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

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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.

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 polysachharides 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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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 polysaccharide

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 polysaccharide 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 polysaccharides 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 polysaccharide, 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: $RSA\ (\%) = A_{DPPH} - (A_{sample} - A_{control})/A_{DPPH} \times 100$ 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 polysaccharide was compared between Silymarine (AA) and polysaccharide, 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

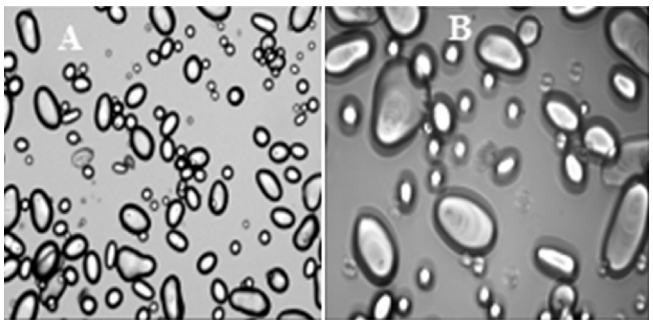

Fig. 1. Photomicrograph of isolated starch (A) 10×, (B) 40×.

polysacharide were administered to group of rats and observed continuously for 1 h and then at half-hourly intervals for 4 h, for any gross behaviour changes further up to 72 h followed by14 days for any mortality.

2.4.3. Thioacetamide-induced hepatotoxicity

The animals were divided into six groups each consisting of six rats. Groups 1 (normal) and 2 (control) received vehicle 10 ml/kg, groups 3, 4 and 5 (test) were given 50,100 and 200 mg/kg of polysacharide and the group 6 (Silymarin, 50 mg/kg, standard) respectively. All the doses calculated with respective body weights of animals and administered orally once daily for 10 days followed by single subcutaneous injection of thioacetamide (100 mg/kg).

2.4.4. Assessment of liver functions

Twenty-four hours after the toxin administration, the rats of each group were anaesthetized and blood was collected directly from the heart. The blood samples were allowed to clot for 20–30 min. Serum was separated by centrifugation at 37 °C and used for estimation of various biochemical parameters. The activities of serum glutamate oxaloacetate transaminase (SGOT) and of serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel (1957). The activity of serum alkaline phosphatase (ALP) was estimated by the method of King and King (1954). The enzyme activity was expressed as U/l. Serum bilirubin (BRN) and total protein (TP) were estimated by the methods of Malloy and Evelyn (1937) and Wooten (1964), respectively. The units were expressed as mg/dl.

2.4.5. Histopathological evaluation

Liver tissue for histopathological analysis was fixed in 10% buffered formalin saline, processed by routine histology procedures and embedded in paraffin. Tissue sections (4–5 μm) were stained with haematoxylin and eosin and examined for possible histopathological changes under high-resolution microscope with photographic facility.

2.4.6. Statistical analysis

Results of the biochemical estimations are reported as mean ± S.D. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA), Student's *t*-test was used for determining significance (Woolson, 1987).

3. Result and discussion

3.1. Characterization of polysacharide

3.1.1. Morphology

Photomicrographs of the isolated starch when observed under microscope (Fig. 1). Shape of granules was found to be irregular, with the diameter ranging between 5 μm and 10 μm. There is presence of eccentric hilum, like that of potato starch.

Table 1
Physicochemical parameters of starch isolated from *Tamarindus indica*.

Sl. no.	Parameters	Result
1	Moisture content (%)	11.21 ± 2.05
2	Ash value (%)	1.02 ± 0.03
3	pH	7.02 ± 0.04
4	Water holding capacity (%)	454.31 ± 5.46
5	Mineral content (PPM)	Ca(14.40),Mg(1.12), Fe(2.46), Mn(0.049), Zn(13.54), Ni(0.017) and Cu(0.034)

3.1.2. Moisture content, ash value, pH, mineral content and water holding capacity

Moisture content, ash value, pH, mineral content and water holding capacity of the isolated starch is shown in Table 1. High moisture content value of isolated starch signifies maximum dry unit weight can be attained as a result of a given compaction effort. The ash content value signifies total amount of minerals present in the starch. From the value of water holding capacity of the starch it can be hypothesize that the higher cohesiveness was caused by the bulky hydrophilic hydroxyl groups that stabilized the gel matrix by interacting with starch chains and water.

