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Original Article

Hepatoprotective effect of *Ficus religiosa* latex on cisplatin induced liver injury in Wistar rats

Yogesh C. Yadav

Department of Pharmacy, Sumandeep Vidyapeeth, Piparia, Vadodara, India

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ABSTRACT

Ficus religiosa L., Moraceae, is widely planted in the tropics. The chemical constituents of *F. religiosa* include tannin, saponin gluanol acetate, β-sitosterol, leucoanthocyanidin, and leucoanthocyanin. These are used for the treatment of pain, inflammation, impotence, menstrual disturbances, and urine related problems, and as uterine tonic. The present study aimed to evaluate hepatoprotective effects of *F. religiosa* latex on cisplatin induced liver injury in Wistar rats. In experimental protocol contained five groups of rats ($n = 6$). In which, group I (control) was administered acacia (2%, w/v) of 5 ml/kg throughout the experiment for 16 days. The group II (cisplatin treated) was administered single dose of cisplatin (7.5 mg/kg *i.p.*) on 1st day. Group III (extract control) was administered 300 mg/kg *p.o.* of extract for 1st to 10th day. Group IV (Protective) was administered extract (300 mg/kg *p.o.*) of *F. religiosa* latex for 1st to 10th day and administered single dose of cisplatin (7.5 mg/kg *i.p.*) on 11th day and group V (Curative) received single dose of cisplatin (7.5 mg/kg *i.p.*) on day 1st, and administered extract (300 mg/kg *p.o.*) from 7th to 16th days. On the 6th day in cisplatin treated, 10th day in extract control and 16th day in control, protective and curative, blood withdrawn from retro-orbital sinus of rats for biochemical estimation for serum and dissected out the livers for estimation of antioxidant enzymes and histopathological works. The cisplatin-treated group 2 showed a significant increase in serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and hepatocytes cells degeneration inflammatory infiltrate and necrosis it's were significantly ($^{**}p < 0.01$) alleviates by protective groups.

Introduction

Ficus religiosa L., Moraceae, is widely planted in the tropics (Bailey and Bailey, 1976). It is a religiously popular bodhi tree and has its own medicinal importance in Indian culture (Prasad et al., 2006). *F. religiosa* has been extensively used in traditional medicine for a wide range of ailments of the central nervous system, endocrine system, gastrointestinal tract, reproductive system, respiratory system, and infectious disorders (Singh et al., 2011). The bark is used as an antibacterial, antiprotozoal, antiviral, astringent, and antidiarrhoeal agents, as well as in the treatment of gonorrhea and ulcers, and the leaves used for skin diseases. The leaves are reported to regulate the menstrual cycle and antivenom activity (Kalpana and Rishi, 2009; Chopra and Chopra, 1958). Fruits are used as laxatives (Shah, 1982); latex is used as a tonic, and fruit powder is used to treat asthma (Singh et al., 2002; Ananda and Kunjani, 2000).

Aqueous extract of *F. religiosa* decreased the fasting blood glucose and exaggerated activity of superoxide dismutase (SOD) in streptozotocin induced type II diabetic rats (Kirana et al., 2009). *F. religiosa* showed *in vitro* anthelmintic activity (Zafar et al., 2001). Aqueous extract showed high antimicrobial activity against selected pathogens such as *B. subtilis* and *P. aeruginosa* (Preethi et al., 2010).

Cisplatin (CDDP) can induce hepatotoxicity after having administered at high doses (Cavalli et al., 1978; Cersosimo, 1993; Pollera et al., 1987). Oxidative stress appears to play an important role in cisplatin induced hepatotoxicity. Metallothionein protects against liver injury induced by high doses of cisplatin in mice (Liu et al., 1998). Selenium and high dose of vitamin E administration protect against cisplatin-induced oxidative damage to liver (Naziroglu et al., 2004).

Nephroprotective and curative effects of *F. religiosa* latex extract can work against cisplatin-induced acute renal failure (Yadav and Srivastava, 2013).

