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ANTI-DIABETIC AND ANTI-HYPERLIPIDEMIC ACTIVITIES OF GLUCOMANNAN ISOLATED FROM *ARAUCARIA CUNNINGHAMII* SEEDS

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ABSTRACT

The aim of the study was to isolate, characterize and to investigate the antidiabetic and anti-hyperlipidemic activity of glucomannan from the seeds of *Araucaria cunninghamii*. Chemical structures, molecular weight, crystalline structure of glucomannan were investigated by FTIR, NMR and X-ray diffraction. The anti-diabetic and anti-hyperlipidemic activity was investigated in streptozotocin induced diabetic rats. Diabetes was confirmed after 72 hrs of single intraperitoneal injection of streptozotocin (60 mg/kg) in albino Wister rats. Glucomannan (25 and 50 mg/kg) and glibenclamide (4 mg/kg, p.o.) orally administered daily for 15 days, blood was withdrawn for glucose determination on 0, 1, 10 and 15 days respectively. On the 15^{th} day, overnight fasted rats were sacrificed and blood was collected for the determination of high density lipoproteins cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), total cholesterol (TC) and total glycerides (TG). The results revealed the presence of glucomannan with a backbone of (1, 4)-linked β-D-mannopyranosyl residues and β-D-glucopyranosyl residues and was demonstrated to be composed of D-mannose and D-glucose in the molar ratio of 1/0.13 and having a relatively low degree of branching at C-6 positions of the hexose residues. The molecular weight of glucomannan was found to be 1.102×10^6. It was found to be amorphous in nature. Glucomannan at doses of 25 and 50 mg/kg showed significant reduction is blood glucose, lipid profiles. We concluded that glucomannan possess anti-diabetic and anti-hyperlipidemic activities.

Key words: *Araucaria cunninghamii*, glucomannan, isolation, characterization, anti-diabetic and anti-hyperlipidemic activity.

INTRODUCTION

Araucaria cunninghamii is a species of Araucaria which is also known as Morton Bay Pine or Hoop Pine. Other commonly used names include Colonial Pine and Richmond River Pine. The species is found in the coastal rainforests of Eastern Australia and in New Guinea. The trees can live up to 450 years and can grow up to a height of about 60 meters. The bark is rough and splits naturally, but does not peel. The leaves on young trees are awl-shaped, 1-2 cm long, about 2 mm thick at the base and scale-like, incurved, 1-2 cm long and 4 mm broad on mature trees. The cones are ovoid, 8-10 cm long and 6-8 cm diameter and takes about 18 months to mature. They disintegrate at maturity to release the nut-like seeds (Ash et al, 1983). It has been used as food and food additives in China and Japan for more than 1000 years. Glucomannan (GM) is a polysaccharide of the mannan family, very abundantly available in nature, specifically in softwoods (hemicelluloses), roots, tubers and many plants bulbs. Despite the variety of sources, the most commonly used type of GM is named as konjac glucomannan (KGM) or konjac flour, which is extracted from tubers of Amorphophallus plants. Irrespective of its origin, GM is composed of β-1, 4-linked D-mannose and D-glucose monomers. There may be certain short side branches at C-3 position of the mannoses and acetyl groups randomly present at the C-6 position of a sugar unit (Kato et al, 1969; Smith et al, 1959). The acetyl group frequently ranges from 1 per 9 sugar units to 1 per 20 sugar units (Kato et al, 1969; Koroskenyi et al, 2001). In addition, there may be some differences in molecular structure of KGM from different species. However, the mannose/glucose monomer ratio may vary depending on the original source of GM. For example, it has been reported that konjac GM has a molar ratio of around 1.6:1, whereas GMs extracted from Scotch pine and orchid tubers with ratios of 2.1:1 and 3.6:1 respectively. These values should be regarded cautiously given the variability observed depending on the studies and in particular, on the analytical procedures (Ishrud et al, 2001).