3.1.3. Micromeritics properties

Micromeritics is the science and technology of small particles. The size, and hence the surface area of a particle, can be related to the physical, chemical and pharmacologic properties of drugs. Clinically, the particle size of a drug can affect its release from dosage forms that are administered orally, parenterally, rectally and topically (Brittain, 1995; Carstensen, 1993; Martin & Patrick, 2006). Micromeritics Properties such as particle size, bulk density, tap density, carr's index, Hausner's ratio and angle of repose were determined. The particle size of powder was found to be within range of 5–25 μm. The tapped density values found to be 1.42 ± 0.12 g/cm^3, while the bulk density found to be 1.17 ± 0.09 g/cm^3. This may be due to the presence of slight high density of powders. The carr's index and Hausners ratio was found to be 17.6% and 1.21 ± 0.10 respectively. Starch powder showed excellent flowability as expressed in terms of angle of repose (25.21 ± 1.20). The better flow property indicates that starch powder is in non-aggregated state.

3.1.4. Total sugars, sulfate, protein and uronic acid

The results of chemical analysis showed that the contents of neutral sugars and protein in isolated starch were determined to be 78.4 and 5.1%, respectively. Notably, it contained relatively high contents of uronic acid (6.3%) and sulfate (7.7%).

3.1.5. UV spectral analysis

UV–vis spectral analysis of isolated starch shows characteristic absorbance at 664.50 nm, 342.00 nm, 309.50 nm, 259.50 nm, 316.00 nm, 290.00 nm and 233.50 nm. In which maximum absorbance obtained at 259.50 nm (Fig. 2). This spectral data explain the polymeric structure of isolated starch, but not able to explain its structure, but the nature of the spectra can be considered as standard parameter for the evaluation of this starch.

3.1.6. Thermal analysis

Differential scanning calorimetry analysis is a very good technique to determine the physical state of drug either amorphous or crystalline after and before the formulation. In the present study DSC of the isolated starch shows melting transition at 98.61 °C and

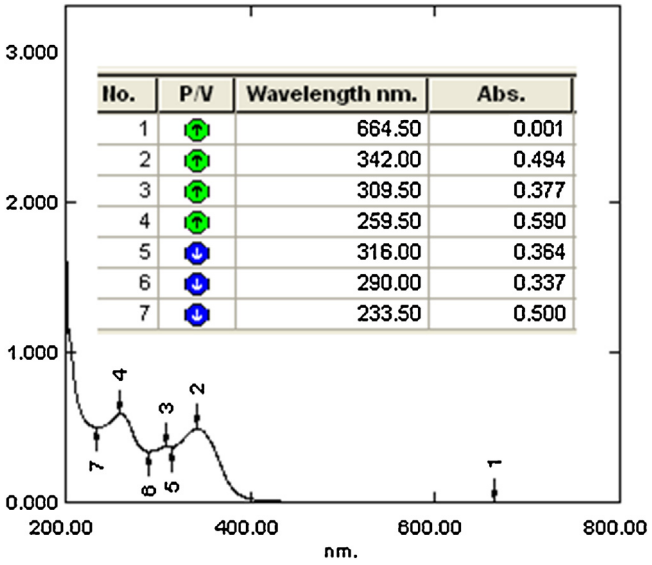

Fig. 2. UV spectral analysis of TIP.

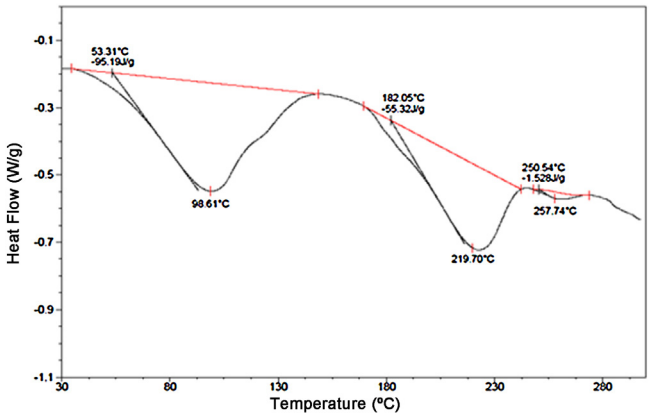

Fig. 3. DSC thermogram of TIP.