With this background, the current study investigated whether oral administration of methanol extract of *F. religiosa* latex has

E-mail: info@sumandeepuniversity.co.in

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possible protective effect on cisplatin induced liver injury in Wistar rats.

Materials and methods

Drug and reagents

Gallic acid, quercetin, DPPH, TPTZ (2,4,6-tripyridyl-*S*-triazine), BHT (Merck Pvt. Ltd., India), cisplatin (VHB, Life Sciences Inc., India), urea and creatinine (Span Diagnosis kits), DTNB (Merck Pvt. Ltd., India), thiobarbuturic acid (Loba Chemicals Pvt. Ltd., India), epinephrine and superoxide dismutase (Sigma Aldrich Chemicals Pvt. Ltd., India), alanine aminotransferase (ALT) and aspartaite aminotransferase (AST) (Simens Healthcare Diagnostic Kit, India), and alkaline phosphatase (ALP) (Lab-care Diagnostic Kits, India) were used.

Plant material

Ficus religiosa L., Moraceae, latex was collected from Piparia village, Vadodara district of Gujarat, Western India in September, 2010. The plant was identified by Dr. Nagar (Professor of Botany), M.S. University, Vadodara (Gujarat) and a voucher specimen of *F. religiosa* latex (DPSV/F/01/2010) was submitted in Department of Pharmacy, Sumandeep Vidyapeeth, Vadodara.

Sample preparation

F. religiosa latex was extracted by maceration process (48 h) in methyl alcohol after defatted with petroleum ether at 72 h at room temperature. The extract was dried by rotatory evaporator under reduced pressure. The yield of latex was 18.56% (w/w). Phytochemical identification and standardization of *F. religiosa* latex were performed by TLC method and high performance TLC (HPTLC) (CAMAG Switzerland, Linomet 5, and Scanner 3, Win Cat Software); mobile phase: butanol:formic acid:water (7.5:1.5:1). HPTLC analysis was performed by using various standard amino acid markers including glutamine, glycine, cysteine, methionine, lysine, arginine tyrosine, leucine, etc. and extract in which many compounds were observed on the extract track. One spot R_f value 0.56 of extract was similar to standard methionine marker. The methionine content of *F. religiosa* latex extract standardized that was found $0.648 \pm 0.0425\%$ of standard methionine.

In vitro antioxidant activity

Determination of DPPH scavenging activity

DPPH radical scavenging activity of methanol extract of *F. religiosa* latex was determined (Mensor et al., 2001). An aliquot of 0.5 ml of each fraction doses (20, 40, 60, 80, 100 and 120 μg/ml) test solution in methanol was mixed with each 2.5 ml of 0.5 mM methanol solution of DPPH. The mixture was shaken well and incubated for 10 min in the dark at room temperature. The absorbance was measured at 517 nm using UV–vis spectrophotometer. Ascorbic acid was used as a positive control and it was mixed in each similar concentration and doses in DPPH as a test solution. All tests and analyses were run in triplicates and the results obtained were averaged. DPPH free radical scavenging ability (%) was calculated by using the formula:

$$\%\,\text{of inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

IC_{50} value of extract was calculated by using formula which was generated by an Excel graph between % DPPH radical scavenging (% of inhibition) and concentration of extract (μg/ml).

Determination of phosphor–molybdenum scavenging activity

The antioxidant activity of the extract was determined by the phosphor–molybdenum method as described by Prieto et al. (1999). The extract (0.3 ml) of each fractions doses (20, 40, 60, 80, 100 and 120 μg/ml) test solution in methanol of was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 °C, for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer (Merck Thermo Spectronic, Model No. UV-1, double beam) against blank. Methanol (0.3 ml) in place of extract was used as the blank. Ascorbic acid was used as a positive control and it was mixed in each similar concentration and doses as a test solution. Phosphor–molybdenum free radical scavenging ability (%) was calculated by using the formula. % of inhibition = absorbance of control − absorbance of sample/ absorbance of control × 100

IC_{50} value of extract was calculated by using formula which was generated by an Excel graph between % phosphor–molybdenum radical scavenging (% of inhibition) and concentration of extract (μg/ml).