The studies on glucomannan revealed that glucomannan has many applications in many fields. Due to its biodegradability and gel-forming ability, konjac glucomannan can be widely used for the manufacture of various dosage forms (Wang et al, 2002). Glucomannan has very good film-forming ability, so that several kinds of transparent blend films of konjac glucomannan with polyacrylamide, gelatin, sodium carboxymethyl cellulose, chitosan, xanthenes, sodium alginate and cellulose were formulated (Li et al, 2000; Xiao et al, 2000; Xiao et al, 2002; Ye et al, 2006). In medicine field konjac glucomannan and its derivatives were used to lower blood cholesterol and sugar level, helps in weight loss; promotes intestinal activity and immune function (Vuksan et al, 1999). Although they had been investigated for so many years, glucomannan and its derivatives still needs to be exploited compared to other polysaccharides such as cellulose, starch etc.

Among the 18 species of *Araucaria* found in the coastal rainforests of Eastern Australia and New Guinea, the cunninghmii species is not yet exploited. Therefore, the present work to study the antidiabetic and anti-hyperlipidemic activity of glucomannan isolated from the seeds of *Araucaria cunninghamii*.

MATERIALS AND METHODS

Material: The seeds of *Araucaria cunninghamii* were purchased from Bharat Vastu Bhandar (Herbs, seeds and plants merchants), Dhamawala bazaar, Dehradun and was authenticated by Prof. Madavachetty, SV university, Tirupathi.

Chemicals: Streptozotocin (STZ) was purchased from Sigma Aldrich chemicals, Germany. Acetic acid and sodium hydroxide was purchased Rankem laboratory, New Delhi. Sodium chlorite was purchased from G. S. chemicals, Bombay. Potassium hydroxide was purchased from M/S Hi-media Ltd., Bombay. Boric acid, barium hydroxide and ethanol were procured from Changshuyangyuan chemicals, China. All other chemicals and reagents used in experiments were of analytical grade.

Isolation and purification of glucomannan from *Araucaria cunninghamii*: The seeds of *Araucaria cunninghamii* were shade dried and powdered. About 100 gm of dry powder was suspended with stirring in water (1.67 L) at 70-80ºC. Acetic acid (11 mL) and sodium chlorite (33.3 gm) were added for every hour for a total period of 7 hours. The reaction mixture was cooled and the solid was washed by decantation with tap water. They were then transferred to a filter and washed with distilled water and ethanol. The white product was dried in the air to yield (78 gm) of holocellulose.

The holocellulose (78 gm) was shaken with aqueous potassium hydroxide (560 mL) at room temperature for 3 hours. The alkaline extract was removed by filtration through whatmann60filter paper and the residue was washed with water. The wet residue was extracted in the same 61way with 17.5% sodium hydroxide to which 4% boric acid had been added. The alkaline 62extracts and washings (745 mL) were poured into ethanol (2.780 mL) containing glacial acetic 63acid (80 mL). The precipitate formed was washed on the centrifuge with 80% aqueous 64ethanol, ethanol and petroleum ether (b.p. 30-60ºC).

Crude glucomannan (4 gm) was dissolved in 10% sodium hydroxide (100 mL) and 5% aqueous barium hydroxide (200 mL) was added with constant stirring over a period of 2 hours. The precipitate formed was collected by centrifuging, washed twice with water, acidified with acetic acid and poured into ethanol (500 mL). The precipitate was recovered in the usual way. Lastly, the glucomannan was dried at 50ºC for 3 hours and used for further characterization. The polysaccharide content (PC) was calculated using the following formula

$$\text{PC } \% = \left(\frac{\text{m1}}{\text{m2}}\right) \text{x} 100$$

Where m_1 and m_2 weight of final white powder and original *Araucaria cunninghamii* seeds, respectively (Jabber mian, Timel, 1960).