219.70 °C (Fig. 3). This justifies polymeric nature of the isolated starch.

3.1.7. FTIR spectroscopy

FTIR spectra of the isolated starch showed characteristic peaks at wave numbers 839.03, 916.19, 997.20, 1026.13, 1051.20, 1076.28, 1111.00, 1149.57, 1203.58, 1224.80, 1296.16, 1338.60, 1373.32, and 1458 cm^{-1} this confirming the polysaccharide nature of the isolated starch. The peaks at 916.19 cm^{-1} and 1149.57 cm^{-1}, indicating the presence of C—O stretching. A prominent band appeared at 1051.20 cm^{-1}, which showed sensitivity to water. Peaks at 1076.28 cm^{-1} and 1149.57 cm^{-1} represents presence of anhydroglucose ring C—O stretch of C—O—H in starch (shown in Fig. 4).

3.1.8. In vitro antioxidant activity (DPPH radical-scavenging activity)

In vitro antioxidant activity of TIP and Silymirin were expressed in terms of %RSA, as shown in Fig. 5. This shows RSA of TIP and Silymirin were increased with respect to increase in concentration shows maximum inhibition of 81.20 ± 3.12% and 88.21 ± 4.26% for the concentration of 300 μg/ml. The EC50 values calculated from the graph shows that the RSA of TIP (EC50 = 99.66 ± 2.34 μg/ml), appeared significantly ($P < 0.05$) lower than that of the Silymarin (EC50 = 5.21 ± 0.27 μg/ml). The EC50 value obtained for TIP is comparable to that of plant/plant parts showing promising hepatoprotective activity.

3.2. Hepatoprotective activity

3.2.1. Acute oral toxicity

For the determination of acute oral toxicity in the groups treated with maximum dose of 2000 mg/kg, there is no death of animals or clinical sign of toxicity.

