

Aloe Vera High Molecular Weight Fractions as Carbohydrate-based Immune Adjuvants

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ABSTRACT

By using the patented hyper-dry system after washing out coloured materials with running water, aloe high molecular fractions (AHM) were obtained in original and natural form containing less than 10 ppm of barbaloin. AHM mainly contained high molecular fractions, such as polysaccharide (acemannan) and glycoprotein (verectin) showing immunomodulatory and anti-inflammatory activities. On the basis of chemical and biochemical properties, AHM were examined for the therapy designed by the implementation of well-controlled clinical trials, and exhibited the efficacy as immunomodulators for viral infection-induced hepatic periportal fibrosis and type 2 diabetic patients, and as wound and ulcer-healing ointment to patients suffering from bedsores. Therefore, AHM were considered to be a novel low-cost and safe drug of natural origin, indicating a possible therapeutic efficacy in prevention of age-related diseases.

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Key words: Aloe vera high molecular weight fractions; Therapeutic efficacy; Hepatic periportal fibrosis; Type 2 diabetes; Bedsores

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INTRODUCTION

Traditional hand-filleted aloe processing is very laborious intensive and does not fully remove aloin and anthraquinones which have laxative effects. Aloe vera gel from parenchyma cells is mucilaginous

jelly and when ground into slurry and exposed to air, the gel is rapidly oxidized and loses its bioactivities by self-enzyme digestion. In order to remove aloin and anthraquinones the gel slurry was passed through carbon-coated plates. And pasteurization by a high temperature short time treatment at 85-95°C for 1-2 min is an effective method to avoid the loss of biological activities of the gel slurry. However, potentially beneficial aloe constituents, such as polysaccharides having over 1×10^6 D molecular weight and glycoprotein having 29×10^3 D molecular weight, were mostly lost or denaturalized, when the removal of aloin and anthraquinones through carbon-coated plates and pasteurization above 60°C were performed.

The review aims to provide potential biological activities of aloe high molecular weight fractions (AHM) prepared by using the patented hyper-dry system in a combination of freeze-dry technique with micro wave and far infrared-ray radiations. The review composed of three parts of clinical studies introduces function of AHM as a natural medicinal plant source without figures and tables, and of contribution of AHM as carbohydrate-based adjuvants to immune responses. Please find figures and tables on internet, in detail, following to the literatures^[1,2,3].

ANTI-FIBROTIC EFFECT OF AHM IN VIRAL INFECTION-INDUCED HEPATIC PERIportal FIBROSIS

The anti-oxidative and anti-fibrotic effects of AHM in patients with liver fibrosis were investigated.

Methods: AHM were prepared from the gel part, by washing with running water using the patented hyper-dry systems in combination of freeze-dry technique with micro wave and far infrared-ray radiations. Barbaloin (aloin) content was less than 10 ppm in the powder form of AHM. The pore size of AHM was 5.090 μm by use of Master size 2000 of Malvern Instrument LTD. AHM contain acemannan having about 1×10^6 D of molecular weight and glycoprotein (verectin) having 29×10^3 D of molecular weight.

Fifteen healthy volunteers as the control group and 40 patients were included. The patients were randomly sub-divided into two equal groups: the conventional group was treated with the conventional treatment with placebo (starch), and AHM group were treated with the conventional treatment with 0.15 g/d AHM, both for 12 consecutive weeks. The patients were investigated before

and after treatment. Serum activity of aspartate aminotransferase (AST), alanine amino-transferase (ALT), alkaline phosphatase (ALP), hyaluronic acid (HA), transforming growth factor- β (TGF- β) and matrix metalloproteinase-2 (MMP-2) were determined. The reduced glutathione (GSH) and malondialdehyde (MDA) levels in liver were assayed and the expression of hepatic α -smooth muscle actin (α -SMA) was identified by immunohistochemistry.

Results: At the start of the study, the hematoxylin and eosin staining revealed fibro-proliferated bile ducts, thick fibrous septa and dense inflammatory cellular infiltration in the patients before treatment. The use of AHM for 12 week significantly ameliorated the fibrosis, inhibited the inflammation, and resulted in minimal infiltration and minimal fibrosis compared to the conventional group. The enzyme activities of the liver (ALT, AST and ALP) were attenuated after treatment in both groups and the decrease in AHM group was more significant as compared with the conventional group. Similar to the AST, the MDA levels were significantly higher before treatment, and were attenuated after treatment in both groups. In contrast, the hepatic glutathione content in the patients were decreased significantly in the AHM group compared to the controls. The serum levels of the fibrosis markers (HA, TGF- β and MMP-2) were also reduced significantly after treatment. The expression of α -SMA was modified in patients before and after treatment as compared with the normal controls. In the conventional group, there was only thin and incomplete parenchymal α -SMA positive septum joining the thickened centrilobular veins, while in the AHM group few α -SMA positive cells were present in sinusoid and lobule after treatment.

Conclusion: Oral supplementation with AHM could be helpful in alleviating the fibrosis and inflammation of hepatic fibrosis patients^[1].

