EFFECT OF 1,3-β-D GLUCAN OF *GANODERMA LUCIDUM* ON FIBRO SARCOMA INDUCED MICE

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Summary

The maximal mycelial growth recorded in glucose amended cuture, and in the Production and isolation of extra cellular carbohydrates and proteins the maximum amount of protein 231 mg/L was record in sucrose amended medium besides *Ganoderma lucidium* produced maximum amount of carbohydrate (975 mg/L) and protein (55.4 mg/L) in synthetic medium. The absorption peaks of polysaccharide isolated from the fruit body of *G. lucidium* coincide with the 1,3-β-D glucan (Sigma). Effect of polysaccharide on fibrosarcoma 180 induced mice. The body weight of sarcoma-induced mice was increased by 11% than the normal mice. In polysaccharide treated mice the body weight significantly decreased and was similar to that of the normal mice, and remarkable increase in the weight of the spleen (336%) and kidney (10.6%) in sarcoma induced mice than normal mice whereas the weight of liver decreased to much as 29.3% in induced mice. In polysaccharide treated mice a remarkable percent of decrease in weigh of the liver (116%), spleen (53.1%) and kidney (35.46%) were observed. The concentration of DNA and RNA were elevated in induced mice.

Key words: Polysaccharide, DNA, RNA, Carbohydrates

Introduction

Ganoderma lucidum an important polypore fungus found all over the world causes white rot on hardwoods, conifers, palm. In the orient, *Ganoderma lucidium* is regarded as the herb of longevity. This fungus has been used in folk medicine for hundreds of years and strains are commercially cultivated for preparation of health tablets. For thousands of years Ganoderma has been considered by the Chinese to be a high quality herbal medicine. Under the attentive research done by several workers in recent years revealed that *G. lucidium* can be used as hemocantharsis, detoxicant, liver

protector, intestine regulator, cardio tonic, blood pressure adjuster, a cold tonic, antitussive and expectorant, a tranquilizer and an anti-tumor drug. *Ganoderma lucidium*, one of the well-known fungus have rich source of bioactive compounds. In the present study, the polysaccharide isolated from fruit body of *Ganoderma lucidium* was bio-assayed subcutaneously in implanted sarcoma 180 tumor in mice. Fungal glucans basically one β-glucans, which one of the cell wall components important rigidly to the cell wall along with chitins. In general β-glucan consist of a backbone chain of 1-3 β D glucopyranos units an 1,6 β D glucopyranose units in the basic chain[1] (Rou et al. 1990) Glucans contain mo than 85% of hexose sugars. Phosphorylatd poly-β-,1,3 glucans isolated from *Saccharomyces cerevisiae* showed immuno stimulation of macrophage and has antitumor effect on adenosarcomas, sarcomas and against lymphocytic leukemia. The fruiting body of *Ganoderma lucidium* called Reishi belonging to the family of poyporaceae, are distributed in the oriental countries, such as China, Japan and Korea. In these regions. Reishi has long been a popular oriental medicine to cure various human diseases.

Kim et al. [2] 1982 isolated an antitumor active complex of polysaccharide (27%) and Protein(72%) from the dilute alkali-extract of the fruiting body These studies suggested that the antitumor activities of *Ganoderma lucidium* polysaccharides were exhibited mainly by the branched (1-3) β-D glucan activity. Water extracts obtained from the fruit bodies of *Ganoderma lucidium*, significantly decreased Plasma sugar in mice [3]. This extracts contain two glucons, Ganoderin A and B Glycons elicited remarkable hypoglycaemic actions in normal and alloxan induced hypoglycaemic mice. Maruyama [4] reported that aqueous extract of *Ganoderma lucidium* is remarkably effective for inhibition of tumor growth, than the better not extract when administered intraperitoneally than oral administration.