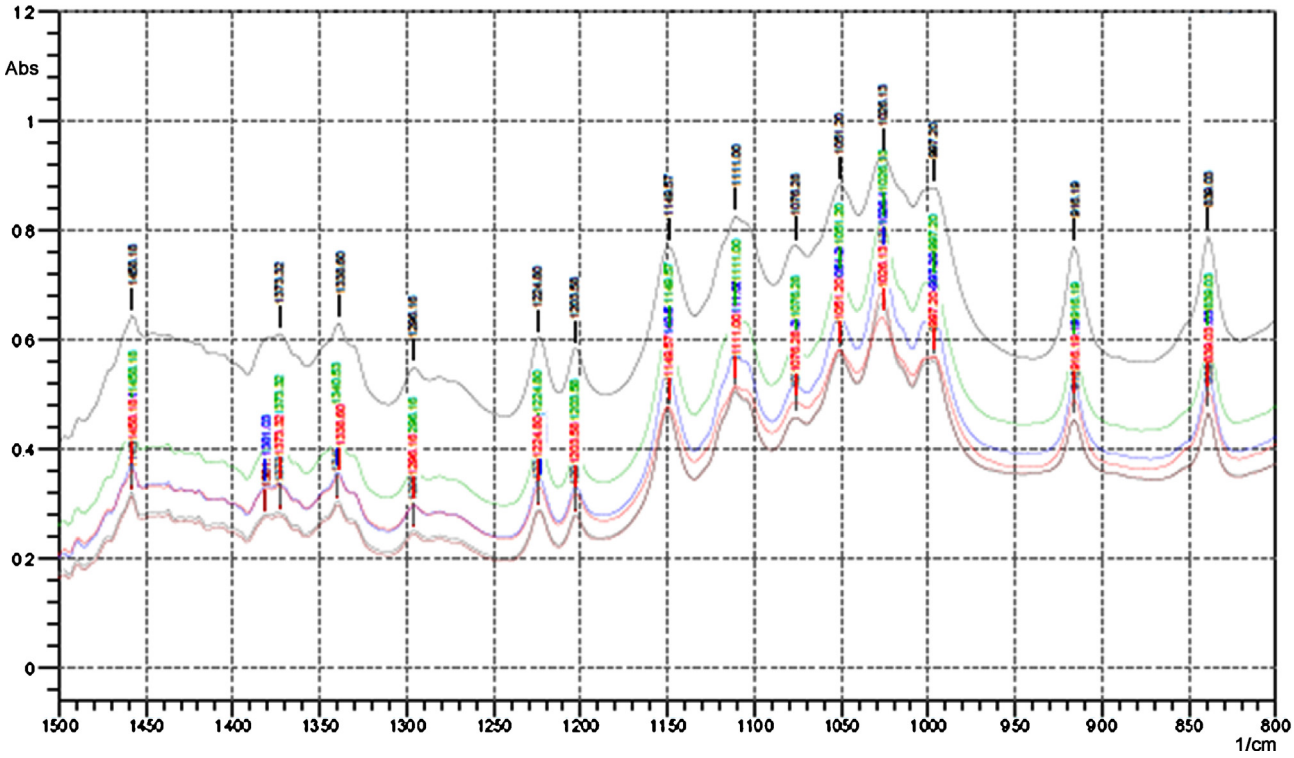

Fig. 4. FTIR Spectra of TIP.

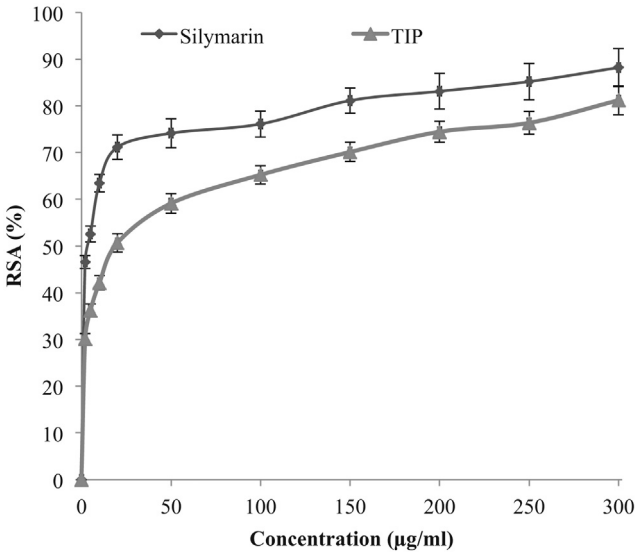

Fig. 5. DPPH radical scavenging activity of TIP.

