

Clinical Trial Research on Immune–Assist

Alcoholic Liver Disease and Hyperlipidemia

John C. Holliday¹

Wang Ruwei, Ji Peijun, Li Shuifu, Xia Jinxing²

Xie Jianjun³

Zhan Hongpeng, Sun Huiling, Lei Lixing, Zhang Guoyong⁴

Li Songhua⁵

Yu Jin⁶

1 Aloha Medicinals Inc., Maui, Hawaii

2 The National Medicinal Academy of Zhejiang Province, China

3 The Sino-Japanese Academy of Plant Resources in Lishui City, China

4 The People’s Hospital in Lishui City, China

5 Daogen Medical College in Japan, Jiangjin Guochi Internal Medicine Hospital Daogen ,Japan

6 The Chinese Medicine Hospital in Hangzhou City, China

Abstract This paper reports on the clinic trial results of the proprietary *Immune–Assist Critical Care Formula* brand of mushroom extract mixture for the treatment of Alcoholic Liver Disease and hyperlipidemia. Through this study the preparation method, the quality control standards, the medicinal function and the safety and toxicity study, we found that this preparation was both safe and effective, and shows great potential as a preventive and health care medicine for treating and curing the disease of alcoholic liver and hyperlipidemia. We found that this polysaccharide mixture could not only restrain the alcohol-induced damage to the liver cells, but also enhances the restoration of liver function and decreases blood lipids. The curative result was significant and safe. This compound shows great promise for use in clinical therapy.

Key words: *Immune-Assist*, polysaccharide, medicinal mushrooms, Alcoholic Liver Disease, Hyperlipidemia

Introduction: Alcohol is used widely throughout society, and often for medicinal purposes such as to stimulate the appetite, cure pain, eliminate fatigue and as a disinfectant. But alcohol use is more often indicated in health problems, for example a wide range of acute and chronic diseases and behavioral dysfunction. Chronic alcoholism has become a global social problem. In America alone, an estimated 100,000 people die annually due to the abuse of alcohol. The economic burden in this one country is estimated to be about 100 billion dollars a year. Chronic alcoholism has become a familiar disease in many other countries as well. Because of this, many experts at home and abroad have been studying effective ways to restrain the damage to liver cells caused by chronic abuse of alcohol, and ways of treating or curing the fibrosis of the liver resultant of chronic alcohol abuse. In recent years, science has

found that the polysaccharides extracted from edible mushrooms have various biologically active functions. The city of Lishui, which lies in the southwest of the Zhejiang province of China, has a rich resource of edible mushrooms, and a long history of the use of medicinal mushrooms. A thorough review of the literature and customs of this area has shown that these mushrooms can protect the liver, decrease blood lipids and improve immune function. According to this long history of folk-usage, a modern extraction method was developed to produce an effective medicinal mushroom polysaccharide combination.

This mushroom extract combination is produced and marketed in America under the brand name ***Immune-Assist Critical Care Formula*** by Aloha Medicinals Inc. P.O. Box 686 Haiku, Hawaii 96708. The components of this formula are alcohol-precipitated, hot-water extracts of *Lentinula edodes*, *Grifola frondosa*, *Coriolus versicolor*, *Agaricus blazei*, *Ganoderma lucidum*, *Cordyceps sinensis*, and the extra-cellular compounds derived from the culture broth of fermented *Cordyceps sinensis*. From April of 2001 to May of 2002, this research group used the ***Immune-Assist*** formula in the treatment of alcoholic liver disease and hyperlipidemia. The tablets used in this study were 500 mg each and contained 400 mg mixture of very complex polysaccharides, mainly (d)beta-glucans of differing structures, primarily 1↔3 main chain structure with 1↔6 side branching. With more than 200 differing polysaccharide structures, there are many other polysaccharides present besides these prototypical and well-understood mushroom-derived immunomodulator compounds. The Government research authorization number granted to this project was 99-118. The primary research results are as follows.

1) Formula and preparation method

1.1 Formula: A proprietary blend of Lentinan, Krestin, Cordycepin, A. blazei PS, Ganoderma PS, Grifolan, Cordyceps extra-cellular PS, and the normal binders and excipients used in the tableting process. (Note 1)

1.2 Preparation method: We prepared the formula and made the tablets according to reference[1]. Each tablet was 0.5g total weight with 0.4g polysaccharide complex total.

2) Quality control and verification standard

2.1 The tablet is composed of granular material, light brown in color and has a characteristic smell.

2.2 Differentiation and authenticity verification:

To two tablets (1.0g) add 2mol/L solution of hydrochloric acid to dissolve. Add ninhydrin approx. 2mg, heat up to $90^{\circ} \pm 5^{\circ}$, so the solution changes from deep blue to light blue.

To two tablets (1.0g) add hot water 20ml to dissolve. Then to 5ml of this solution add silver nitrate 2.5ml. A black deposit of silver should appear.