POSSIBLE HYPOGLYCEMIC EFFECT OF AHM ON TYPE 2 DIABETIC PATIENTS

AHM containing less than 10 ppm of barbaloin, polysaccharide (molecular weight 1×10^6 D) and glycoprotein, verectin (molecular weight: 29×10^3 D), were prepared by patented hyper-dry system in combination of freeze-dry technique with micro wave and far infra-red radiation. AHM produced significant decrease in blood glucose level sustained for 6 weeks of the start of the study. Significant decrease in triglycerides was only observed 4 weeks after treatment and continued thereafter. No deteriorate effects on kidney and liver functions were apparent.

Treatment of diabetic patients with AHM may relief vascular complications probably via activation of immune system^[2].

CASE REPORTS OF BEDSORE USING AHM

It has been reported that the Japanese have higher rate of life expectancy and that Japan is rapidly becoming a super aging society. Almost 5 per cent of the aged, who develop diseases such as cerebrovascular related disorders, mental problems, fractures, cancer and infections, also end up suffering from bedsores. It has been found that patients often opt for treatment, when the bedsores become very severe and adding more complications in their treatment. It may be presumed that prevention and early detection are vital for the treatment of bed sores. In our present study, we have tested herbal formulations having Aloe vera gel powder for its efficacy and activity on bedsores. AHM was prepared from the gel part, by washing with running water using the patented freeze-drying under micro wave and far infra red irradiations in which barbaloin content was less than 10

ppm in powder form.

The clinical treatments were given by application of the macromolecule aloe (AHM) ointment for bedsores from I degree to II degree ulcer patients. The results have shown that AHM in the ointment form indicated a high possibility to cure bedsores. It is very difficult to cure, due to the patients' peculiar conditions such as old age, inability of the patients to turn by himself/herself and also due to complications caused by other symptoms. We were able to confirm the effectiveness of the macromolecule aloe ointment in four cases of bedsores with two cases of positive control, using the Design Score and by checking the side effects. Our hypothesis is that polysaccharides as adjuvants can physically absorb and hold the exudates through the osmotic pressure differences, so that the desirable moisture condition to cure wounds is maintained.

Present report showed efficacy of the preclinical trials for bedsores by the external use of AHM ointment^[3].

CONTRIBUTION OF AHM AS CARBOHYDRATE-BASED ADJUVANTS TO IMMUNE RESPONSES

AHM with 5.09 μ m pore-size obtained by patented process, were reported to have anti-fibrotic effect which could be attributed to its ability to attenuate oxidation stress and to enhance the collagenolytic activity. A main ingredient, neutral poly mannan (acemannan), was obtained at a concentration of about 90% in AHM.

The adjuvant properties of a poly dispersed β -(1-4) linked acetylated mannan (ACE-M) were evaluated by Chinnah, *et al*^[4] showing that ACE-M has the effect on flock immunity to Newcastle disease virus and infectious bursal disease virus. Acemannan also stimulated the adjuvant activity due to its capacity to promote differentiation of immature dendritic cells^[5]. Intranasal immunization using HBsAg-acemannan formulations demonstrated the enhancement of the immune responses to nasally administered hepatitis B surface antigen, generating IgG antibody-titers in high level in mice serum^[6]. Acemannan was able effectively and durably to increase the activity of macrophages from the systemic immune compartment in chicken, especially nitrogen oxide (NO) production^[7]. Also human clinical data demonstrating increased bioavailability of vitamins C and E when taken in combination with aloe vera gel powders were presented^[8]. Aloe vera gel products improved the absorptions of vitamins C and E as an adjunct and arginine as a precursor of NO for people who take vitamins and amino acids supplements.

Verectin, composed of carbohydrate and protein in a ratio of 10.7 and 82.0%, respectively, with molecular weight of 29×10^3 D, was obtained in a ratio of 20% in AHM by enzyme-linked immunosorbent assay. Verectin was stable at least for 4 years at 4°C under desiccative conditions and was destroyed completely under pasteurization at 80°C for 1 h^[9]. Verectin, a radical scavenging glycoprotein in AHM, inhibited cyclooxygenase-2 and thromboxane A₂ synthase, suggesting the participation to human immune system^[10]. The verectin-derived N-terminal octapeptide (Asp-Glu-Asp-Asn-Val-Leu-Leu-Thr) exerted a significant in vivo inhibitory effect on the growth of tumor (Ehrlich ascites carcinoma cells progression), which correlated well with the prolongation of animals' life span^[11].

CONCLUSIONS

The remarkable point of AHM with 5.09 μ m pore-size, processed by new technology is that it does not lose the high molecular structure

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with acetyl groups in poly (1-4)-linked mannan and verectin of an octapeptidyl moiety linked-polysaccharide first time.

The study of AHM with verectin and acemannan provides evidence that AHM could be used as biodegradable safe and non-toxic adjuvants, and as an enhancer, through prevention of hepatocellular damage in hepatic fine periportal fibrosis, activation of immune systems in type 2 diabetic patients, and wound-healing property in bedsore patients.

The finding of aloe vera plant extract to immune response systems points out novel low-cost drug discoveries of natural origin, which can be afforded by developing countries.

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