gain and average tumor weight. Present study showed that the artificially grown *Ganoderma lucidum* extract has anticancer activity and regressed 39.46% tumor growth in mice within 9 days treatment. This finding is consistent with previously published data of Janardhana et.al. (2005, 06). Further investigation by several worker like Ahmadi and Riazipour (2007), CAO Li Zhen *et.al.* 2003; Wang Sy *et.al.* 1997;: Wang*et.al.*(2007) etc. [8,9,10,11,12]

Preliminary photochemical screening of extract showed the presence of triterpenoid especially *ganoderic*

Table 1 : The results

Treatment Groups	Av. Body weights (g) of animals on days			Day 13		%Tumor Growth Inhibition	Mortality
	1	5	9	Av. Body weights (g)	Av. Tumor weights (mg)		
Ganoderma lucidum extract (125 mg/kg i/p)	21.85	22.28	22.0	21.28	1140.78	39.46	0/7
Positive control5 FU (22 mg /Kg i/p)	21.85	20.14	19.66	28.16	759.75	59.68	0/7
Normal Control NS (0.2 ml i/p)	22.8	23.3	24.9	25.33	1884.42	--	0/10

acid, polysaccharide – , D-glucan and a special protein LZ-8. These compounds has mitogenic and immune modulating tendency. Treatment with these compounds resulted in an inhibition of tumor proliferation and the progression of inflammation. Moreover,β, D-glucan have a chemo preventive role in cancer through their effects on macrophages, T-helper natural killer (NK) and other effectors cells. All of these increase the production of cytokines such as tumor necrosis factor (TNF-β) intertenkins (IL) and interferon (IFN), nitric oxide (NO) and antibodies by the activated effectors cells. This may be the possible reason of tumor regression.[13,14,15,16,17,18]

In addition inhibition of DNA polymerase and post translational modification of oncoprotiens may contribute to the antitumor activity of *Ganoderma lucidum*. The present study points to the potential anticancer activity of *G. lucidum* and might be a promising chemotherapeutic agent against solid tumor. Further studies to characterize the active principles and to elucidate the mechanism of the action of *G. lucidum* extract are in progress.[19,20]

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