Ganoderma lucidium is the only known sources of group of tri-terpene known as Ganoderic acids which have molecular structure similar to steroid harmones six novel triterpenoids were isolated from the fruit body of *G. applanatum* [5]. The polysaccharides of Ganoderma (G1-B) markedly enhanced the cytotoxicity of cytotoxic T lymphocytes [6,7] reported two new sterol esters, ergosta-7, 22-dien-3 bta-Y *Ganoderma lucidium* linoleat and 5 alpha, 8 alpha-epidioxyergosta-6,22-dien-3 btayl linoleat [8] (Balis 1986) and a novel steroid, ergostal –7,22-dine-3 bta, 3 alpha-triol [9] have been isolated from the fruiting bodies of Gormosan *Ganoderma lucidium* and these compounds exhibited potent inhibition of KB cells and human PLC\PRF\5 cells in vitro. Administration of hot water soluble extracts of *Ganoderma lucidium* (G1) decreased pain dramatically in two patients with postheraupetic neuralgia recalcitrant to standard therapy and two other patients with severe pain due to herpes Zoster infection[10].

Ganoderma japonicum (Fr.) Lloyed mixture has anti-thrombotic effect, blood coagulation and platelet activation were inhibited, and the ability of vascular endothelial cells against the process of thrombosis was enhanced[11]. In Indian medicine plant kingdom serves as an excellent source for pharmacological principles. One of the dreadful diseases of the modern world is cancer, generally, which is symptom less till its advanced stage. All over the world scientist are actively involved in exploring the naturally occurring resources for treatment of cancer without side effect Pharmacologists are in search of means of early diagnosis, proper treatment and preventive measures for cancer, hence search for new drugs to treat cancer still continues. *Ganoderma lucidium*, has been known as an oriental medicine is South-Easter countries The anti cancerous nature of the polysaccharide isolated from the fruit body has already been established.

Therefore it is an worth full attempt to work out the effect of the polysaccharide of *Ganoderma lucidium* on fibrosarcoma 180 induced experimental mice.

The following investigations were carried out

- 1. Extraction, estimation and isolation of extracellular polysaccharide from the culture and fruit body of *Ganoderma lucidium*
- 2. Effect of polysaccharide on fibrosarcoma induced mice in terms of tumor weight and nucleic acids levels.

Materials and Methods

Collection identification of *Ganoderma lucidium*

The basidiocarps of *Ganoderma lucidium* were collected from IIT, Villivakkam- Chennai, Tamil Nadu, India. on *Cassia emarginata* tree and were identified by Pro. PT.Kalaichelvan A piece of fruit body from the pileus region was transferred aseptically to PDA medium and the pure culture and maintained at 30'C+1C. A myclelial disc of 5mm taken out and inoculated into the Czaped Dox Broth medium (6disc/100ml) containing different carbon sources. After 7^{th}, 14^{th} and 21^{st} dayt he mycelium was harvested by filtering through pre-weighed whatmann No1 filter paper, dried at 80'C for 48 hours in an oven for recording dry biomass. The culture filtrate was checked for pH under pH meter.

Effect of culture media on polysaccharide production

Synthetic medium and semisynthetic medium amended with glucose were tested for production of Polysaccharide form *G. Lucidium*

Estimation of extracellular carbohydrate

The total carbohydrate was estimated according to the method of [12] Dubois et al. (1956). The mycelium of pure culture of *Ganoderma lucidium* was cultured on potato dextrose agar medium for seven days and then transferred to CDB amended with different sources (2%) like glucose, sucrose, maltose and mannitol at pH 6.5. The mycelium was harvested on 7th, 14th and 21st days and dry weight was measured. One ml of culture filtrate was added to 1ml of 5% phenol 5ml of 96% con Sulphuric acid was added ra pidely along the sides of the tubes After 10minutes incubation the mixture was in water bath at 25°C-30°C for 20 minutes and read in Beckman Spectrophotometer at 490nm Reagent with glass distilled water served as blank. The estimation was done by using Glucose as standard.

Estimation of protein

The protein estimation was determined according to the method of Bradford (1976) [13] method. Commassie Brilliant blue g-250 (100mg) was dissolved in 50ml of 95% ethanol. To this solution 100ml of 85%(WV) phosphoric acid was added, and final volume was up to mode one litre using standard flask. The concentration in the reagent was (001% v CBBG-250, 4.7% (VV) ethanol and 8.5%(WV) phosphoric acid. Assay was carried out by taking 1ml of culture filtrate in a test tube and adding 5ml of Commassie Brilliant blue mixed thoroughly and read in Beckmann Du20 Spectrophotometer at 595nm. Reagent with glass distilled water served as blank. The estimation was done by using Bovine Serum Albumin as standard.