Animals

Wistar adult male rat 12–13-weeks-old (180–210 g) obtained from Sun Pharma Advanced Research Company (Gujarat, India) were allowed access to water and food *ad libitum*, and maintained under constant (25 ± 1 °C), humidity ($65 \pm 10\%$) and a 12 h light/dark cycle. The experiment carried out in accordance to the guidelines mentioned in the CPCSEA, and IAEC approved the experiment protocols (SVU/PH/IAEC/26.03.2010/02).

Acute toxicity study

Each group ($n = 3$) of Wistar rats fasted overnight prior to the experiment. Each group of rats was given a single dose of *F. religiosa* latex extract, 5, 50, 300, 2000 mg/kg per oral (*p.o.*) body weight. The animals were observed continuously for 2 h and then every 2 h up to 24 and 72 h for gross behavior changes (OCED 423).

Cisplatin-induced hepatic injury

Five groups of rats ($n = 6$) were used, in which group I (control) was administered acacia (2%, w/v) of 5 ml/kg throughout the experiment for sixteen days. The group II (cisplatin treated) was administered single dose of cisplatin (7.5 mg/kg *i.p.*) on 1st day. Group III (extract control) was administered 300 mg/kg *p.o.* of extract for 1st to 10th day. Group IV (Protective) was administered extract (300 mg/kg *p.o.*) of *F. religiosa* latex for 1st to 10th day and administered single dose of cisplatin (7.5 mg/kg *i.p.*) on 11th day and group V (Curative) received single dose of cisplatin (7.5 mg/kg *i.p.*) on day 1st, and administered extract (300 mg/kg *p.o.*) from 7th to 16th days (Mansour et al., 2006). On the 6th day in cisplatin treated, 10th day in extract control and 16th day in control, protective and curative, animals were anesthetized under light diethyl ether and withdrawn blood sample from retro-orbital sinus of rats by using glass capillaries, for biochemical estimation. The liver was retrieved, dissected, washed with saline for estimation of antioxidant enzymes and preserved in a formalin solution (10%) for further histological analysis.

Biochemical assays

The collected blood centrifuged using the table top centrifuge (REMI) at $3000 \times g$ to get serum. Serum urea and creatinine were

Table 1
Total phenolic and flavonoids content of the methanol extract of *Ficus religiosa* latex.

Extract	Total phenolic content (mg GAE/g)	Total flavonoids content (mg QE/g)
Methanol extract of *Ficus religiosa* latex	2.76 ± 0.84 mg	1.84 ± 0.5 mg

estimated using diagnostic kit from Span Diagnostic, Kolkata on chemical analyzer (microlab 3000) for the assessment of liver toxicity. Later, liver was removed, homogenized and centrifuged at 10,000 × *g* at 0 °C for 20 min. The supernatant was used for estimation of different antioxidant enzyme level by calorimetric method using spectrophotometer (Merck Thermo Spectronic, Model No. UV-1, double beam), reduced glutathione (GSH) was estimated by method of Sedlak and Lindsay (1968), and lipid peroxidation by thiobarbuturic acid-reactive substances (TBARS) by Uchiyama and Mihara (1978) method. Superoxide dismutase (SOD) was determined by method Mishra and Fridovich (1972); ALT and AST in serum were determined in accordance with the U.V. Kinetic IFCC method provided by the Simens healthcare diagnostic kits (Bergmeyer et al., 1986) in which 50 μl serum was dispensed with 500 μl working reagent (prepared by kits reagent 1 and 2 ratio 4:1) in tube mixed well and incubated 60 s at 37 °C then recorded first reading of absorbance at 340 nm, and performed other two reading at 60 s intervals. It was calculated by changing absorbance per minute and multiplied by factor.