Characterization of glucomannan: ^{1}H NMR and ^{13}C NMR spectra of *Araucaria cunninghamii* glucomannan were recorded on the 400MHz Bruker spectrometer. The sample concentrations being about 5 and 20 gm/L, respectively, in D_2O at 300 K. X-ray diffraction pattern for the *Araucaria cunninghamii* glucomannan was analyzed using a Siemens D5000 (Japan) diffractometer equipped with a Cu Kα target at 40 kV and 30 mA with a scan rate of 4º/min. The diffraction angle ranged from 2θ =5º to 2θ =65º. FTIR spectrum of the *Araucaria cunninghamii* glucomannan was recorded on the Perkin Elmer Spectrum1 FT-IR spectrometer under dry air at room temperature using KBr pellets in the range between 450-4000 cm−1. The peaks were assigned by comparison with the data reported in the literature (Zhang et al., 2001). The intrinsic viscosity of *Araucaria* glucomannan was measured by Ubbehold viscometer according to methods of Li and Wanchun.

Animals: Male Swiss Albino Wistar rats weighing 150–250g were acclimatized to the experimental room at temperature 23±2°C, controlled humidity conditions (50–55%) and12 hrs light and dark cycle. They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water ad libitum. All the studies conducted were approved by the Institutional Animal Ethical Committee of Ratnam Institute of Pharmacy (Reg.No.1558/PO/a/11/CPCSEA), Pidathapolur, Andhra Pradesh, according to prescribed guidelines of CPCSEA, Government of India.

Experimental induction of diabetes: The animals were fasted for 12 hrs prior to the induction of diabetes (Adaramoye et al, 2006). The rats were injected intraperitoneally with streptozotocin dissolved in ice cold citrate buffer (pH 4.3) at a dose of 60 mg/kg body weight. 5% glucose solution was administered orally for 24 hrs to prevent mortality due to initial hypoglycemia induced by streptozotocin. After 72 hrs of streptozotocin injection, fasting blood glucose levels were tested using glucose oxidase-peroxidase method. Rats showing fasting blood glucose more than 200 mg/kg were considered diabetic and used for further study.

Experimental design: The rats were randomized into five groups comprising of six animals in each groups after the induction of streptozotocin diabetes.

Group I: Normal control rats received citrate buffer daily for 15 days
Group II: Diabetic control rats received citrate buffer daily for 15 days
Group III: Diabetic rats received glucomannan (25mg/kg/day. p.o.) for 15 days
Group IV: Diabetic rats received glucomannan (50mg/kg/day. p.o.) for 15 days
Group V: Diabetic rats received with glibenclamide (4mg/kg/day, p.o.) for 15 days

Blood samples were collected from retro-orbital plexus of each rat under mild anesthesia at 0, 1, 10 and 15th day of the experiment from all the groups of experimental rats and serum glucose was estimated by enzymatic glucose oxidase method. On 15th day of the study, blood samples were collected for biochemical estimations. Later animals were sacrificed and liver was removed, cleaned and washed in ice-cold normal saline for biochemical study.

Biochemical analysis: Serum total cholesterol (Demacker et al, 1980), total glycerides (Foster et al, 1973), LDL-C, VLDL-C (Friedwald et al, 1972) and HDL-C (Assmann et al, 1983) were estimated using standard enzymatic kits (ERBA diagnostic Mannheim GMBH, Germany) spectrometrically. Total protein was estimated by the method of Lowery et al, (1951) using bovine serum albumin as a standard.

Statistical analysis: Results were expressed as the mean ± S.E.M. for statistical analysis of the data group means, were compared by one-way analysis of variance (ANOVA) followed by Dunnets test. $p < 0.001$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Isolation of glucomannan from *Araucaria cunninghamii*: In recent years, many studies targeted on research on glucomannan and its applications. The isolation and purification of glucomannan from Araucaria plants were the important steps in the study process. According to the traditional methods, the isolation and purification process were often carried out in the dried Araucaria seeds (Ye et al, 2006). In this isolation process, the glucomannan was isolated from *Araucaria cunninghamii* species without using hazardous chemicals that pollutes the environment and limits the ability of applying of this product especially as a food and medicine for human. The yield of glucomannan was found to be 3.5 gm. The isolated compound was characterized by FTIR, ^{1}H NMR, ^{13} C NMR, GC-Mass spectroscopy and X-ray diffraction. The molecular weight of glucomannan isolated from *Araucaria cunninghamii* was 1.102×10^{6}.