Isolation of polysaccharides from the fruit body

The basidiocarp of *G. lucidium* washed thoroughly with distilled water and air dried. 150g of dried fruit body was make into small pieces and mixed in 15L of 0.1M Sodium phosphate buffer of pH-7 and kept in a shaker for 48 hours. The supernatant was discarded after centrifugation. The residue after PBS extraction was suspended in distilled water and heated at 120°C for 20 minutes. After cooling the content were transferred to conical flask and placed in shaker for 24 hours. The supernatant was collected after centrifugation at 8000xg for 30minutes, concentrated and precipitated with 12 v/v of ethanol to recover the polysaccharide (Plate 1b) The precipitate was lyophilised to powder under reduced pressure and finally read in an IR spectrophotometer and compared with 1,3-B-D glucan (sigma).

Assay of antitumor activity

The male swiss albino kmice weighing about 20grams were obtained from Cancer Institute, Chennai used for this investigation. The animals were maintained in well ventilated polypropylene cage. The experimental mice were divided into 3 groups namely.

1 Normal – Without inducing cancer

2. Induced-Induced with cancer

3. Treated-Induced with cancer and treated with isolated polysaccharide

Induction of fibrosarcoma

The mice were injected with fibrosarcoma according to Nagarajan and Shankaran (1973) using 3-Methylecholanthrene(20) (100mg in 0.1% saline) administered into the auxiliary region under aseptic condition. After a week the mice was scarified and tumor gall was dissected and mince with 0.1% saline. From that 0.2% ml was subcutaneous injected into the experimental mice. After a week the induced mice begins to palpate. The experimental control were treated with isolated polysaccharide (10mg/kg of body weight/day) for a period of 10 days with dosage of 0.1ml as intra peritoneal injection. After treatment the mice were sacrificed by cervical decapitation. The liver, spleen and kidney were dissected, placed in a ice cold saline for further analysis.

Estimation of nucleic acids

The nucleic acids were extracted by method of Schneider (1945) [14]. Fresh tissue of 100mg was weighed and homogenized in 5ml of cold distilled water using homogeniser with a Teflon pestle Five ml of 10% TCA was added to the homogenate and this was kept in ice for 30 minutes, to allow complete preparation o proteins and nucleic acids. The mixture was centrifuged and washed with ice cold 10%TCA thrice. The precipitate was then treated with absolute alcohol to remove lied materials and was collected by centrifugation. The precipitate free of lipids, were suspended in 5ml of 5% TCA and kept in a water bath maintained at 90°C for 15 minutes, with occasional stirring, which facilitated the quantitative separation of nucleic acids from eh precipitated proteins. The supernatant after centrifugation was used for the estimation of RNA and DNA.

Estimation of RNA and DNA

RNA was estimated by measuring the intensity of green colour produced with orcinol at 660nm as described by Schneider (1957) [14]. An aliquot of the nucleic acid extract was taken and made up to 2ml with water. A blank having only water and standard were also taken and made up to 2ml. To this 3ml of ordinal ferric chloride reagent was added. The contents were mixed well and kept on a boiling water bath for 20minutes. The colour developed was read against the reagent blank in a spectrophotometer at 660nm. The values are expressed as mg per gram of wet tissue.

DNA was estimated according to the method of Burton (1956) [15] using diphenylamine. A known volume of nucleic acid extract was made up to 3ml of 1N perchloric acid. This was mixed with 3ml of diphenylamine reagent. A reagent blank and standards were also carried through the same procedure. This was kept in a boiling water bath for 10 minutes and blue colour developed was read at 600nm in a spectrophotometer.

Results

Effect of different carbon sources on growth of *Ganoderma lucidum*

Among the four different carbon sources, the maximal mycelial growth recorded in glucose amended couture was 1.093, 1.473 and 2.319 mg/L on 7th, 14th and 21st days respectively. The minimum growth was observed in mannitol. During the mycelial growth considerable change has not been observed in 7th day. Change in pH from 6.5 to 56, 6.0 to 5.5 and 6.5 to 59 was observed on 14th and 21st day with glucose and sucrose as carbon source. (Table 1).

