Indian Journal of Natural Products and Resources
Vol. 14(1), March 2023, pp. 67-74
DOI: 10.56042/ijnpr.v14i1.1139

NISCPR
सीएसआईआर–निस्पर

Antioxidant potential and *in vitro* cytotoxicity study of *Saraca indica* extract on lead-induced toxicity in HepG2 and HEK293 cell lines

Brijesh Shivhare[1], Maneesha Pandey[1]* and Ramesh Kumar[2]*

[1]Discipline of Biochemistry, School of Sciences, IGNOU, New Delhi 110068, India

[2]Department of Biochemistry, Bundelkhand University, Jhansi 284128, Uttar Pradesh, India

Received 16 November 2022; revised received 22 February 2023; accepted 27 February 2023

Saraca indica is an important medicinal plant and the compounds present in this plant are very much helpful in preventing various diseases. Therefore, the aim of the present study was to investigate the antioxidant activity and *in vitro* investigation of *S. indica* extract on lead-induced toxicity in HepG2 and HEK293 cell lines. The antioxidant assay was performed by two methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging assay. MTT assay was used for *in vitro* cytotoxic activity of human hepatocellular liver cell (HepG2) and human embryonic kidney (HEK293) cell lines. IC_{50} values of the DPPH radical scavenging assay for aqueous and ethanolic extracts were 380 and 350 µg/mL, respectively. IC_{50} values of $ABTS^+$ radical cation scavenging assay for aqueous and ethanolic extracts were 200 and 350 µg/mL, respectively. Cell viability for HepG2 and HEK293 cell lines was found to be 50% at 800 µg/mL concentration for aqueous extract and 1000 µg/mL for ethanolic extract. Data obtained from the MTT assay indicated that *S. indica* extract significantly increased the viability of the HepG2 and HEK293 cell lines in a dose-dependent manner. Both the aqueous and ethanolic extracts of *S. indica* are involved in the protection against lead-induced toxicity. Therefore, *S. indica* extract may be used as a salvage therapy for lead-induced toxicity. Further studies are required to isolate the bioactive compounds from plants for advanced investigation.

Keywords: Antioxidant activity, Cytotoxicity, HepG2 and HEK293 cell line, MTT assay, *Saraca indica*

IPC code; Int. cl. (2021.01)- A61K 36/00, A61K 36/48, A61P 39/00

Introduction

Saraca indica is commonly known as the Ashoka tree which belongs to the family Caesalpiniaceae. It is an evergreen and consecrated tree. Ashoka is one of the hallowed and holy trees of Hindus for whom it is venerated each year on December 27. It is appropriated all over in Indian woodlands up to a rise of around 750 meters. In the northern region of India, the Himalayas, this tree is found at Khasi, Garo, and Lussi slopes, and in the Kerala locale, such as Palakkad and Kannur regions[1]. The plant contains many phytochemicals such as glycosides, phenols, flavonoids, tannins, and saponins[2]. It is mostly used to treat gynaecological problems in uterine latency, uterine agony, urinary math, dysuria, and so on. It also has other health benefits such as treatment of menstrual cycle disorders exhibiting uterotonic, oxytocic, anti-progestational, anti-estrogenic and anti-tumour activity[3]. Moreover, the plant is helpful in dyspepsia, fever, consuming sensation, colic, ulcer, menorrhagia, leucorrhoea, and pimples[4-6]. The dried bark of *S. indica* has been utilized in menorrhagia disease in India[7-9]. In India, the dried bark of *S. indica* is given as a tonic to women to treat uterine disarranges. Also, the stem bark is utilized on account of all issues related to the menstrual cycle[10]. Ashoka is a blood purifier and beneficial in all skin sicknesses, amenorrhea, dysmenorrhoea menopause, menorrhagia, agonizing monthly cycle blood course and cleansing, malignant growth, jaundice, loss of motion, looseness of the bowels, coronary illness, hepatitis, herpes, joint torment, kidney and nerve stones, rheumatoid joint pain, deterrents in urinary entries[11]. The leaves of *S. indica* have been assessed for anti-helminthic activity[12,13], CNS depressant action[14], and pain-relieving and antipyretic activity[15,16]. Therefore, keeping in view the above beneficial effects of *S. indica*, the phytochemical screening, antioxidant activity, total phenolic content, total flavonoid content and lead-induced *in vitro* cytotoxicity in HepG2 and HEK293 cell lines were evaluated. This study provides information

*Correspondent author
Email: maneesha@ignou.ac.in; drramesh.kumar34@gmail.com
Mob.: 9999103224; +91 7860412141

about the medicinal and phytochemical importance of *S. indica*.