ALP in serum was determined in accordance with the optimized IFCC method and procedure was followed by using the lab-care diagnostic kit (Tietz, 1995).

Histopathological examination

The Wistar rats were killed on the day of blood withdrawal. Livers were isolated, processed, and embedded in paraffin wax. The sections stained in hematoxylin and eosin and permanently were mounted for viewing and reporting (Chandrasekaran et al., 1978).

Statistical analysis

Results were expressed as one-way analysis of variance (ANOVA) followed by Dunnett's test and $p < 0.05$ was considered as significant.

Results and discussion

F. religiosa latex was revealed for the presence of glycoside, alkaloids, methionine, tannin (phenolic compound) and flavonoids. *In vitro* evaluation of *F. religiosa* for its antioxidant potential revealed DPPH and phosphor–molybdenum scavenging effect observed that significant decreased in the concentration of DPPH and phosphor–molybdenum radical due to scavenging potential of the extract. IC_{50} values for DPPH and phosphor–molybdenum were 31.75 ± 0.12 and 18.35 ± 0.48 μg/ml, respectively.

Orally administered dose ranged from 50–2000 mg/kg of extract did not produce any significant changes in the autonomic or behavior responses. Lethal dose cut-off was considered at 2000 mg/kg. The mortality of rate was unlikely at the highest starting dose level (2000 mg/kg body weight). When there was no information on a substance to be tested, for animal welfare reasons it was recommended to use the starting dose of 300 mg/kg (regarding OCED 423) (Table 1).

The cisplatin-treated group II showed significant decrease in serum total protein, total bilirubin and albumin levels (Table 2), GSH, and SOD and (Figs. 1 and 2), increases lipid peroxidation (Fig. 3), ALT, AST, ALP (Table 3), on the 6th day as compared to

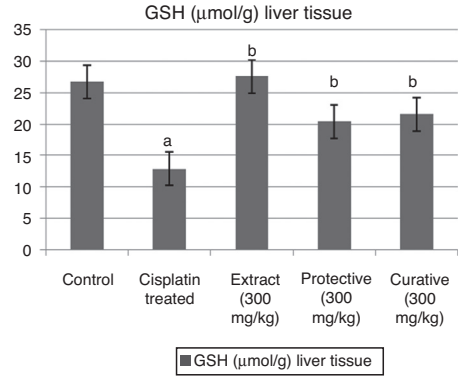

Fig. 1. Effects of the methanol extract of *Ficus religiosa* latex on the GSH level of liver tissue (^{a} $^{**}p < 0.01$ as compared to the control and ^{b} $^{**}p < 0.01$ as compared to the cisplatin treated).

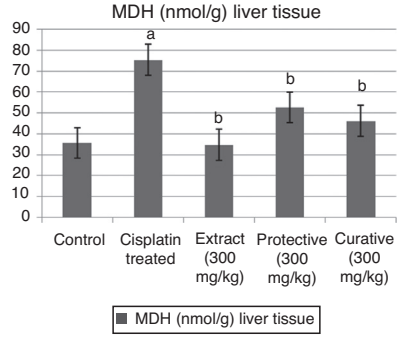

Fig. 2. Effects of the methanol extract of *Ficus religiosa* latex on the MDH level of liver tissue (^{a} $^{**}p < 0.01$ as compared to the control and ^{b} $^{**}p < 0.01$ as compared to the cisplatin treated).

the group I (control). They were significantly ($p < 0.01$) monitored in both protective and curative regimens with treated dose at 300 mg/kg of latex extract.

Histopathological sections of the livers showed marked hepatocytes cells degeneration of inflammatory infiltrate and necrosis was also observed in plates 1(B) (Fig. 4), and in the protective and curative regimens, treated with extract (300 mg/kg body wt., *p.o.*) which were reduced hepatocytes cells. Degeneration of inflammatory infiltrate and necrosis in Fig. 4 (plates D and E) respectively and extract control regimen, treated with extract (300 mg/kg body wt., *p.o.*) was normal histology as a control group in plates A and C.