Table 1: Growth of *Ganoderma lucidum* in liquid medium amended with various carbon source

Carbon sources	7thday g/L	pH	14th day g/L	pH	21st day g/L
Glucose	1093	6.5	1.473	5.6	2.319
Sucrose	0.810	6.2	1.367	6.0	1.615
Maltose	0.594	6.3	1.272	7.5	1.390
Mannitol	0.731	6.5	1.281	6.2	1.662

Production and isolation of extracellular carbohydrates and proteins

The culture filtrate was used for the determination of extracellular carbohydrates by phenol-sulphuric acid method Maximum amount of carbohydrate was observed in glucose amended 684, 1430 and 1192 mg/L on 7^{th},14^{th} and 21^{st} days respectively followed by sucrose and maltose. Decrease in carbohydrate has been recorded on day 21, whereas in biomass there has not been any reduction. Extra cellular protein was determined by Coomassie Brilliant Blue dye binding method. The amount of extra cellular protein consistently increase corresponding to the mycelial growth The maximum amount of protein 231 mg/L was record in sucrose amended medium followed by mannitol and maltose an the minimum amount 59.0mg/L was observe in glucose amended culture. (Table 2).

Table 2: Effect of different carbon source on production of extra cellular carbohydrate and protein of *G. lucidum* culture in liquid medium

Carbon sources	7thday		14^{th} day		21th day	
	Carbohydrates	Protein mg/mL	Carbohydrates	Protein mg/L	Carbohydrates	Protein mg/L
Glucose	684	19.5	1430	28.0	1192	59.0
Sucrose	620	14.0	1834	29.0	1100	23.1
Maltose	532	13.0	1218	27.0	984	128.1
Mannitol	480	7.0	1200	17.2	960	205.6

Effect of culture medium on production of extracellular polysacccharide by *Ganoderma lucidium*

Synthetic medium and semi synthetic medium amended with glucose were tested for the production of extra cellular polysaccharide by *Ganoderma lucidium* in semisynthetic medium. *Ganoderma lucidium* produced maximum amount (975 mg/L) and protein (55.4 mg/L) than in synthetic medium. (Table 3).

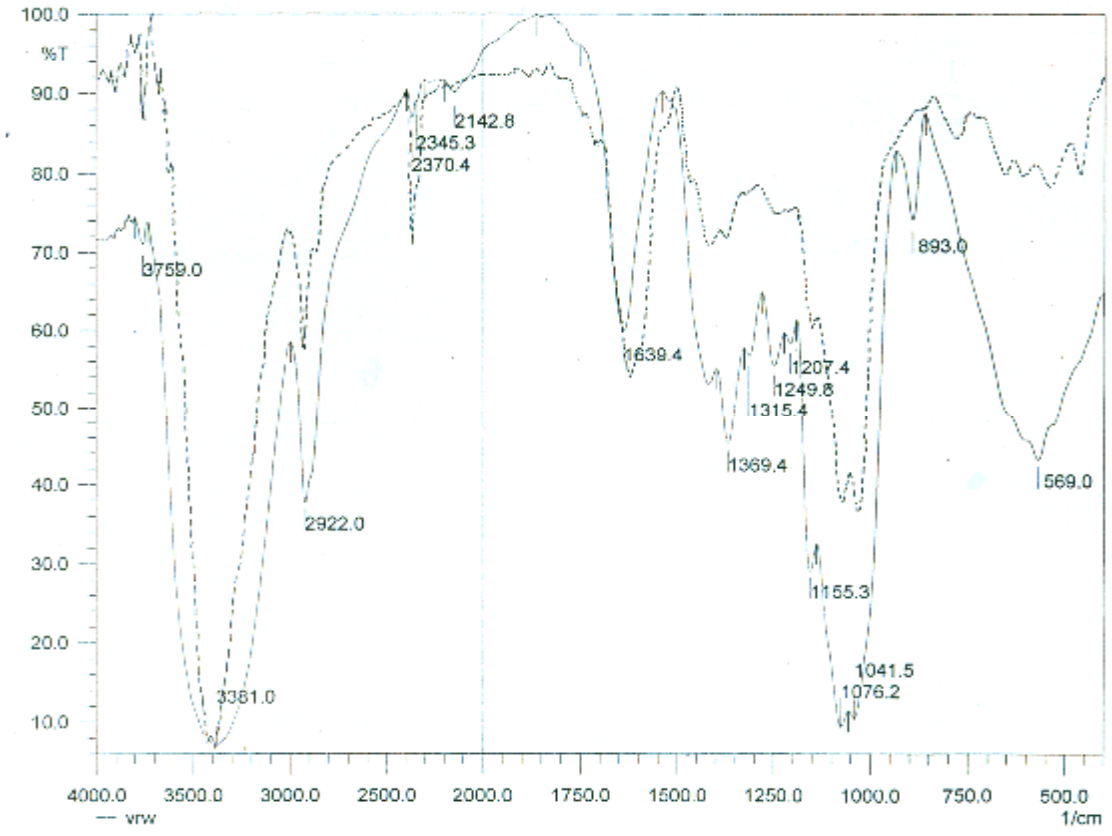
Table 3: Extra cellular polysaccharide, protein of *G.lucidum* mycelium and fruit body