Materials and Methods

Sample collection, identification and extract preparation

The fresh plant parts such as leaves, bark, and flowers of *S. indica* were collected in March 2019 from a garden in Jhansi. The collected plant materials were brought to the laboratory on the same day. The plant samples were identified by Dr. Prem L Uniyal, from the Department of Botany, University of Delhi, New Delhi and a voucher specimen (DUH14788) has been deposited in the Delhi University Herbarium.

The samples were extracted using the Soxhlet method[17]. The plant samples were pre-washed with tap water and then again washed with distilled water and left for air-drying at room temperature for 7-10 days. Finally, it was kept for oven-drying at 40°C to remove the residual moisture. The dried part of the bark, leaves, and flowers were powdered using a mixer grinder and stored in an air-tight container for future use. Water and ethanol (80%) were used for extraction. About 15 g of dry powder from each of the plant samples was extracted with water and ethanol at room temperature using the Soxhlet and the extraction process was continued until the liquid was clear. The extract was then filtered out and concentrated to get a dry mass under vacuum using a rotatory vacuum evaporator. The sample was kept in an airtight container at 4°C for further use. The per cent extract yield (%) was calculated by using the formula.

$$\% \text{ Yield} = \frac{\text{Weight of dry extract obtained}}{\text{Weight of dry plant sample used for extraction}} \times 100$$

Phytochemical analysis

All of the extracts, including the leaves, bark, flowers, and whole plant (mixture of leaves, bark, and flowers) were subjected to a thorough phytochemical examination as described in earlier studies[18].

Determination of total phenolic content

The Folin-Ciocalteu technique was used to determine the total phenolic content as described by Bari *et al.*[19], and Sharma *et al.*[20]. The gallic acid was used as standard with different concentrations (20, 40, 60, 80, and 100 µg/mL). 0.5 mL of the plant extract having a concentration of 100 µg/mL was mixed with 1.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) with the addition of 3 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was kept in dark at room temperature for 30 min with continuous shaking for colour development. The absorbance of the blue coloured solution was taken concerning standard by using a double beam UV-Vis spectrophotometer at a wavelength of 765 nm. The amount of gallic acid equivalent per g of dry extract (GAE/g dry extract) was used to measure the total phenolic content.

Estimation of total flavonoid content

The determination of the total flavonoid content was done as described by Kumar *et al.*[21]. As a reference, quercetin was used as standard with different concentrations (50, 100, 150, 200, and 250 µg/mL). Quercetin equivalent per g of dry extract (QE/g dry extract) was used to calculate the flavonoid content. 500 µL of distilled water, 100 µL of 5% sodium nitrate, and 100 µL of different dilutions were combined and left to stand for 6 min. Following the addition of 150 µL of 10% aluminium chloride solution, the mixture was allowed to stand for an additional 5 min before 200 µL of 1 M sodium hydroxide solution was added. The absorbance was measured at 510 nm, the flavonoid content was calculated as Mean±SD (n=3) and expressed as mg/g of quercetin equivalent (QE) of dry extract.

DPPH radical scavenging activity

The DPPH radical scavenging activity was estimated by the method as described by Kähkönen *et al.*[22]. 0.1 mM DPPH solution in 1 mL of methanol was thoroughly combined with various extract concentrations (0.5 mL each). The mixture was allowed to stand in the dark for 30 min. The multimode plate reader was used to measure the absorbance at 523 nm. As the standard and blank, identical amounts of DPPH and methanol were utilised. The scavenging activity was calculated using the following formula:

DPPH scavenging activity (%) = $(A_{control} - A_{sample})/A_{control}) \times 100$

Where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of control.

ABTS radical scavenging assay

ABTS assay was carried out using the method described by Suseela *et al.*[23]. The stock solutions were prepared using 7 mM ABTS solution and 2.45 mM potassium persulfate/ ammonium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at 30°C in the dark. The solution was then diluted by mixing 1 mL ABTS solution with

60 mL methanol to obtain an absorbance of 0.706±0.001 at 734 nm using a Shimadzu UV spectrophotometer. Plant extracts (1 mL) were treated with 1 mL of the ABTS solution and incubated for 7 min. The absorbance was taken at 734 nm using the spectrophotometer. All the experiments were performed in triplicates. The ABTS scavenging capacity of the extract was calculated as:

ABTS radical scavenging activity (%) = ($A_{control}$ – A_{sample})/ $A_{control}$) x 100

Where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of control.

Determination of cytotoxicity

The Human hepatocellular liver cell (HepG2) and Human embryonic kidney (HEK293) cell lines were purchased from the National Center for Cell Sciences, Pune, India. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS from Gibco Life Technologies, Bangalore, India, two mM L-glutamine, 100 IU/mL penicillin, and 100 µg/mL streptomycin. In the experiment, 1 x 10^5 cells using the Trypan blue exclusion method were plated in each well of 96-well plates, and were placed in the humidified 5% CO_2 incubator at 37°C to allow them to attach to the substrate for 24 h period. Cells were exposed to lead acetate at different concentration for IC_{50} value and after that different concentrations of aqueous and ethanolic extract and placed in the humidified 5% CO_2 incubator for 24, 48 and 72 h. Cells incubated in culture medium alone served as a control for cell viability (untreated wells).