In the present study, cisplatin-induced liver impairment was evidenced by an increase in serum ALT, ALP and AST and

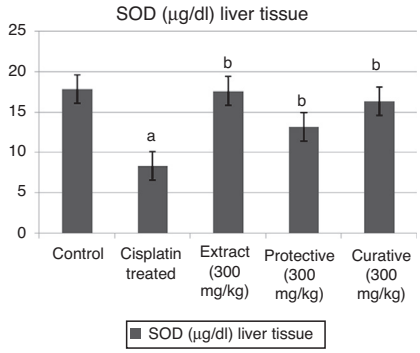

Fig. 3. Effects of the methanol extract of *Ficus religiosa* latex on the SOD level of liver tissue (^{a} $^{**}p < 0.01$ as compared to the control and ^{b} $^{**}p < 0.01$ as compared to the cisplatin treated).

Table 2
Effects of the methanol extract of *Ficus religiosa* latex on the total protein, total bilirubin and albumin level of blood serum.

Groups	Total protein (mg/dl)	Total bilirubin (mg/dl)	Albumin (mg/dl)
Control	9.08 ± 0.32	0.99 ± 0.06	5.33 ± 0.08
Cisplatin treated	5.86 ± 0.11[a]	2.75 ± 0.07[a]	4.31 ± 0.090[a]
Extract (300 mg/kg)	8.51 ± 0.13[b]	1.13 ± 0.10[b]	5.98 ± 0.04[b]
Protective (300 mg/kg)	7.71 ± 0.08[b]	1.41 ± 0.06[b]	5.88 ± 0.32[b]
Curative (300 mg/kg)	8.43 ± 0.24[b]	1.39 ± 0.03[b]	6.12 ± 0.34[b]

[a] $^{**}p < 0.01$ as compared to the control.
[b] $^{**}p < 0.01$ as compared to the cisplatin treated.

hepatocytes cells degeneration inflammatory infiltrate and necrosis. These changes persisted on the 6th day due to single dose of 7.5 mg/kg cisplatin. The methanol extract of *F. religiosa* latex normalized serum ALT, ALP and AST and lipid peroxidation, GSH, SOD of liver.

According to previous findings by Dubskaia et al. (1994), Saad et al. (2001), Kadikoylu et al. (2004) and Klukowska et al. (2001), which reported that CDDP administration induced significant increase in serum ALT, AST and ALP and significant decrease in serum total bilirubin, total protein, and albumin levels. The ability