Mycelium	Carbohydrates(mg/L)	Protein(mg/L)
Synthetic medium +Glucose	745	28.0
Semisynthetic medium+Glucose	975	55.4
Fruit body	631	6.32

Isolation and identification of polysaccharide from fruit body of *Ganoderma lucidium*

From the mature fruit body of the *Ganoderma lucidium* polysaccharide was extracted by using phosphate buffer saline and hot water. The isolated polysaccharide was read in an Infra Red spectrophotometer and compared with pure 1,3-β-D glucan (Sigma). The IR spectrum of isolated crude polysaccharide resembles with spectrum of pure glucan sample (Figure 1).

Figure 1: IR Spectrum of 1,3-B-D glucan and polysaccharide isolated from *G. lucidum*



Effect of polysaccharide on fibrosarcoma 180 induced mice

Fibrosacoma was induced in experimental mice and induced mice were treated with isolated polysaccharide form the *Ganoderma lucidium* fruit body. The various physiological parameters observed in the present investigation are presented in Figures 2, 3 and table 4. Within seven days the tumor developed in sarcoma 180 implanted mice the body weight of normal, treated and untreated mice are presented in. The body weight of sarcoma-induced mice was increased by 11% than the normal mice. In polysaccharide treated mice the body weight significantly decreased and was similar to that of the normal mice. After, 10 days of administration of polysaccharide to the tumor induced mice and untreated mice were sacrificed The liver, kidney and spleen were removed and weighed a remarkable increase in the weight of the spleen (336%) and kidney (10.6%) in sarcoma induced mice than normal mice whereas the weight of liver decreased to much as 29.3% in induced mice. (Table 4). In polysaccharide treated mice a remarkable percent of decrease in weigh of the liver (116%), spleen (53.1%) and kidney (35.46%) were observed (Table 5). The nucleic acid contents, determined in liver and spleen of normal, induced and treated mice revealed that the level of DNA doubled increased in liver of sarcoma induced mice than normal mice but there was no change in RNA content Similarly, the total DNA and RNA contents doubled in spleen of the sarcoma induced mice. Considerable decrease of total DNA and RNA levels I polysaccharides treated mice was observed.