Cell viability was determined using the MTT assay[24,25]. In brief, 50 µL aliquots of MTT solution (5 mg/mL in PBS) were added to each well and re-incubated for 30 min at 37°C. Then, the supernatant culture medium was carefully aspirated and 200 µL aliquots of dimethyl sulfoxide (DMSO) were added to each well to dissolve the formazan crystals, followed by incubation for 10 min to dissolve air bubbles. The culture plate was placed on a Biotex Model micro-plate reader and the absorbance was measured at 550 nm. The amount of colour produced is directly proportional to the number of viable cells. All assays were performed in six replicates for each concentration and means±SD values were used to estimate the cell viability. Cell viability rate was calculated as the percentage of MTT absorption as follows:

% survival = (mean experimental absorbance/mean control absorbance) × 100.

Statistical analysis

Statistical analysis was done using one-way analysis of variance (ANOVA Dunnett's test) for multiple samples and Student's t-test for comparing paired sample sets. Data were presented as means±SDs. *P* values less than 0.05 were considered statistically significant.

Results and Discussion

The per cent yield of extract of various plant samples in ethanol was 16, 9.68, 15.93, and 12% for leaves, bark, flower and whole plant, respectively whereas the per cent yield extract in aqueous was 9.7,5.03, 4.94, and 6.52% for leaves, bark, flower and whole plant, respectively.

Phytochemical analysis

Preliminary screening was done for detecting the presence or absence of secondary metabolites. The screening was done for leaves, bark, flower and whole plant part (mixture of leaves, bark and flower) using two different solvents i.e. water and ethanol (80%). The presence of flavonoids, carbohydrates, glycosides, saponins and steroids was observed in the aqueous extract of the whole plant whereas carbohydrates, flavonoids, saponins, glycosides and steroids were present in the 80% ethanolic plant extract. The extracts were subjected to preliminary phytochemical analysis using standard chemical methods which mainly showed the presence of carbohydrates, flavonoids, phenols, glycosides, tannins, and saponins that were present in the bark, leaves, and flower of *S. indica* shown in Table 1.

Total phenolic content and total flavonoid content

The total phenolic content observed in the ethanolic extracts of leaves was high as compared to the bark and flower which may be because of the presence of higher amounts of polyphenols in leaves. Therefore, the result shows that ethanol is a better solvent as compared to water extraction. Fig. 1a, shows the standard curve of Gallic acid and Fig. 1b, the quercetin standard curve. A more useful explanation for the activity of organic extract can be ascribed to the enzyme polyphenol oxidase, which degrades polyphenols in water extracts, whereas in ethanol and methanol, they are inactive. The higher concentrations of more bioactive flavonoid compounds were detected in ethanol (80%) (Fig. 2). Ethanol has higher polarity than water. By adding water to the pure

Table 1 — Qualitative phytochemical analysis of aqueous and ethanolic extracts *Saraca indica*

Phytochemical tests	Aqueous extract				Ethanolic extract			
	Leaf	Bark	Flower	Whole plant	Leaf	Bark	Flower	Whole plant
Test for Carbohydrates								
Benedict's test	+	+	+	+	+	+	+	+
Fehling's test	+	+	+	+	+	+	+	+
Iodine test	-	-	-	-	-	-	-	-
Test for Flavonoids								
Alkaline reagent test	+	+	+	+	+	+	+	+
Test for Saponins								
Foam test	+	+	+	+	+	+	+	+
Test for Phenol & Tannin								
Ferric chloride test	+	+	+	+	+	+	+	+
Test for Glycosides								
Liebermann's test	-	-	-	-	-	-	-	-
Salkowski's test	+	+	-	+	+	+	-	+
Keller-kilani test	+	+	+	+	+	+	+	+
Test for Steroids								
Salkowski's test	+	+	-	+	+	+	-	+
Test for Phenolic compounds								
Ferric chloride test	+	+	+	+	+	+	+	+