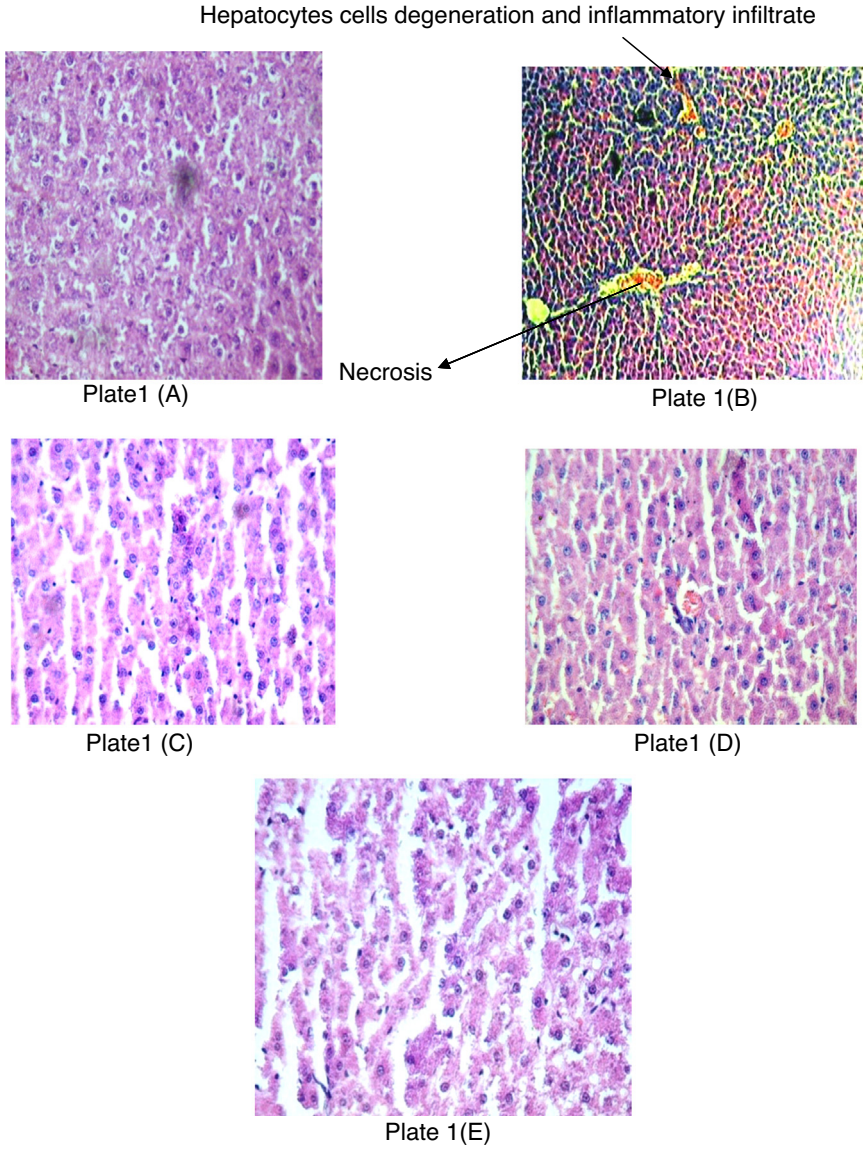

Fig. 4. Histopathology sections of liver plate normal liver 1(A), plate 1(B) Cisplatin-treated rats (7.5 mg/kg, 6 days), plate 1(C) extract (300 mg/kg), and plate 1(D) protective with extract (300 mg/kg), plate (E) Curative with extract (300 mg/kg), magnification 20× for each section. *Histopathology*: Cisplatin treated group (plate B) shown, heptocytes cells degeneration inflammatory infiltrate and necrosis. Treated groups with extract, extract + cisplatin (protective) and cisplatin + extract (curative) reduced significantly cisplatin induced liver injury.

Table 3
Effects of methanol extract of *Ficus religiosa* latex on the ALT, AST and ALP level of blood serum.

Groups	ALT (U/l)	AST (U/l)	ALP (U/l)
Control	42.83 ± 1.53	41.16 ± 1.07	85.83 ± 1.92
Cisplatin treated	135.17 ± 2.50[a]	81.16 ± 2.05[a]	146.50 ± 2.82[a]
Extract (300 mg/kg)	49.50 ± 1.40[b]	43.83 ± 1.51[b]	81.83 ± 1.51[b]
Protective (300 mg/kg)	71.83 ± 2.37[b]	57.66 ± 1.47[b]	109.8 ± 1.81[b]
Curative (300 mg/kg)	51.33 ± 1.16[b]	50.66 ± 1.47[b]	90.66 ± 2.07[b]

[a] $^{**}p < 0.01$ as compared to the control.
[b] $^{**}p < 0.01$ as compared to the cisplatin treated.

of cisplatin to cause alterations in the activity of these enzymes could be a secondary event following CDDP-induced liver damage with the consequent leakage from hepatocytes.