Figure 2: Growth of *Ganoderma lucidum* in liquid medium in different carbon source

Figure 3: Effect of different carbon on production of polysaccharide by *G.lucidum*

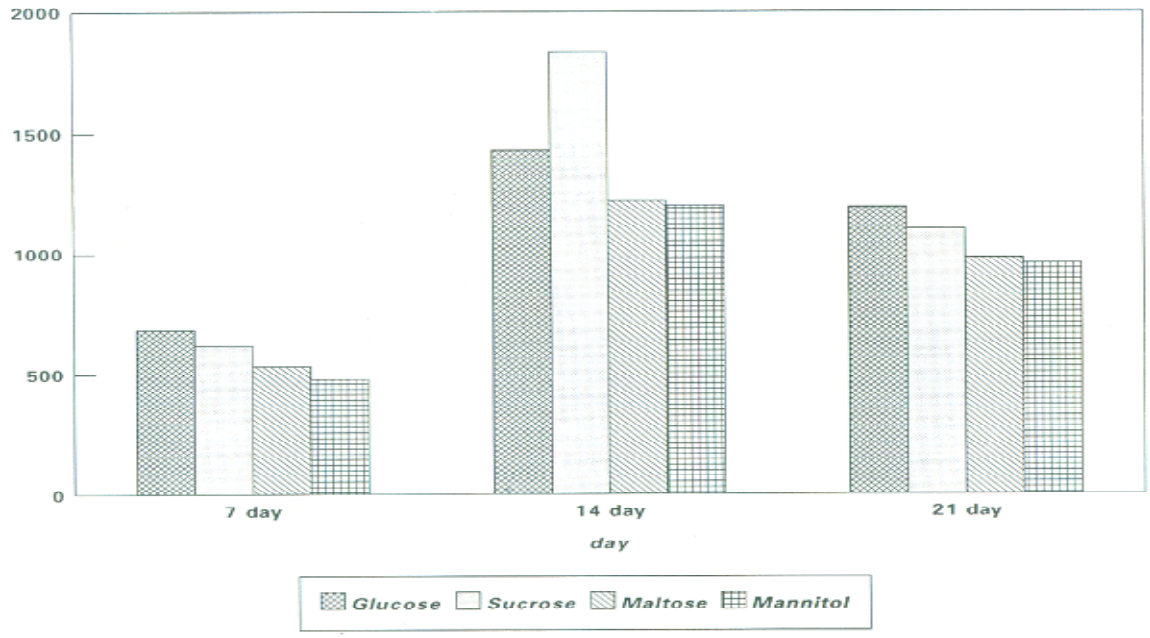


Figure 4: Levls of extracellular protein in *G. lucidum* culture

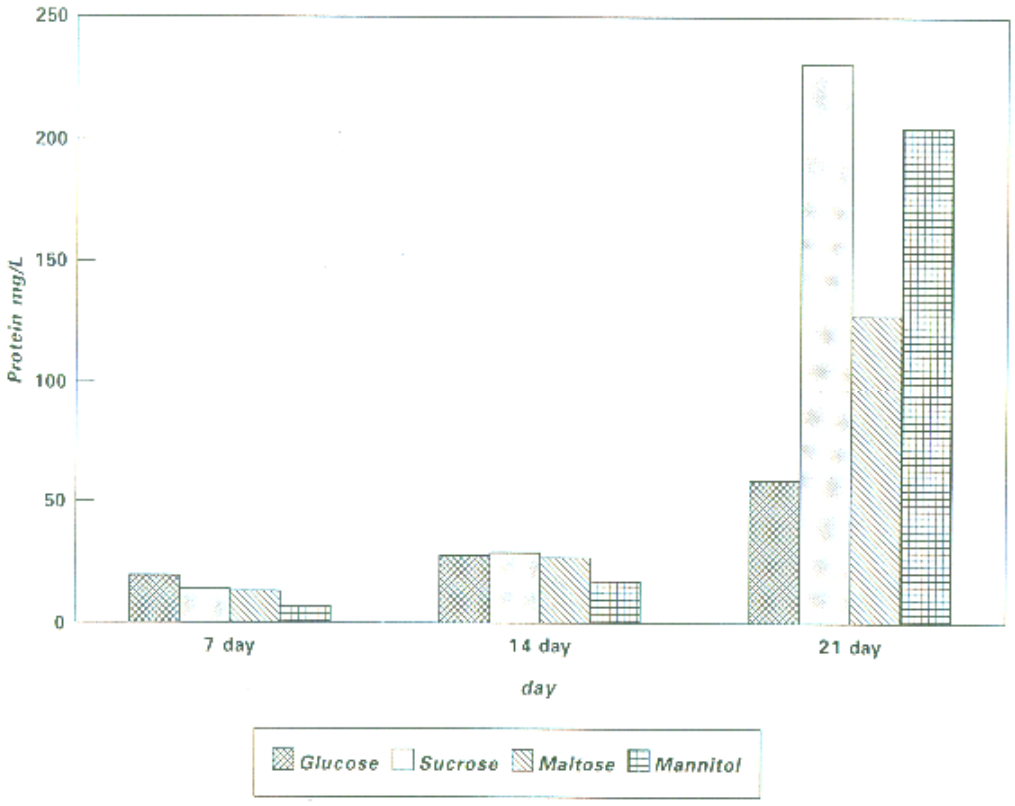


Table 4: Comparative account of the body weight, liver weight, Spleen weight, Kidney weight in normal, sarcoma induced mice and treated mice (g)

Carbon sources	Normal	Induced	Treated
Body weight	23.8	26.4	24.2
Liver	1.249	0.8821	0.7798
Spleen	0.1865	0.7232	0.3389
Kidney	0.2091	0.2313	0.2082

Table 5: Comparative account of DNA and RNA amount in Liver and Spleen of normal, induced and treated mice (mg/g of fresh weight)

Mice	Liver		Spleen	
	DNA	RNA	DNA	RNA
Normal	6.17	7.05	2.0	1.32
Induced	13.48	7.06	7.31	2.73
Treated	10.4	7.6	6.05	1.83

Discussion

Production of exo-polysaccharides by *Ganoderma lucidium* has been reported by Yoshiaki [16] and it has also been isolated from fresh fruiting bodies of *Ganoderma lucidium* and used to treat the tumor Wang [17]. In our study, *Ganoderma lucidium* mycelium has been screened under different carbon sources an polysaccharide was extracted from fruit body and read I IR spectrophotometer. The IR spectrum of absorption peaks it coincides into the spectrum of 1,3βD glucon (sigma) [18].

Amount of extra cellular polysaccharide increased on 14th day and decreased on 21st day. Under carbon source limiting conditions organisms may use their own exo-polysaccharides as their carbon sources Pison [19] reported that *Acremonium pericimum* produces β-glucanases, which can degrade their won glucans. In the present investigation the above report is considerable since the intracellular protein content was maximum on 21st day. In *Ganoderma lucidium* culture Tumor weight and volume measurements are commonly used to valuate the anticancer of any drug. The tumor volume has been observed after 10days treatment of *Ganoderma lucidium* which showed that thee was a reduction in tumor volume when compared to that of fibrosarcoma induced mice.

