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RESEARCH ARTICLE

IMMUNOSTIMULANT AND FREE RADICAL SCAVENGING STUDIES OF *GANODERMA APPLANATUM*

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Abstract: Objective: The present animal experimentation study is planned to screen the immunostimulant efficacy, free radical scavenging potential and the acute toxicity of the bracket fungus, Ganoderma applanatum . **Methods:** The study analyse the invitro immunostimulant activity by phagocytic index determination using Candida albicans, acute toxicity study by brine shrimp assay and the free radical scavenging activities of G. applanatum solvent extracts. **Conclusion:** The percentage immunostimulation was found to be 88% for the chloroform-methanol (1:1) Ganoderma applanatum extract (GAE). The free radical scavenging activity studies were performed by utilising invitro model of hydroxyl, superoxide and lipid peroxide radical generating system. **Results** indicated that GAE at 100 mcg/ml showed 88.1, 89.19, and 81.87 % scavenging activity of hydroxyl, superoxide and lipid peroxide radical respectively. The LC50 was determined using brine shrimp assay method and calculated as 875 mcg /ml. The present study shows that G. applanatum can be utilised as an effective natural supplement to impart immunostimulation.

KEYWORDS: *Ganoderma applanatum, Immunostimulant activity, free radical scavenging activity*

INTRODUCTION:

In Chinese traditional medicine, *G. applanatum* has been used commonly as haemostatic, immunostimulant, tumour inhibitor, and also for the treatment of rheumatic tuberculosis and oesophageal carcinoma[1,2]. The common synonyms are artist's bracket, bear bread, artist's conk etc[3]. *G. applanatum* is a parasitic and saprophytic fungus lives inside the living or dead tree wood as mycelium. Compounds like applanoxidic acid and sugars like arabitol, ribose, fucose, mannitol, sorbitol, glucose, sucrose, maltose, uronic acid etc were isolated and reported previously[5,6,7]. Mohammad S.H *et al* reported[8] the usefulness of *G. applanatum* in the management of diabetes meletus, hyperlipidemia etc. The present study is designed to evaluate the invitro immunostimulant activity by phagocytic index determination using *Candida albicans,* acute toxicity study by *brine shrimp assay* and the free radical scavenging activities of GAE.

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MATERIALS AND METHODS:

The dried and matured fruiting bodies of *Ganoderma applanatum* (Ganodermataceae) were collected from Agriculture University, Trivandrum, Kerala in January 2003. The specimen was identified at the Dept. of Plant Pathology, Agriculture University, Vellayani, Trivandrum. A voucher specimen deposited at the herbarium of the department of Pharmacognosy, SRIPMS, Coimbatore -641044, India.

The 'chloroform: methanol soxhleted extract of *G. applanatum* (yield: 8.0%w/w) shows positive reactions for steroids and triterpenoids upon phytochemical screening[4].

Studied activities

Immunostimulant activity was conducted by phagocytic index determination (Table-4, Fig-1) and free radical scavenging activity studies (for hydroxyl, superoxide and lipid peroxide radicals) by invitro studies. The LC_{50} determination was performed by Brine shrimp assay (BSA) method[10].

Acute toxicity studies: *Brine Shrimp Assay method*

Brine shrimp assay method[10] was followed to find out the LC_{50} for the extract GAE. The brine shrimp eggs were hatched in a rectangular chamber filled with artificial sea water and five numbers each were transferred to vials using a 9-inch disposable pipette. The survival rate of the shrimps was observed after 24 h for different concentrations of GAE. The LC50 was found from the dose -response graph. The results are tabulated in Table 1.

Table -1. Toxicity studies

No.	Con:(mcg/ml)	% Inhibition	LC_{50} mcg/ml
1	250	0	
2	500	20	
3	750	40	875
4	1000	60	

Free radical scavenging activity studies

- **Hydroxyl radical scavenging activity[11]**

This study was conducted by measuring the inhibition of deoxyribose degradation in presence of the test drug extracts. Hydroxyl radical was generated by Fe EDTA and H_2O_2 in presence of ascorbic acid. The extract GAE was added in various concentrations. (10mcg/ml – 100mcg/ml) to a reaction mixture containing deoxyribose (3mM), $FeCl_3$ (20mM/pH 7.4) to make a final volume of 3ml. To this mixture, trichloroacetic acid and thiobarbituric acid (0.5ml each) were added and measured the absorbance at 532nm. The percentage of hydroxyl radical inhibition and IC_{50} of the test drug extracts were determined by the method of Halliwell et. al (1987). A 0.01 mM copper sulphate solution was used as a reference standard. The results are tabulated in Table 2.

