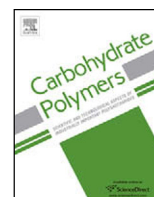
Carbohydrate Polymers 102 (2014) 1–7

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol



Isolation, preliminary characterization and hepatoprotective activity of polysaccharides from *Tamarindus indica* L.

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ARTICLE INFO

Article history:
Received 5 July 2013
Received in revised form 17 October 2013
Accepted 18 October 2013
Available online 27 October 2013

Keywords:
Tamarindus indicus
Hepatoprotective
Polysacharide
SGOT
SGPT

ABSTRACT

Polysaccharide was isolated from *Tamarindus indica* L. (TIP) and was characterized in terms of moisture and ash content, pH, water holding capacity, particle size, tapped density, bulk density, carr's index, Hausners ratio, angle of repose, content of glucose, uronic acid and sulfate. Morphological, spectral (UV–vis, FTIR) and DSC thermal analysis reveals polysaccharide nature of the isolated starch. DPPH radical scavenging activity of TIP shows RSA comparable to that of silymarin. Hepatoprotective potential of TIP in terms of biochemical parameters, SGOT, SGPT, ALP and BRN were significantly increased ($P < 0.05$) and reduction of serum Total protein in the group of rats given thioacetamide (100 mg/kg s.c.). Histopathology reveals that TIP under antagonize the effect of thioacetamide by acting, either as membrane stabilizer, thereby preventing the distortion of the cellular ionic environment associated with thioacetamide intoxication, or by preventing interaction of thioacetamide with the transcriptional machinery of the cells.

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1. Introduction

Liver is the major site for metabolism, elimination and detoxification of drugs and chemicals (Akindele, Ezenwanebe, Anunobi, & Adeyemi, 2010, Mihailovic et al., 2013). The metabolism of toxic chemicals, drugs, and virus infiltration from ingestion or infection leads to generation of reactive oxygen species within hepatocytes results in hepatic damage, gross cellular change and cell death causing hepatotoxicity or liver damage (Jain et al., 2011). Further Hepatitis and drug related hepatotoxicity is the leading cause of acute liver failure (Wang et al., 2008). Hepatic problems are responsible for a significant number of liver transplantation and death. Available pharmacotherapeutic options for liver diseases are very limited and there is a great demand for the development of new effective drugs (Akindele et al., 2010). There is absence of a reliable liver protective drug in the modern system of medicine, still the current treatment scenario for liver disease include pharmacotherapy, surgery as well as liver transplantation, all of which have shown limited therapeutic benefits and are associated with serious complications. Treatment with steroids, vaccines, and antiviral drug has met with poor therapeutic success and is associated with serious risks of toxicity, especially if administered chronically or sub-chronically. Clearly there exists a critical need for exploring novel and alternative approaches for the treatment of liver disease (Bishayee, Darvesh, Politis, & McGory, 2010; Srivastava & Shivanandappa, 2010).

Tamarindus indica L. (TI) is a tree-type of plant which belongs to the Leguminosae, family. Its fruit is rich with polysacharides having wide pharmacological activities such as digestive, carminative, laxative, expectorant, blood tonic, antioxidant, antihepatotoxic, antiinflammatory, antimutagenic and antidiabetic (Martinello et al., 2006) activities. Further traditional healers of Chhattisgarh state of India use powder of TI seeds as treatment of Liver disorders. The natural hydrocolloid composed of complex carbohydrate macromolecules has a wide scale of physicochemical properties which are essential in the traditional medicine and pharmacy. Polysaccharides can have a number of effects including anti-inflammatory, immunostimulating, complement activation, antitrombotic, antidiabetic, and infection protective activities (Thakur, Bhargava, Praznik, Loeppert, & Dixit, 2009). Further Polysaccharides and natural compounds widely existed in plants, animals and microorganism, have been demonstrated to possess potent antioxidant activity and to protect liver injury induced by various chemicals. So a systematic attempt has been taken to isolate polysaccharide from *T. indica* L. seed (TIP) and its characterization and exploration of hepatoprotective activity.

2. Materials and method

2.1. Isolation of polysacharide

Water soluble starch was isolated as per the method of Deepika, Kumar, and Anima (2013a) with slight modification. Seed kernel of

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http://dx.doi.org/10.1016/j.carbpol.2013.10.059

TI was obtained from tribal belt of Chhattisgarh, India and shade dried. One Kilogram of kernel was extracted with petroleum ether at room temperature for 72 h with occasional stirring in order to remove any fat present in it. Then steeped in to citric acid solution (0.3%, w/w) to separate the starch from mucilage and then milled with distilled water using a blender. Resulting paste was then thoroughly washed using distilled water until washing water got clear. Starch suspension was allowed to settle and water was decanted before drying.

Crude starch thus obtained was allowed to dry in air for 48 h.

2.2. Characterization of polysacharide

2.2.1. Morphology of starch granules

The external morphology of the starch was analyzed by optical microscope (Carl Zeiss, Primostar). The isolated starch was stained with water and the photograph was taken at 10× and 40×.