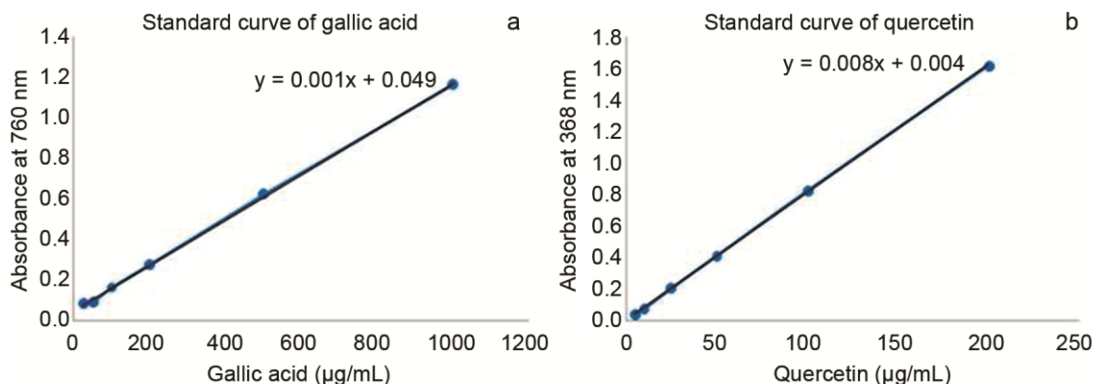

Fig. 1 — Graph of concentration versus absorbance, a) Gallic acid; and b) Quercetin standard curve.

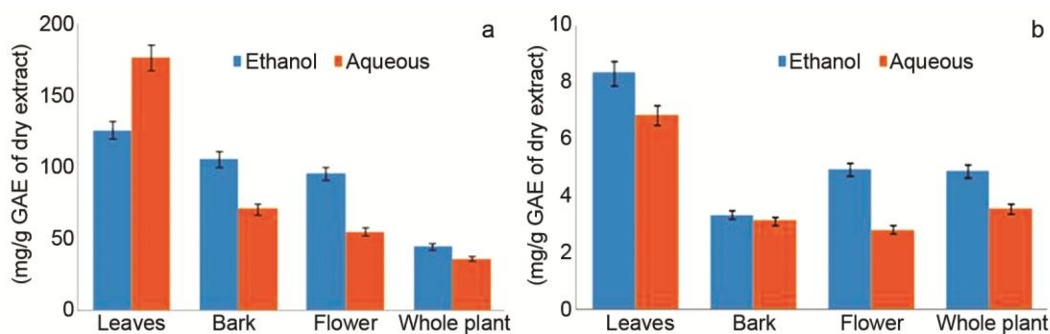

Fig. 2 —a) The total phenolic content (mg/g GAE of extract); and b) total flavonoid content (mg/g QE of extract) of aqueous and ethanolic extract of *S. indica* leaves, bark, flower, and whole plant.

ethanol up to 20%, the polarity of the solvent is increased[26,27]. Additionally, ethanol is found to be easier to penetrate the cellular membrane to extract the intracellular active components from the plant material[28].

DPPH radical scavenging activity

Natural plant materials have good antioxidant properties such as electron donation capacity which can be easily measured by the purple-coloured solution of DPPH. This method is based on the

scavenging of DPPH through the addition of a free radical or antioxidant that decolorizes the DPPH solution. The degree of colour change can be proportional to the concentration and potency of the antioxidants[29]. In the present study, the DPPH radical scavenging activity of *S. indica* was high at 95.51 and 98.15% in aqueous and ethanolic extract respectively as compared to reference control i.e. ascorbic acid (Fig. 3a). IC_{50} values of DPPH radical scavenging assay for aqueous and ethanolic extracts were 380 and 350 µg/mL, respectively. Higher phenolic and flavonoid content in the plants can also lead to an increase in antioxidant activity[30].

ABTS radical scavenging activity

The ABTS radical cation is generated by the oxidation of ABTS with ammonium persulfate and its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 734 nm (Saeed *et al.*)[31]. At higher concentrations, the ABTS scavenging activity of *S. indica* aqueous and ethanolic extract was more as compared to reference control i.e. ascorbic acid. However, at a lower concentration, the ABTS scavenging activity was comparable with the reference control (Fig. 3b). IC_{50} values of $ABTS^{+}$ radical cation scavenging assay for aqueous and ethanolic extracts were 200 and 350 µg/mL, respectively. These results were similar to the study performed on the whole extract of *Torilis leptophylla*[32].