Table-2. Hydroxyl radical scavenging activity

No.	Con:(mcg/ml)	% Inhibition	EC_{50} mcg/ml
1	10	10.04±0.5185	
2	30	30.43±0.08576	56
3	50	45.08±0.0989	
4	70	62.34±0.01416	
5	100	88.179±0.0476	
6	$CuSO_4$	95.6±0.05185	

- **Superoxide radical scavenging activity[12,14]**

Superoxide radical scavenging activity was studied according to the method reported in the literature. Alkaline dimethyl sulphoxide (1% in 5mM NaOH) was added to the reaction mixture containing nitro-blue tetrazolium (NBT 0.1mg) and the test drug GAE at various concentrations. The absorbance was determined at 560nm. The reduction of NBT by the superoxide radical generated was calculated in the presence and absence of test drugs. In this study, thio urea (20mM) was used as the reference standard. The results were tabulated in Table 3.

Table -3. Superoxide radical scavenging activity

No.	Con:(mcg/ml)	% Inhibition	EC_{50} mcg/ml
1	10	17.37	
2	30	35.59	
3	50	53.38	
4	70	70.33	
5	100	89.19	28
6	Thiourea	90.04	

- **Lipid peroxide scavenging activity**

In this study, the liver tissue homogenate[13] of albino rats was prepared in phosphate buffer saline of pH 7.4. The protein content of the homogenate was adjusted to 10mg/ml. The effect of the test compounds on lipid peroxide was estimated as malondialdehyde by thiobarbituric acid (TBA) method. To the reaction mixture containing test drug extracts at various concentrations, 1ml of liver tissue homogenate and 1ml of "HCl thiobarbituric acid-trichloro acetic acid reagent" was added. The mixture was warmed gently for 5min in a water bath at 37^0C. After cooling the flocculent precipitate was removed by centrifugation at 1000rpm for 10min. The absorbance of the supernatant liquid was measured at 532nm against blank and the lipid peroxide content was determined using the extinction coefficient $1.56 \times 10^5 m^{-1}cm^{-1}$. The final result was expressed as nanomoles of malondialdehyde per mg of protein. Vitamin E (50mcg/ml) was used as the standard reference in this study. The results are tabulated in Table 4.

Table-4. Lipid peroxide scavenging activity

No.	Con:(mcg/ml)	% Inhibition	EC_{50} mcg/ml
1	10	16.1	
2	30	32.55	
3	50	50.16	51
4	70	65.43	
5	100	81.87	
6	Vit E	88.08	

- **Immunostimulant activity studies[9]**

The immunostimulant activity study was conducted by phagocytic index determination using *Candida albicans*. Human blood (2-3 drops) was taken by finger prick method from a healthy volunteer with prior informed consent and documented with the institutional ethics committee (IEC/SRIPMS/11/18). The slide with the blood collected was kept on a cotton pad in a sterile petridish and incubated at 37°C for 25 min. After incubation the clot was removed very gently and the slide was slowly drained with sterile normal saline taking care not to wash the adhered neutrophilis. The slide was flooded with predetermined concentration of the test drug, incubated at 37°C for 15 min and flooded with a suspension of Candida albicans in Hank's balanced salt solution and human serum and incubated at 37°C for Ih. After this, the slide was drained, fixed with methanol and stained with Giesma stain. The mean number of phagocytised cells on the slide was determined microscopically for 100 granulocytes. This number was taken as the phagocytic index (PI) and was compared with the basal phagocytic index of control. The results are tabulated in Table 5. The percentage immunostimulation was calculated by using the equation.

$$\% \text{ immunostimulation} = PI_{(T)}\text{-}P\ I_{(c)} / P\ I\ (C) \times 100$$

$PI_{(T)}$ – Phagocytic index of test

$PI_{(C)}$ – Phagocytic index of control

Table-5. Immunostimulant activity studies

Drug Groups (n)	Dose mg/ml	Phagocytic index	% Immunostimulation
GAE	50	41 ± 0.816	64 ± 3.26
GAE	100	47 ± 0.47	88 ± 0.87**
Control (normal saline)	-	25 ± 0.8164	-

n=5 p value < 0.01**, < 0.05* GAE: *Ganoderma applanatum* extract

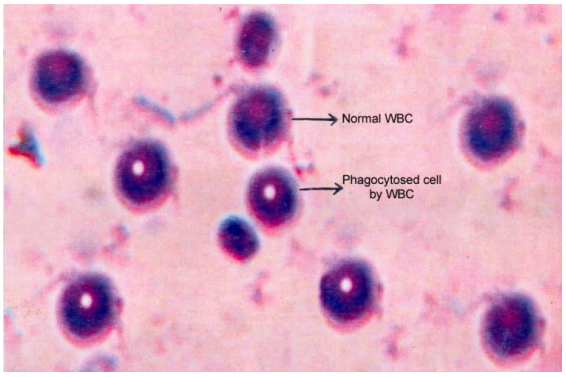

Figure 1. Phagocytosis of WBC with *Candida albicans*

CONCLUSION :

The qualitative analysis of GAE showed the presence of steroidal triterpenes. The extract showed significant scavenging of superoxide, hydroxyl, and lipid peroxide radicals, when compared to the standards $CuSO_4$, Vit.E, and Thiourea respectively. The study showed that the extract obtained from *Ganoderma applanatum* (GAE) can be used as an effective natural immunostimulant and free radical scavenging agent with less toxicity for the affected individuals in the post Covid-19 pandemic period.

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