2.3 This product should measure up to all of the standards and medicinal rules in the first addendum of "The Codex of the People's Republic of China" (Edition 1).

3) Toxicity study (presided over by Dr. Li Songhua at Daogen Medical College, Japan)

3.1 Acute toxicity experiment: 20 baby mice (20 ± 1 g each, half male half female), administer P.O. a

solution of the polysaccharide tablet 3 times / 24 hours (0.5g for each mouse every time), total dose is 75g/kg/day. Maintain this dosage for 7 days and otherwise feed according to normal. At the end of the seven days all the mice are healthy and none show any signs of toxicity or abnormality. This short-term overdose is approximately 835 times the normal adult dosage.

3.2 Long-term toxicity experiment: Choose 80 healthy adult mice, administer the solution P.O. at 10g/kg/day for 90 days. The mice have no abnormal characteristics and the tissues show no toxicity changes. This confirms that this product has little toxicity for long-term administration

3.3 These result shows this product is very safe.

Clinical Research

4.1 Objectives and methods

Subjects: The experimental group includes 48 cases of Alcoholic Liver Disease (some of whom also have Hyperlipidemia), all the cases are patients confined in the analytical facility and all have abnormal indexes after medical examination. The patients are all male and their ages are from 31 to 70, with an average age of 54. Among these cases, there are 26 cases of alcoholic fatty liver, 11 cases of alcoholic hepatitis, 10 cases of alcoholic fibrosis of liver and 1 case of hepato-cirrhosis. The diagnoses all comply with the well-accepted medical standards. The control group has 17 cases that are patients in the analytical facility at the same time. The cases of the control group have no obvious differentiation to those of experimental group for age, history of drinking alcohol or state of illness according to statistical methods. (All patients came from Lishui City. Four cooperative medical units referred both groups of patients for this study).

Methods: The experimental group is treated with *Immune-Assist* tablets, 3 times a day, 2 tablets each time for the full 13-month period. The comparison group is treated with vitamin E, 400 IU, 3 times a day for the same period.

4.2 Result

4.2.1 HA and LN: Research the anti-fibrosis function of the *Immune-Assist* tablets according to the analytical changes of blood serum HA and LN. The results are shown in Table 1 and Graph 1A.

Table 1 Analysis of blood serum HA and LN between experiment group and comparison group

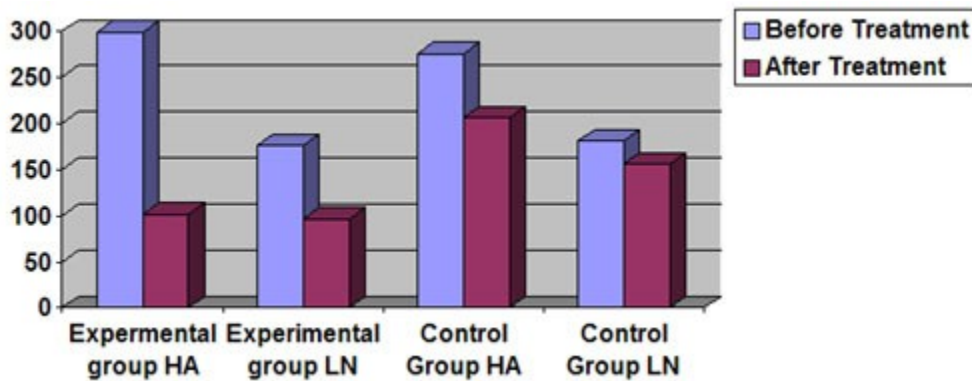
Group	HA (µg/L)	LN (µg/L)
Experimental group	Before treatment 296.81±23.36	176.30±12.37
	After treatment 102.29±19.83	97.45±17.50

Control group	Before treatment 276.12±25.20	181.27±13.51
	After treatment 206.31±21.56	156.17±16.79

The content changes of blood serum HA and LN before and after curing (X±SD)

The comparisons to before treatment for the same group are P<0.01, after treatment for the same group are *P<0.001