X-ray diffraction: The X-ray curve of cunninghamii glucomannan is shown in **Figure 1**. As observed, the pattern of polysaccharide (Figure 1), exhibited a non crystalline state and only had a very broad peak around 2θ = 20–45º, which was consistent with the data reported by Xu, Li, Kennedy, Xie, and Huang (2007).

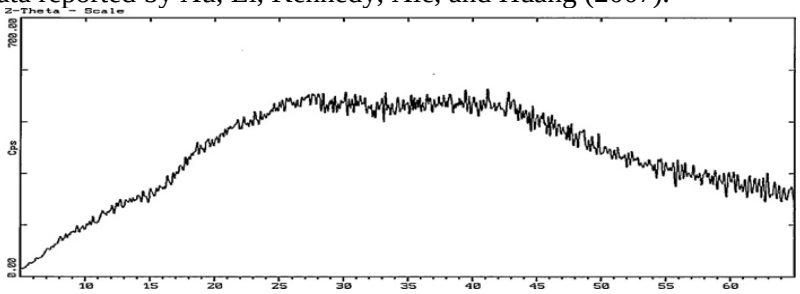

Figure1: XRD pattern of glucomannan isolated from *Araucaria cunninghamii*

FTIR analysis: The FTIR spectrum of the glucomannan in the wavelength range of 4000–400 cm^{-1} is shown in **Figure 2**. In the spectrum of polysaccharide, the wide band observed at 3000–3700 cm^{-1} could be attributed to the O–H stretching of the glucomannan. The band at 2922 cm^{-1} was attributed to the asymmetric stretching of C–H, while the band at 1577 cm−1 was ascribed to adsorbed water and the bands at 1410 and at 1280 cm^{-1} due to the angular deformation of C–H. The C–O ether bond has shown stretching at about 1162 cm^{-1}, while the C–O alcohol bond stretching was shown at 1112 and 1048 cm^{-1}. The characteristic peaks observed at 808–900 cm^{-1} were assigned to the β-pyranose between mannose and glucose unit. The peak at 1628 cm^{-1} was attributed to carbonyl of acetyl group. These results were in agreement with the data reported by Zhang et al. (2001).

^{1}H NMR analysis: The ^{1}H NMR spectrum of the glucomannan from *Araucaria cunninghamii* was shown in **Figure 3**. The peaks were assigned by comparison with chemical shift data reported in the literature (Ishurd et al, 2006). Figure 3 showed that the signals attributed to the hydrogen have linked to C2–C6 of both glucose and mannose units were not well separated. This was due to the complex nature of the spectra of polysaccharides. Meanwhile, the signals attributed to the hydrogen- H1 linked to the carbon-C1 of both glucose unit (4.993ppm) and mannose unit (5.047 ppm) were well separated. Therefore, the mannose/glucose ratio in glucomannan molecule could be calculated using

the integrals of proton in the ^{1}H NMR spectrum. According to this method, the mannose/glucose molar ratio in glucomannan molecule from *Araucaria cunninghamii* was found to be 1/0.13.

