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Immunostimulant and Free Radical Scavenging Studies of *Ganoderma applanatum*

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Abstract

The immunostimulant activity of the chloroform/methanol extract of *Ganoderma applanatum* was investigated by determining the phagocytic index. The percentage immunostimulation was found to be 88% for the $CHCl_3$: MeOH (1:1) *Ganoderma applanatum* Extract (GAE). The free radical scavenging activity studies were performed by utilizing *in vitro* 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 LC_{50} was determined using brine shrimp assay method and calculated as 875 mcg/ml.

Keywords:* *Ganoderma applanatum; Immunostimulant activity; Free radical scavenging activity; Peroxide; scavenging activity of hydroxyl

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]. It has synonyms like 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 [5-7]. Mohammad SH, et al. reported the usefulness of *G. applanatum* in the management of diabetes mellitus, hyperlipidemia etc. [8].

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 by Dr. Geetha, 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 [9].

Studied activities

Immunostimulant activity was conducted by phagocytic index determination and free radical scavenging activity studies (for hydroxyl, superoxide and lipid peroxide radicals) by *in vitro* studies. The LC_{50} determination was performed by Brine Shrimp Assay (BSA) method (Figure 1) [10].

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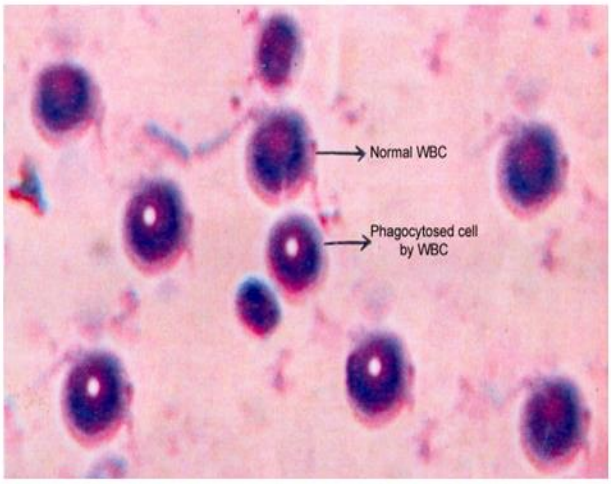

FIG. 1. **Phagocytosis of WBC with *Candida albicans.***

Acute toxicity studies: Brine shrimp assay method

Brine shrimp assay method 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 LC_{50} was found from the dose response graph. The results are tabulated in Table 1 [11].

TABLE 1. **Toxicity studies.**

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

Results and Discussion

Free radical scavenging activity studies

Hydroxyl radical scavenging activity: 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 (10 mcg/ml-100 mcg/ml) to a reaction mixture containing deoxyribose (3 mM), $FeCl_3$ (20 mM/pH 7.4) to make a final volume of 3 ml. To this mixture, trichloroacetic acid and thiobarbituric acid (0.5 ml each) were added and measured the absorbance at 532 nm. The percentage of hydroxyl radical inhibition and IC_{50} of the test drug extracts were determined by the method of Halliwell, et al. 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	56
2	30	30.43 ± 0.08576	
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: Superoxide radical scavenging activity was studied according to the method reported in the literature. Alkaline dimethyl sulphoxide (1% in 5 mM NaOH) was added to the reaction mixture containing nitro blue tetrazolium (NBT 0.1 mg) and the test drug GAE at various concentrations. The absorbance was determined at 560 nm. The reduction of NBT by the superoxide radical generated was calculated in the presence and absence of test drugs. In this study, thio urea (20 mM) 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	28
2	30	35.59	
3	50	53.38	
4	70	70.33	
5	100	89.19	
6	Thiourea	90.04	

Lipid peroxide scavenging activity: In this study, the liver tissue homogenate of albino rats was prepared in phosphate buffer saline of pH 7.4. The protein content of the homogenate was adjusted to 10 mg/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, 1 ml of liver tissue homogenate and 1 ml of "HCl thiobarbituric acid-trichloro acetic acid reagent" was added. The mixture was warmed gently for 5 min in a water bath at 37°C. After cooling the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant liquid was measured at 532 nm 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 (50 mcg/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	51
2	30	32.55	
3	50	50.16	
4	70	65.43	
5	100	81.87	
6	Vit E	88.08	

Immunostimulant activity studies: The immunostimulant activity study was conducted by phagocytic index determination using *Candida albicans*. Human blood (2-3 drops) was taken by finger prick method and placed in a sterile glass slide. The slide 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 neutrophils. 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 1 h. 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 percentage immunostimulation was calculated by using the equation:

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

$PI_{(T)}$-Phagocytic Index of test.

$PI_{(C)}$-Phagocytic Index of control.

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 $CuSO_4$, Vitamin E and Thiourea respectively. The study showed that the extract obtained from *Ganoderma Applanatum* (GAE) can be used as a good immunostimulant and free radical scavenging agent with less toxicity.

Conflicts of Interest

Nil.

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