3.2.2. Thioacetamide-induced hepatotoxicity

Several studies have shown that polysaccharides from invertebrates possess significant free radical scavenging activities and have protective effect against liver injury in mice (Jiang et al., 2013; Liao et al., 2013; Qiao et al., 2009). In the present study, we investigated the hepatoprotective effects of TIP on liver injury in mice. Silymarin, an antioxidant flavonoid complex derived from milk thistle (*Silybum marianum*), has long been used in the treatment of liver diseases. It has been proved to have a protective effect against experimental hepatotoxicity by regulating the actions of the ultrastructures of the liver cells and improving the activities of hepatocellular enzymes (Hagymasi, Kocsis, Lugasi, Feher, & Blazovics, 2002). Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver into blood. Toxicity experienced by the liver during thioacetamide poisoning results from the production of a metabolite, thioacetamide s-oxide, which is a direct hepatotoxin. This leads to Hepatic injury. Hepatic injury causes leakage of cellular enzyme into plasma (Schmidt & Schmidt, 1967; Schmidt et al., 1975; Wilkinson, 1962). Estimation of enzymes, ALP and BRN in the serum is a useful quantitative marker for the extent and type of hepatocellular damage (Ansari, Tripathi, Patnaik, & Dhawan, 1991) as damaged liver cells leads to releases these into the blood stream. Further activation of the functions of reticuloendothelial system (Gruen, Leihr, Gruen, Rasenack, & Branswig, 1974) or inhibition of protein biosynthesis (Castro et al., 1977) are some of the mechanisms which can reduce the hepatotoxicity of thioacetamide (Dwivedi, Rastogi, Sharma, Garg, & Dhawan, 1991; Iwu, Igboko, Elekwa, & Tempesta, 1990). In the present study biochemical parameters SGOT, SGPT, ALP and BRN were significantly elevated ($P < 0.05$) in the group of rats given thioacetamide (100 mg/kg s.c.). The pretreatment of TIP and Silymarin exhibited inhibition of thioacetamide induced increase in the levels of all the four biochemical parameters, resulting in significant restoration towards their control values (shown in Table 2). Further reduction of serum TP brought towards normal values by pretreatment TIP and Silymarin. According to the results stated above and some other literatures, it is believed that the hepatoprotective effect of polysaccharide is related to its antioxidant activity,which is dependent on the chemical and monosaccharide composition, molecular weight and function groups of polysaccharides (Liang et al., 2011; Liu, Luo, Ye, & Zeng, 2012; Olafsdottir & Ingolfsdottir, 2001). Polysaccharides with relatively low molecular weight and high protein content are found to possess high antioxidant activity (Wang & Luo, 2007). The high uronic acid and sulfate content has been proved to be beneficial for antioxidant activity of polysaccharide (Chen, Zhang, & Xie, 2004; Qi et al., 2005). The probable mechanism by which TIP exerts its protective action against thioacetamide-induced hepatocellular metabolic alterations could be by the stimulation of hepatic regeneration through an improved synthesis of protein or accelerated detoxification and excretion. The maximum protection against thioacetamide induced hepatic aberrations was achieved with the higher dose of the TIP and 50 mg/kg of Silymarin (comparable).

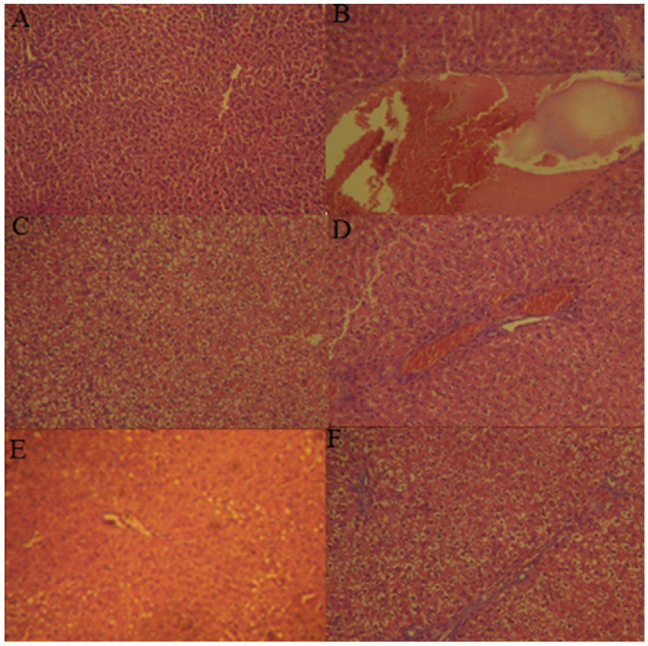

Fig. 6. (A). Liver histopathology of normal control. (B) Group treated with thioacetamide (100 mg/kg), (C) group treated with thioacetamide (100 mg/kg) along with Silymarin (50 mg/kg), (D) group treated with thioacetamide (100 mg/kg) along with TIP (50 mg/kg), (E) Group treated with thioacetamide (100 mg/kg) along with TIP (100 mg/kg) and (F) group treated with thioacetamide (100 mg/kg) along with TIP (200 mg/kg).