2.2.2. Moisture content, ash value, pH and mineral content

For the determination of moisture content 5 g of sample was kept at 105 °C in a hot air oven till constant weight. Ash content was determined by incinerating a known amount sample in a muffle furnace at 500 °C for 12 h, till a complete white mass was obtained. The value was expressed in %. For determination of pH of 1% suspension of sample was prepared in water and was determined by using digital pH metre. Further 0.2 g of sample was first digested with HNO_3/H_2O_2 mixture in microwave digester. The clear solution obtained was filtered and used for determination of mineral contents with the help of a inductive coupled plasma optical emission spectrometer (ICP-OES) (optical 2100DV, Perkin Elmer, USA).

2.2.3. Water holding capacity

For determination of water holding capacity, 1 g of polysacharide in 15 ml of distilled water was stirred for 1 h. The free water was poured off from wet starch. After draining for 10 min, the wet starch was weighed and the result was expressed as percent (w/w) on dry basis (Deepika, Kumar, and Anima, 2013b).

2.2.4. Micromeritics properties

The isolated starch was characterized by their micromeritic properties such as particle size, bulk density, tapped density, carr's index, Hausner's ratio and angle of repose. For the determination of particle size about 1 mg of starch is dispersed in 10 ml of dispersed media and particle size was observed with the help of SALD particle size analyzer (Shimadzu). Bulk density was determined by taking accurately weighed amount of sample in a 100 ml graduated cylinder, the powder level was noted. The bulk density was calculated in g/cm^3 by the formula. Bulk density = mass of powder taken/volume. The tapping method was used to determine the tapped density by using the formula tapped density = mass of powder after tapping/volume of powder after tapping. After determination of tap density and bulk density carr's index and Hausner's ratio was calculated using the formula. C.I = tap density − bulk density/tap density × 100, Hausner's ratio = tapped density/bulk density. For the determination of angle of repose a glass funnel was held in place with a clamp on a ring support over a glass plate. The glass plate is placed on a stand. Approximately 50 g of powder was transferred into funnel keeping the orifice of the funnel blocked by the lower thumb. As the thumb is removed, the particles are emptied from funnel, and the angle of repose is determined by above mentioned formula and calculated as $\tan\theta = 2H/D$, where 2H/D is the surface area of the free standing height of the microspheres heap that is formed on a graph paper after making the microspheres flow from the glass funnel.

2.2.5. Estimation of total sugars, sulfate, protein and uronic acid

The total sugar content of CCPS was determined using the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The protein content was determined by the method of Bradford using bovine serum albumin as the standard (Bradford, 1976). The content of uronic acid was determined according to the method of Blumenkrantz and Asboe-Hansen (1973) using D-glucuronic acid as the standard. The content of sulfate radical was determined according to the reported method (Doigson & Price, 1962).

2.2.6. UV spectral analysis

Starch sample of 10 μg/ml was prepared with water; UV scanning was done between 200 and 400 nm using UV-double beam spectrophotometer-1800 (Shimadzu, Japan).

2.2.7. Thermal analysis

Thermal behaviour of the isolated starch was determined from Differential Scanning Colorimetry (DSC) thermogram. The instrument used for analysis is DSC-50 SHIMADZU, Japan. Weighed amount of starch was taken in aluminium pans and sealed. Empty closed aluminium pan was used as the reference cell. Samples were scanned from 20 °C to 300 °C at a heating rate of 10 °C/min. All the samples were equilibrated for 15 min at the starting temperature. The thermal analysis was carried out in nitrogen atmosphere.

2.2.8. FTIR spectroscopy

IR spectra of the isolated starch was taken with the help of a FTIR Spectrophotometer (Shimadzu-IR Affinity^{-1}) scanned with wave number range of 400–4000 cm^{-1}. The characteristic peaks were determined.

2.3. In Vitro antioxidant activity (DPPH radical-scavenging activity)

The free radical-scavenging activity of isolated polysacharides was evaluated using the stable radical DPPH, according to the method of Grzegorczyk et al., 2007. 2, 5, 10, 20, 50,100, 150, 200, 250, and 300 μg/ml of polysacharide, prepared in water. One ml of these solutions were added to 1 ml of a 0.1 mM methanolic solution of DPPH followed by stand for 30 min at 27 °C. The absorbance of the sample was measured at 517 nm with the help of UV-Visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). Same procedure has been followed for Silymarin. DPPH radical-scavenging activity (RSA), expressed as percentage, calculated using the following formula: $\text{RSA }(\%) = A_{DPPH} - (A_{sample} - A_{control})/A_{DPPH} \times 100$ where A_{DPPH} is the absorbance of DPPH solution without sample extract, A_{sample} is the absorbance of sample extract mixed with DPPH solution and $A_{control}$ is the absorbance of the sample extract tested without DPPH. DPPH RSA of polysacharide was compared between Silymarine (AA) and polysacharide, with the same concentration.

2.4. Hepatoprotective activity

2.4.1. Experimental animals

Wistar albino rats (100–150 g) of either sex were used in the study. The study was carried out on mixed sex of Wistar albino rats. The rats were obtained from Departmental animal house of Guru Ghasidas University, Bilaspur. The rats were kept in standard environmental conditions (temperature 25–28 °C and 12 h light/12 h dark cycle).

2.4.2. Acute oral toxicity

Acute toxicity study was performed according to OECD guideline-420. Different doses (50–2000 mg/kg, p.o.) of

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