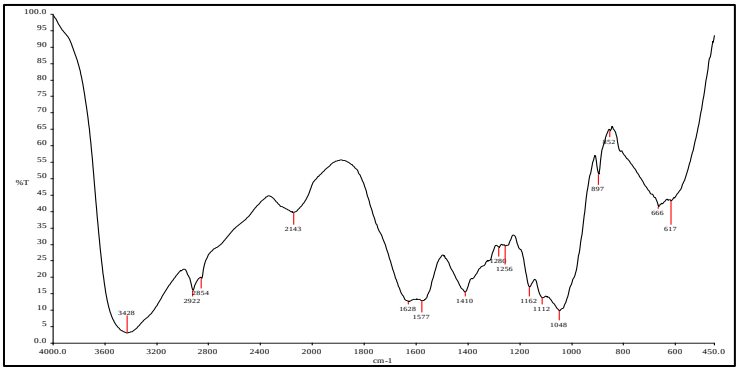

Figure 2: FTIR spectrum of glucomannan isolated from *Araucaria cunninghamii*

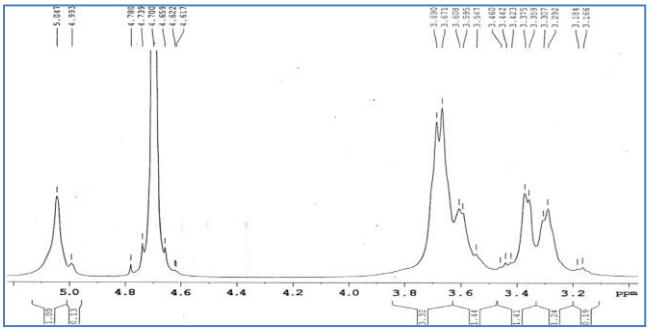

Figure3: ^{1}H NMR spectrum of glucomannan isolated from *Araucaria cunninghamii*.

^{13}C NMR analysis: The ^{13}C NMR spectrum of glucomannan from *Araucaria cunninghamii* was shown in the **Figure 4**. The ^{13}C NMR spectrum showed characteristic anomeric signals at 181.52 ppm due to C-1 resonances of β -D-glucose residues at 176.82 ppm due to C-1 resonances of D-mannose residues. The C- 4 chemical shifts of the glucosyl and mannosyl units involved in glycosidic linkages appeared at 137.88 and 135.40 ppm, respectively. The signals at 101.41, 73.81 and 72.34 ppm were assigned to C-5, C-3 and C-2 of mannose residues, respectively. The characteristic resonances of C-5, C-3 and C-2 of β-1, 4-linked glucose residues were observed at 101.41, 76.52 and 72.85 ppm, respectively. The signals in the high magnetic field at 59.89–59.91 ppm were generated by the resonances of non substituted C-6 of glucosyl and mannosyl residues. Besides, a low intensity signal at 63.10 ppm could be assigned to substituted C-6 of glucosyl or mannosyl residues. Thus, the results confirmed a linear structure of glucomannan composed of 1,4-linked D-mannosyl and D-glucosyl units in the mole ratio of 1/0.13 respectively. The β configuration of glycosidic bond in the main chain and the presence of short side chains at C-6.

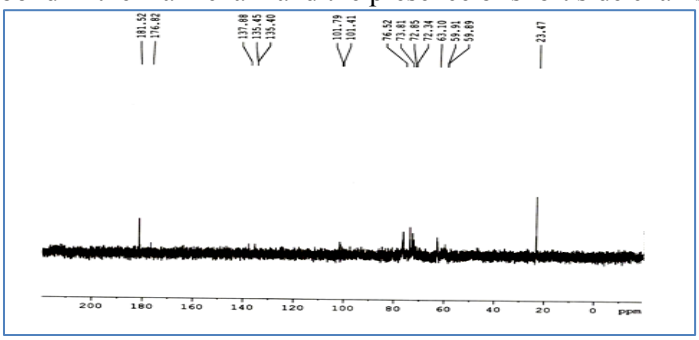

Figure 4: ^{13}C NMR spectrum of glucomannan isolated from *Araucaria cunninghamii*.

Anti-diabetic effect of glucomannan : Glucomannan (25 and 50 mg/kg) and glibenclamide (10 mg/kg) were given orally to the different groups and showed significant ($p < 0.01$) percentage reduction in glycemia. **Table 1** shows the effect of glucomannan on blood glucose levels of diabetic and animals after the daily treatment (25 and 50 mg/kg) for 15 days. Showed significant ($p < 0.001$) percentage fall in blood glucose levels with the doses of 25, 50 mg/kg of glucomannan and 4 mg/kg of glibenclamide as compared to diabetic control group.