The present study was revealed that significantly decrease level of ALT, ALP and AST in blood serum due to its antioxidant effect and its ability to act as a free radical scavenger, thereby protecting membrane permeability (Ramadan et al., 2002) after treatment with extract indicate hepatoprotective and curative effect. Yadav and Srivastava (2013) reported nephroprotective and curative activity of methanol extract of *F. religiosa* latex at a dose of 200 mg/kg against cisplatin-induced renal failure in rats. *In vitro* studies of *F. religiosa* latex evaluated for its good antioxidant potential because it has revealed that DPPH and phosphor–molybdenum free radical scavenging effect with lower IC_{50} values due to observed rich sources of flavonoids, poly phenol, and methionine.

Yalcinkaya et al. (2007) reported methionine-supplemented diet augments hepatotoxicity and prooxidant status in chronically ethanol-treated rats, and reported that hepatoprotective properties did show with treatment of methionine against experimentally induced liver injury in rats. The present phytochemical screening data was observed methionine which was antagonized cisplatin hepatotoxicity.

Actually cisplatin contributed various mechanisms to liver dysfunction consist of cellular toxicity, vasoconstriction in the renal microvasculature, and proinflammatory effects, by producing free radicals oxidative stress which participated for decline antioxidant enzyme level of liver and lead to liver injury. Hence the possible mechanism of hepatoprotection and curative effect of *F. religiosa* latex could be due to its good antioxidant potential and amino acid content it might contribute free radicals scavenging and antagonism hepatotoxicity those were produce by cisplatin in liver injury.

Conclusion

Finally on the basis of these investigations, we could conclude that the administration of methanol *F. religiosa* latex caused a generally protective and ameliorative effect against cisplatin induced liver injury. The protective effect of *F. religiosa* latex is associated with its content of methionine and good antioxidant properties, as it possibly acts as a free radical scavenger, lipid peroxidation inhibitor and glutathione levels preservation.

Conflicts of interest

The author declares no conflicts of interest.