Graph 1A Changes in Blood Serum HA and LN



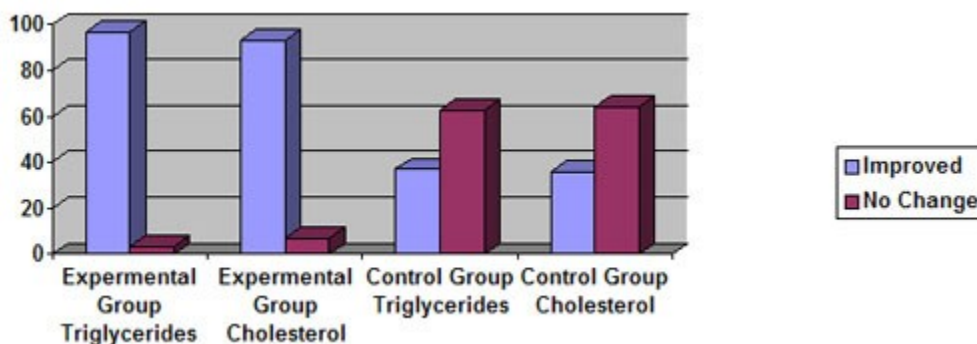
These result shows that the content of blood serum HA and LN decreased significantly during and after treatment for the experimental group, but did not so obviously decrease with the control group. According to the comparison of HA and LN of the experimental group and those of control group after treatment, there is a great differentiation. The lesser changes in blood serum shown by the control group can be entirely attributed to the cessation of alcohol consumption by that group during the duration of the test, while the greater improvement noted in the experimental group must be due to other factors. That is to say that this *Immune-Assist* product has great functionality for anti-fibrosis and protection of liver in these advanced liver dysfunction cases.

4.2.2 ALT and AST: Among 48 patients in the experimental group, 30 had abnormal levels of ALT, and 27 had abnormal levels of AST. After 4 weeks of treatment with *Immune-Assist*, the patients were analyzed and the following results were found: For the 30 abnormal cases of ALT, 10 cases had changed to normal (33.3%), 20 cases have no clear changes. For the 27 abnormal cases of AST, 7 cases changed to normal (25.9%), 20 cases had no clear changes. After 4 weeks treatment for the control group, among 17 abnormal cases of ALT, 6 cases changed to normal (35.3%) and 6 abnormal cases of AST changed to normal (35.3%), 11 cases had no clear changes. There are no apparent differences in the changes in ALT and AST between these two groups.

These results suggest the changes of ALT and AST in the experimental group have no obvious differentiation to the control group before treatment and after treatment. This indicates that this product has no apparent effect on the degradation of these enzymes.

4.2.3 Triglycerides and Cholesterol: Among 48 cases in the experimental group, after 4 weeks of treatment, for the 31 abnormal cases of triglyceride, 30 cases changed to normal (96.7%) and 1 case had no clear changes. Among 28 abnormal cases of cholesterol, 26 cases changed to normal (92.9%). After 4 weeks treatment for the control group, among 16 abnormal cases of triglyceride, 6 cases changed to normal. (37.5%) Among 14 abnormal cases of cholesterol, 5 cases changed to normal (35.7%) and 9 cases have no apparently differentiation. Data shown in Graph 2 below

Graph 2- Hyperlipidemia results after 4 weeks treatment with Immune-Assist



These result suggests the changes in serum triglyceride and cholesterol in the experimental group have obvious differentiation to the control group after treatment. These results would indicate that this product shows effectiveness in normalizing triglycerides and cholesterol.

Efficacy Research in the Laboratory

The Hygienic Food College of the National Medicinal Academy of Zhejiang Province, China was entrusted to research the two main functions of this product to protect the liver and to reduce abnormal levels of blood lipids. The Disease Control Center of Nanjing, China did the animal experimentation. All the results gathered suggest that this mushroom polysaccharide combination has very apparent and profound function in decreasing the blood cholesterol and triglycerides, and at the same time has obvious effect for the chemical protection and repair of the damaged liver, but the effect on degradation of some of the liver enzymes (ALT and AST) was not as apparent. The above results have been reported to the Health Bureau of China for further study.

Discussion

In recent years the diseases of the Alcoholic Liver and high-blood fat have increased quickly. These diseases are not only a great economic burden for the family and society, but also have a great influence on the patients' jobs and quality of life. It is very important for us to look for a reliable food or nutritional supplement to help prevent this alcohol damage and where possible to reliably treat the disease of Alcoholic Liver and the associated hyperlipidemia. According to the references [2-4], the medicinally active mushrooms have a number of beneficial medicinal functions and bio-active

compounds present. It has long been observed that they can improve the patient's overall resistance and disease dormancy period, increase the appetite, ameliorate fatigue, regulate and enhance the patients immunity, etc. By consuming these mushrooms the body's nonspecific immunity is enhanced, there is a measurable improvement in the secretion of IgA, an increase in the function of mononuclear-phagocytes and in the activity of the NK cells, regulation of the immune balance, resistance to the alcoholic damage of liver cell efficiency and acceleration of the restoration and regeneration of damaged liver tissue cells. This study shows that edible fungi have certain curative effect on chemically induced liver damage and in blood lipid reduction. Edible fungi can play an important role in the clinical treatment for these conditions.

Reference:

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Note 1 – The original *Immune-Assist Critical Care Formula* manufactured Aloha Medicinals Inc. is marketed in the form of capsules containing no inactive ingredients or additives, but for the purposes of this study it was decided to administer the compound in the form of tablets to make them indistinguishable from the placebo. Otherwise, the formula used in this study was identical to that of the commercially available products.