Cytotoxicity assay

The dose-dependent Pb-acetate treated cells showed cell viability with an IC_{50} value at 160 µM. We observed that lead acetate is cytotoxic to HepG2 and HEK293 cell lines at 60 µg/mL at different time intervals i.e., 24, 48, and 72 h. Hence, to examine the effect of *S. indica* plant extract on lead acetate-induced cytotoxicity, cells were treated with aqueous and ethanolic extract at 100, 200, 400, 600, 800, 1000, 1200 µg/mL plus 60 µg/mL lead acetate. It was found that treatment of cells with lead acetate decreases cell viability to about 52% (Fig. 4) whereas the treatment of HepG2 and HEK293 cells by extract increases the cell viability in a dose-dependent manner (Fig. 5). The viability of HepG2 and HEK293 cells exposed at 160 µM of lead acetate resulted in cell growth and proliferation compared to cells treated with lead acetate alone, indicating the stimulatory effect of this antioxidant (Fig. 4). For instance, in cells treated with extracts, the viability was 122% and 160% compared to lead acetate alone. Similar results were also obtained for the trypan blue exclusion test. The result

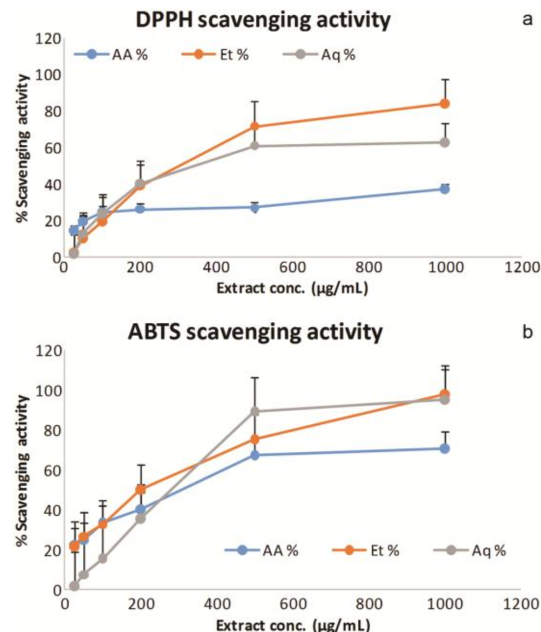

Fig. 3 — a) The scavenging activity on DPPH radical of aqueous and ethanolic extract of *S. indica*; and b) The scavenging activity on ABTS radical of aqueous and ethanolic extract of *S. indica.*

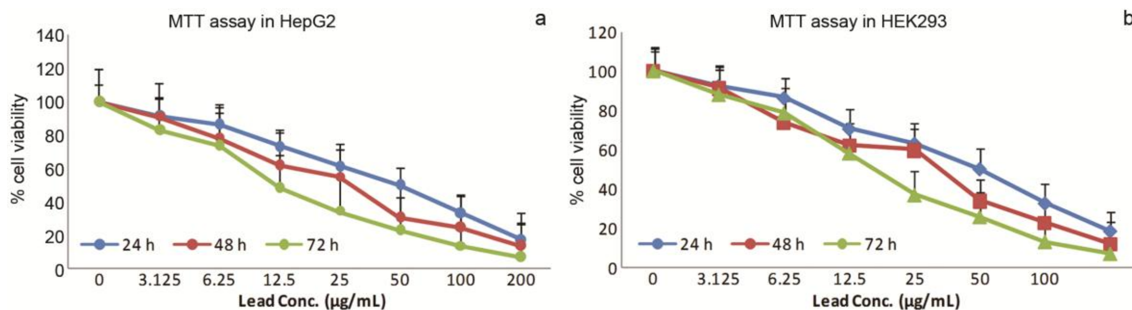

Fig. 4 —a) Effect of Lead acetate ($PbAc_2$) on human hepatocellular liver cell (HepG2) cells; and b) Effect of Lead acetate ($PbAc_2$) on Human embryonic kidney (HEK293) cells. Cells were cultured with different doses of lead (Pb) for 24, 48 and 72 hours as indicated in figure. Cell viability was determined based on the MTT assay. Each point represents a mean value and standard deviation of experiments with 3 replicates per dose. *Significantly different from the control by ANOVA Dunnett's test; $P < 0.05$.