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References

Ananda, R.J., Kunjani, J., 2000. Indigenous knowledge and uses of medicinal plants by local communities of the kali Gandaki Watershed Area, Nepal. J. Ethnopharmacol. 73, 175–183.
Bailey, L.H., Bailey, E.Z., 1976. Hortus, third ed. MacMillan General Reference, New York.
Bergmeyer, H.U., Horder, M., Rej, R., 1986. IFCC methods for L-aspartate aminotransferase. J. Clin. Chem. Biochem. 24, 497–510.
Cavalli, F., Tschopp, L., Sonntag, R.W., Zimmermann, A., 1978. Cisplatin-induced hepatic toxicity. Cancer Treat. Rep. 62, 2125–2126.
Cersosimo, R.J., 1993. Hepatotoxicity associated with cisplatin chemotherapy. Ann. Pharmacother. 27, 438–441.
Chandrasekaran, S.K., Benson, H., Urquhart, J., 1978. Methods to achieve controlled drug delivery: the biochemical engineering approach. In: Robinson, J.R. (Ed.), Sustained and Controlled Drug Delivery Systems. Marcel Dekker, New York, p 557–593.
Chopra, R.N., Chopra, S., 1958. Indigenous Drugs of India, second ed. Dhur and Sons, Calcutta, p. 606.
Dubskaia, T.L.U., Vetoshkina, T.V., Goldberg, V.E., 1994. The mechanisms of the hepatotoxicity of complex platinum compounds. Eksp. Klin. Farmakol. 57, 38–41.
Kadikoylu, G., Bolaman, Z., Demir, S., Balkaya, M., Akalin, N., Enli, Y., 2004. The effects of desferrioxamine on cisplatin induced lipid peroxidation and the activities of antioxidant enzymes in rat kidneys. Hum. Exp. Toxicol. 23, 29–34.
Kalpana, G., Rishi, R.B., 2009. Ethnomedicinal knowledge and healthcare practices among the Tharus of Nawalparasi district in central Nepal. For. Ecol. Manage. 257, 2066–2072.
Kirana, H., Agarwal, S.S., Srinivasan, B.P., 2009. Aqueous extract of *Ficus religiosa* Linn reduce oxidative stress in experimentally induced type 2 diabetic rats. Indian J. Exp. Biol. 47, 822–826.
Klukowska, L., Nadulska, A., Dyba, S., 2001. The influence of cisplatinum and goserelinum on the magnesium and calcium level in rat serum. Ann. Univ. Mariae Curie Sklodowska Med. 56, 483–486.
Liu, J., Liu, Y., Habeebu, S.S., Klaassen, C.D., 1998. Metallothionein (MT)-null mice are sensitive to cisplatin-induced hepatotoxicity. Toxicol. Appl. Pharmacol. 149, 24–31.
Mansour, H.H., Hafez, H.F., Fahmy, N.M., 2006. Silymarin modulates cisplatin-induced oxidative stress and hepatotoxicity in rats. J. Biochem. Mol. Biol. 39, 656–661.
Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., dos Santos, T.C., Coube, C.S., Leitão, S.G., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15, 127–130.
Mishra, H.P., Fridovich, I., 1972. The role superoxide anion in the auto-oxidation of epinephrine and a simple assay of SOD. J. Biol. Chem. 247, 3170–3175.
Naziroglu, M., Karaoglu, A., Aksoy, A.O., 2004. Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. Toxicology 195, 221–230.
Pollera, C.F., Meglio, F., Nardi, M., Vitelli, G., Marolla, P., 1987. Cisplatin-induced hepatic toxicity. J. Clin. Oncol. 5, 318–319.
Prasad, P.V., Subhakthe, P.K., Narayana, A., Rao, M.M., 2006. Medico historical study of "asvattha" (sacred fig tree). Bull. Indian Inst. Hist. Med. Hyderabad 36, 1–20.
Preethi, R., Devanathan, V.V., Loganathan, M., 2010. Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. Adv. Biol. Res. 4, 122–125.
Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantization of antioxidant capacity through the formation of a phosphor–molybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341.
Ramadan, L., Roushdy, H.M., Abu Senna, G.M., Amin, N.E., El-Deshw, O.A., 2002. Radioprotective effect of silymarin against radiation induced hepatotoxicity. Pharmacol. Res. 45, 447–452.
Saad, S.Y., Najjar, T.A., Noreddin, A.M., Al-Rikabi, A.C., 2001. Effects of gemcitabine on cisplatin-induced nephrotoxicity in rats: schedule-dependent study. Pharmacol. Res. 43, 193–198.
Sedlak, M., Lindsay, R., 1968. Estimation of total, protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25, 192–205.
Shah, N.C., 1982. Herbal folk medicines in northern India. J. Ethnopharmacol. 6, 293–301.

Singh, A.K., Raghubanshi, A.S., Singh, J.S., 2002. Medical ethnobotany of the tribals of Sonaghati of Sonbhadra district, Uttar Pradesh, India. J. Ethnopharmacol. 81, 31–41.

Singh, D., Singh, B., Goel, R.K., 2011. Traditional uses, phytochemistry and pharmacology of *Ficus religiosa:* a review. J. Ethnopharmacol. 12, 565–583.

Tietz, N.W., 1995. Clinical Guide to Laboratory Tests, third ed. W.B. Saunders, Philadelphia, PA, p 76–77.

Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbuturic acid test. Anal. Biochem. 86, 271–278.

Yadav, Y.C., Srivastava, D.N., 2013. Nephroprotective and curative effects of *Ficus religiosa* latex extract against cisplatin induced acute renal failure. Pharm. Biol. 51, 1480–1485.

Yalcinkaya, S., Unlucerci, Y., Uysal, M., 2007. Methionine-supplemented diet augments hepatotoxicity and prooxidant status in chronically ethanol-treated rats. Exp. Toxicol. Pathol. 58, 455–459.

Zafar, I., Qazi, K.N., Khan, M.N., Akhtar, M.S., Waraich, F.N., 2001. In vitro anthelmintic activity of *Allium sativum*, *Zingiber officinale*, *Curcurbita mexicana* and *Ficus religiosa*. Int. J. Agric. Biol. 3, 454–457.