3.2.3. Histopathological evaluation

The protective effect of the TIP was further confirmed by histopathological examination of the livers of the control, thioacetamide induced, TIP treated groups and Silymarin treated groups (Fig. 6). The livers of the thioacetamide treated rats showed irregular perilobular cloudy swelling, dilated sinusoidal spaces, diffuse hyaline necrosis with blood pooling in sinusoidal spaces and central venule. The histopathological pattern of the livers of the rats treated with TIP and Silymarin showed a normal lobular pattern with minimal pooling of blood in the sinusoidai spaces. The present study reveals the hepatoprotective activity of TIP against thioacetamide induced liver toxicity. Toxicity due to thioacetamide poisoning results from the production of a metabolite, thioacetamide S-oxide which is a direct hepatotoxin (Neal & Halpert, 1982). Thioacetamide induces centrilobular necrosis within 3 h of administration. It has also been observed that thioacetamide causes specific changes in the nucleolus and increased synthesis of guanine and cytosine-rich RNA, with concomitant decrease in ribosomal RNA in the cytoplasm (Zimmerman, 1976). It is quite likely that TIP under study 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. Furthermore, protective mechanism not specific to thioacetamide may be responsible for

Table 2
Effect of TIP on rats biochemical parameters intoxicated with thiocetamide.

Group	SGOT	SGPT	ALP	BRN	TP
G-I (normal)	147.24 ± 8.40	102.46 ± 14.52	82.14 ± 9.45	0.94 ± 0.06	10.14 ± 0.54
G-II (TCM)	345.5 ± 20.45	267.45 ± 18.42	164.85 ± 13.46	4.23 ± 0.09	6.63 ± 0.48
G-III (TCM +50 mg/kg, TIP)	247.46 ± 7.56	187.49 ± 11.25	128.46 ± 9.36	2.79 ± 0.09	7.59 ± 0.76
G-IV (TCM +100 mg/kg, TIP)	198.87 ± 9.15	145.49 ± 12.59	104.25 ± 9.86	1.45 ± 0.08	8.49 ± 0.78
G-V (TCM +200 mg/kg, TIP)	154.63 ± 9.43	107.89 ± 12.41	85.29 ± 6.87	1.05 ± 0.08	9.75 ± 0.74
G-VI (TCM + 50 mg/kg, SYL)	155.46 ± 7.89	105.15 ± 13.12	83.19 ± 8.41	0.96 ± 0.05	9.87 ± 0.63

Values are mean ± S.D., $n = 6$ animals per group.
G-II Compared with G-I; $P < 0.05$ in all values.
G-III,G-IV, G-V and G-VI are compared with G-II; $P < 0.05$ in all values.

hepatoprotective activity of the TIP. Thus, the stimulation of hepatic regeneration known to cause the liver to become more resistant to damage by toxins (Lesch, Reutter, Keppler, & Decker, 1970) could explain the hepatoprotective effect of the TIP. Likewise, activation of the functions of reticuloendothelial system (Gruen et al., 1974) or inhibition of protein biosynthesis (Castro et al., 1977) are some of the mechanisms which can reduce the hepatotoxicity of thioacetamide (Dwivedi et al., 1991; Iwu et al., 1990). It is quite likely that the extract under study prevents the thioacetamide-induced hepatotoxicity due to multiple mechanisms.

4. Conclusion

In the present study, TIP was preliminarily characterized by moisture content, ash value, pH, mineral content, water holding capacity, micromeritics properties, content of total sugars, sulfate, protein and uronic acid followed by UV spectral analysis, Thermal analysis and FTIR study. Content of neutral sugars and protein in isolated starch were determined to be 78.4 and 5.1%, respectively with relatively high contents of uronic acid (6.3%) and sulfate (7.7%). UV spectral analysis, Thermal analysis and FTIR study reveals the polysaccharide nature of TIP. In the present study biochemical parameters SGOT, SGPT, ALP and BRN were significantly elevated ($P < 0.05$) in the group of rats given thioacetamide (100 mg/kg s.c.). The pretreatment of TIP and Silymarin exhibited inhibition of thioacetamide induced increase in the levels of all the four biochemical parameters, resulting in significant restoration towards their control values. Further reduction of serum TP brought towards normal values by pretreatment TIP and Silymarin. The livers of the thioacetamide treated rats showed irregular perilobular cloudy swelling, dilated sinusoidal spaces, diffuse hyaline necrosis with blood pooling in sinusoidal spaces and central venule. The histopathological pattern of the livers of the rats treated with TIP and Silymarin showed a normal lobular pattern with minimal pooling of blood in the sinusoidai spaces. The present study reveals the hepatoprotective activity of TIP against thioacetamide induced liver toxicity.

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