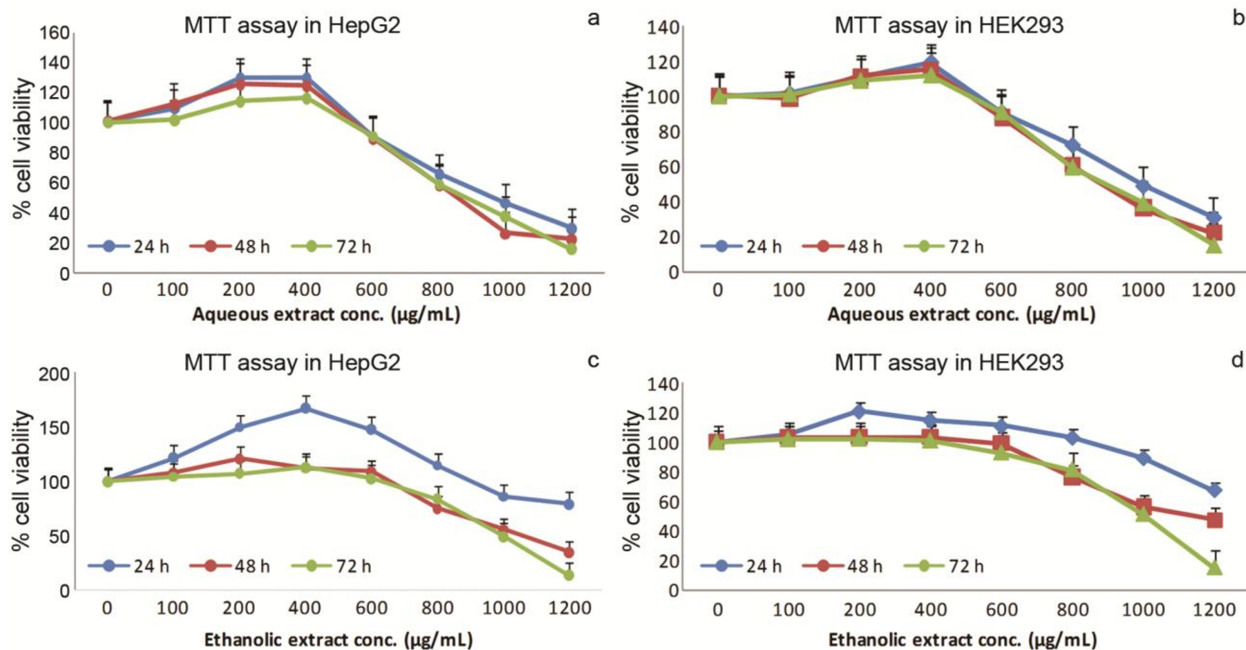

Fig. 5 — a) Potential effect of *S. indica* aqueous extract on human hepatocellular liver (HepG2) cells; b) Potential effect of *S. indica* aqueous extract on Human embryonic kidney (HEK293) cells; c) Potential effect of *S. indica* ethanolic extract to human hepatocellular liver (HepG2) cells; and d) Potential effect of *S. indica* ethanolic extract on Human embryonic kidney (HEK293) cells. Cells were cultured in the absence of lead acetate and *S. indica* aqueous extract for 24, 48 and 72 hours as indicated in figure. Cell viability was determined based on the MTT assay. Each point represents a mean value and standard deviation of 3 experiments with 3 replicates per dose. *Significantly different from the control by ANOVA Dunnett's test; *P*<0.05.

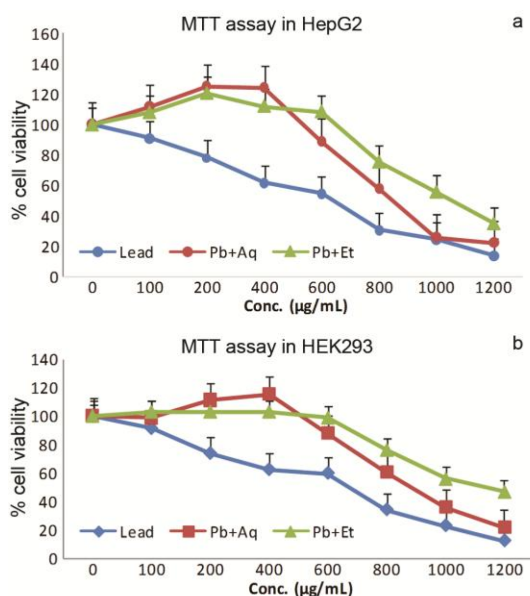

Fig. 6 —a) Protective effect of *S. indica* aqueous and ethanolic extract on lead acetate induced oxidative stress in HepG2 cells; and b) Protective effect of *S. indica* aqueous and ethanolic extract on lead acetate induced oxidative stress in HEK293 cells.

obtained from the present study indicates that lead acetate at 60 µg/mL of exposure is highly cytotoxic to human hepatocellular cell and Human embryonic kidney (HepG2 and HEK293) cells. These results are in conformity with other cytotoxicity studies on HepG2 and HEK293 cells where concentration and time-dependent decrease in cell viability based on the MTT assay is reported[32-36]. In the present study, the protective effect of *S. indica* extracts (aqueous and ethanolic) on lead acetate-treated HepG2 and HEK293 cells was also investigated. We found that cells co-exposed to both compounds resulted in a significant (P <0.05) increase in cell growth and proliferation. These findings clearly showed evidence that *S. indica* extracts (aqueous and ethanolic) act as a potential chelator of heavy metal that attenuates lead acetate-induced toxicity in HepG2 and HEK293 cells (Fig. 6). Recent studies have suggested that *S. indica* extracts (aqueous and ethanolic) may have the potential as an anti-metallic agent with efficacy in preventing initial damage[37,38].