The clearly proves the anticancer activity of the drug. Nucleic acids play on important rule during neoplastic transformation previous reports revealed that the concentrations of RNA and DNA were increased in liver, lung and spleen cancers. Hilf [20] have demonstrated an alteration in nucleic acid pattern in the adrenals and liver of mice bearing sarcoma 180.

The levels of DNA and RNA of liver, spleen and kidney were found to be progressively increasing in fibrosarcoma induced mice and among nucleic acids, DNA exhibited prominent increase have observed that in mice bearing sarcoma the ratio of RNA to DNA in tumor tissue varied. During the period of tumor growth there was a rapid increase in DNA content when compared to RNA various investigations were carried out on Fibro sarcoma induced and treated mice to understand the antitumor effect of the polysaccharide isolated from *Ganoderma lucidium.* The results clearly indicated the ant tumor nature of the isolated compound.

Cerecedo and Bresnick (1961) [21] have reported that in mammary cancer the concentration of DNA and RNA were elevated in liver, lung and spleen. In the present work increase in DNA and RNA of spleen were observed in untreated mice and only DNA levels increased in liver. Whereas it has been brought to near normal level I polysaccharide treated mice.

Conclusion

These studies indicate that the glucons (polysaccharides) extracted from *Ganoderma lucidium,* can be formulated as an effective anti-cancerous drug. However a detailed investigation into its mode of action is yet to be explored in higher mammals.

Acknowledgments

The authors kindly thank CLRI –Chennai, Tamil Nadu, for their assistance with IR spectrum analysis.

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