Table 1: Effect of glucomannan from *Araucaria cunninghamii* on blood glucose level in STZ induced diabetic in rats

Groups	Treatment	Blood glucose levels mg/dl			
		0 day	1 day	10^{th} day	15^{th} day
1	Normal control	84.57± 1.12	87.21± 0.91	79.30 ±1.47	83.64 ±2.2
2	Diabetic control	310.34 ±5.21	352± 4.83	379.4± 5.07	343.11± 3.09
3	Diabetic control+ Glucomannan (25 mg/kg)	260.7± 1.91*	220.4± 2.68*	85.66± 1.14*	84.27± 3.8*
4	Diabetic control+ Glucomannan (50 mg/kg)	245.49± 1.6*	189.72± 3.4*	81.53± 1.08*	82.8± 1.3*
5	Diabetic control+ Glibenclamide (4 mg/kg)	275.4 ±4.2*	181.2 ±0.95*	73.61± 4.8*	80.7± 5.2*

Values are expressed as Mean ± S.E.M; n=5. Statistical significance: (*p<0.01). One way ANOVA followed by Dunnett test.

Antihyperlipidemic effect of glucomannan: Streptozotocin treatment resulted in significant ($p < 0.01$) elevation of TG, TC, VLDL-C, LDL-C, and reduction of HDL-C levels as compared to the normal control rats. Glucomannan (25 and 50 mg/kg) and glibenclamide (4 mg/kg) significant ($p < 0.01$) reduction in elevated TG, TC, VLDL-C, LDL-C and HDL-C level was restored respectively when compared to diabetic control, the results are shown in **Table-2.**

Table 2: Effect of glucomannan from *Araucaria cunninghamii* on lipid profile in STZ induced diabetic in rats

Groups	Treatment	HDL-C	LDL-C	VLDL-C	TC	TG
1	Control	34.12± 0.87	22.27± 0.33	13.81 ±0.76	61.92± 0.63	65.23± 1.24
2	Diabetic control	13.69± 1.4	108.48± 2.61	36.98± 1.47	180.24 ±1.71	161.48± 1.59
3	Drug control+Glucomannan (25 mg/kg)	21.22± 0.79*	54.72± 1.19*	23.8± 0.52*	120.4± 0.42*	95.06 ±1.62*
4	Drug control+Glucomannan (50 mg/kg)	28.8± 0.15*	24.51± 1.73*	14.66± 0.33*	63.9 ±2.12*	67.7± 0.33*
5	Drug control+Glibenclamide (4 mg/kg)	30.6 ±0.9*	27.12± 0.82*	12.5± 1.91*	62.7 ±1.93*	68.8± 1.29*

Values are expressed as Mean ± S.E.M; n=5. Statistical significance: (*p<0.01). One way ANOVA followed by Dunnett test.

Conclusion

A simple method without using toxic chemicals for isolating cunninghamii glucomannan from *Araucaria* seeds has been investigated. Glucomannan was white, hard to dissolve in water and its content was about 5–9% (w/w). The structure and molecular weight of glucomannan have been investigated by FTIR, NMR and Mass spectroscopy. The crystalline natures of glucomannan were investigated by X-ray diffraction. The results showed that the structural component of glucomannan from *Araucaria cunninghamii* consisted of β-1, 4-linked D-mannosyl and D-glucosyl units in the mole ratio of 1/0.13. The presence of short side chains at C-6 was also observed. The molecular weight of glucomannan from these above Araucaria species was 1.102×10^6. Glucomannan at doses of 25 and 50 mg/kg showed significant reduction is blood glucose, lipid profiles. The cunninghamii glucomannan from *Araucaria* species in Australia exhibited a potential application as both a food and medicine for humans.

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