Conclusion

In the Ayurvedic system of medicine, herbal extracts are used instead of purified compounds. The present study revealed that the various phytochemical components such as carbohydrates, flavonoids, saponins, phenols, tannins, glycosides, and steroids, are present in the leaves, bark, and flower of *S. indica*.

Ashoka has many medicinal uses and is a traditional medicinal plant. The compounds present in the plant are used in the development of the modern drug from *S. indica* should be emphasized for the control of various diseases such as in treating against lead acetate induced hepatotoxicity and nephrotoxicity. The findings proves that aqueous and ethanolic extract of *S. indica* seems to produce a protective effect against lead acetate-induced toxicity in HepG2 and HEK293 cells. According to these findings the *Saraca indica* extract is useful in the treatment against lead induced toxicity in liver and kidney.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

The authors would like to acknowledge the Department of Biosciences, Jamia Milia Islamia University, New Delhi for providing the necessary facilities to carry out the study.

Source of funding

The author has no funding Source.

References

1 Pal T K, Bhattacharyya S and Dey A, Evaluation of antioxidant activities of flower extract (fresh and dried) of *Saraca indica* grown in West Bengal, *Int J Curr Microbiol App Sci*, 2014, **3**(4), 251-259.
2 Pradhan P, Joseph L, Gupta V, Chulet R, Arya H, *et. al.*, Saraca asoca (Ashoka): A review, *J Chem Pharm*, 2009, **1**(1), 62-71.
3 Srivastava G N, Bagchi G D and Srivastava A K, Pharmacognosy of Ashoka stem bark and its adulterants, *Int J Crude Drug Res*, 1988, **26**(2), 65-72.
4 Peña Jr R A D, Gracilla D E, Pangilinan C R and Bagunu J V, *Gynuranepalensis* DC: A potential wonder medicinal plant, *Philippine BIOTA*, 2018, **48**, 1-6.
5 Middlekoop T B and Labadie R P, An *in vitro* examination of bark extracts for *Saraca asoca* (Roxb.) de Wilde and *Rhododendron arboreum Sm.* for oxytocic activity, *Int J Crude Drug Rec*, 1986, **24**(1), 41-44.
6 Bhandary M J, Chandrashekar K R and Kaveriappa K M, Medical ethnobotany of the siddis of Uttara Kannada district, Karnataka, India, *J Ethnopharmacol*, 1995, **47**(3), 149-158.
7 Wink M, Medicinal plants: A source of anti-parasitic secondary metabolites, *Molecules*, 2012, **17**, 12771-12791.
8 Škerget M, Kotnik P, Hadolin M, Hraš A R, Simonič M, *et al.*, Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities, *Food Chem*, 2005, **89**(2), 191–198.
9 Middelkoop T B and Labadie R P, Proanthocyanidins in the bark of *Saraca asoca* Roxb. de Wilde, *Zeitschriftfür Naturforschung B*, 1985, **40**(6), 855-857.
10 Nadkarni K M, The Indian Materia Medica, 3rd edn, (Popular Book Depot. Bombay), 1975, 1075.
11 Kulkarni R V, *Saraca asoca* (ashoka): A review, *World J Pharmaceut Res*, 2018, **7**(19), 536-544.
12 Nayak S, Sahoo A M and Chakraborti C K, Anthelmintic activity study of *Saraca indica* leaves extracts, *Int J Appl Biol Pharm*, 2011, **2**, 37.
13 Rice-Evans C A, Miller N J and Paganga G, Antioxidant properties of phenolic compounds, *Trends Plant Sci*, 1997, **2**(4), 152–159.
14 Verma A, Jana G K, Sen S, Chakraborty R, Sachan S, *et al.*, Pharmacological evaluation of *Saraca indica* leaves for central nervous system depressant activity in mice, *J Pharm Sci Res*, 2010, **2**(6), 338-343.
15 Pradhan P, Joseph L, George M, Kaushik N and Chulet R, Pharmacognostic, phytochemical and quantitative investigation of *Saraca asoca* leaves, *J Pharm. Res*, 2010, **3**(4), 776-780.
16 Sasidharan S, Chen Y, Saravanan D, Sundram K M and Latha L Y, Extraction, isolation and characterization of bioactive compounds from plants' extracts, *Afr J Tradit Complement Altern Med*, 2011, **8**(1), 1-10.
17 Singh V and Kumar R, Study of phytochemical analysis and antioxidant activity of *Allium sativum* of Bundelkhand region, *Int J Life Sci Res*, 2017, **3**(6), 1451-1458.
18 Kumar R, Sharma S and Devi L, Investigation of total phenolic, flavonoid contents and antioxidant activity from extracts of *Azadirachta indica* of Bundelkhand region, *Int J Life Sci Res*, 2018, **4**(4), 1925-1933.
19 Bari M S, Khandokar L, Haque E, Romano B, Capasso R, *et al.*, Ethnomedicinal uses, phytochemistry, and biological activities of plants of the genus Gynura, *J Ethnopharmacol*, 2021, **271**, 113834.
20 Sharma S, Verma O, Devi L and Kumar R, Antioxidant activity, total phenolic and flavonoid contents of *Aloe vera* leaves extract, *J Phytol Res*, 2021, **34**(1), 49-56.
21 Kumar D, Sharma S and Kumar R, Free radical scavenging activity of the mixture of different medicinal plants of Bundelkhand region, *World J Pharm Res*, 2021, **10**(2), 1008-1019.
22 Kähkönen M P, Hopia A I, Vuorela H J, Rauha J P, Pihlaja K, *et al.*, Antioxidant activity of plant extracts containing phenolic compounds, *J Agric Food Chem*, 1999, **47**(10), 3954–3962.
23 Suseela V, Gopalakrishnan V K and Varghese S, *In vitro* antioxidant studies of fruits of *Artemisia nilagirica* (Clarke) Pamp, *Indian J Pharm Sci*, 2010, **72**(5), 644.
24 Mosmann T, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, *J Immunol Methods*, 1983, **65**, 55-63.
25 Tchounwou P B, Wilson B, Schneider J and Ishaque A, Cytogenic assessment of arsenic trioxide toxicity in the Mutatox, Ames II and CAT-Tox assays, *Metal Ions Biol Med*, 2000, **6**, 89-91.
26 Bimakr M, Rahman R A, Taip F S, Ganjloo A, Salleh, *et al.*, Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Menthaspicata* L.) leaves, *Food Bioprod Process*, 2011, **89**(1), 67-72.
27 Onwukaeme D N, Ikuegbvweha T B and Asonye C C, Evaluation of phytochemical constituents, antibacterial activities and effect of exudate of *Pycanthus Angolensis* Weld Warb (Myristicaceae) on corneal ulcers in rabbits, *Trop J Pharm Res*, 2007, **6**(2), 725-730.

28 Hazra A and Das S, Comparative metabolomics analysis and radical scavenging activity of *Saraca asoca* (Roxb.) de Wilde flowers in different stages of maturity, *Jordan J Biol Sci*, 2021, **14**(3), 513-522.
29 Gupta R, Sharma A K, Dobhal M P, Sharma M C and Gupta R S, Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia, *J Diabetes*, 2011, **3**(1), 29-37.
30 Osuntokun O T, Oluduro A O, Idowu T O and Omotuyi A O, Assessment of nephrotoxicity, anti-inflammatory and antioxidant properties of epigallocatechin, epicatechin and stigmasterol phytosterol (synergy) derived from ethyl acetate stem bark extract of *Spondias Mombin* on Wistar rats using molecular method of analysis, *J Mol Microbiol*, 2017, **1**(1), 103.
31 Saeed N, Khan M R and Shabbir M, Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L., *BMC Complement Altern Med*, 2012, **12**(1), 1-2.
32 Ghatak A A, Chaturvedi P A and Desai N S, Indian grape wines: A potential source of phenols, polyphenols, and antioxidants, *Int J Food Prop*, 2014, **17**(4), 818-828.
33 Tchounwou P B, Yedjou C G, Foxx D, Ishaque A and Shen E, Lead induced cytotoxicity and transcriptional activation of stress genes in human liver carcinoma cells, *Mol Cell Biochem*, 2004, **255**, 161-170.
34 Nirmal S A, Pal S C, Mandal S C and Patil A N, Analgesic and anti-inflammatory activity of β-sitosterol isolated from *Nyctanthes arbortristis* leaves, *Inflammopharmacol*, 2012, **20**(4), 219-224.
35 Chai J W, Kuppusamy U R and Kanthimathi M S, Betasitosterol induces apoptosis in MCF-7 cells, *Malaysian J Biochem Mol Biol*, 2008, **16**(2), 28-30.
36 Tchounwou P B, Wilson B A, Abdelgnani A A, Ishaque A B and Patlolla A K, Differential cytotoxicity and gene expression in human liver Carcinoma (HepG2) cells exposed to arsenic trioxide and monosodium acid methanearsonate (MSMA), *Int J Mol Sci*, 2002, **3**, 1117-1132.
37 Khandel P, Yadaw R K, Soni D K, Kanwar L and Shahi S K, Biogenesis of metal nanoparticles and their pharmacological applications: Present status and application prospects, *J Nanostruct Chem*, 2018, **8**, 217–254.
38 Dash S S, Majumdar R, Sikder A K, Bag B G and Patra B K, *Saraca indica* bark extract mediated green synthesis of polyshaped gold nanoparticles and its application in catalytic reduction, *Appl Nanosci*, 2014, **4**, 485-490.