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Original Article

OXIDATIVE DNA DAMAGE PROTECTIVE AND HEPATOPROTECTIVE ACTIVITY OF *TERMINALIA BELLERICA* (BAHEDA)

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ABSTRACT

Objective: To investigate the ethanolic extracts of *Terminalia bellerica* for its *in vitro* DNA damage protective activity against hydroxyl radical ($OH^{\bullet}$) and hepatoprotective activity against CCl_4 induced toxicity in HepG2 liver cells.

Methods: The DNA damage assay was performed using supercoiled pBR322 plasmid DNA with Fenton's reagent. Protection of human liver-derived HepG2 cells against CCl_4 induced damage was determined by trypan blue exclusion assay and Tetrazolium bromide salt MTT assay. Liver cells were pre-exposed to 0.5 µmol/ml of CCl_4 for 60 min at room temperature and screening assay was carried out in HepG2 cells to evaluate the cytotoxicity of tested extracts with concentration 0.001 to 100 µg/ml.

Results: The low dose (0.001-0.1 µg/ml) of leaf and bark has an effect on the percentage viability (101±4.04) and 97±4.04) for leaf and bark extracts respectively) of HepG2 cells. Whereas, the percentage cell viability 99±2.89 and 103±4.04 were found for fruit pulp and seed extracts respectively. The uniform DNA damage protective activity was observed in a dose-dependent manner for leaf, fruit pulp, seed and bark extracts of *T. bellerica.*

Conclusion: Leaf and bark of *T. bellerica* exhibit 10 fold less toxicity compared to fruit pulp and seed during CCl_4 exposure on HepG2 cells suggesting that leaf and bark has more therapeutic potential against hepatotoxicity.

Keywords: DNA damage, CCl_4, HepG2, Hepatoprotective, *Terminalia bellerica*

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INTRODUCTION

Liver is a key organ regulating homeostasis in the body. It plays a major role in detoxification and excretion of many endogenous and exogenous compounds and injury of its function may lead to several implications on human health [1]. It has been reported that hepatotoxic chemicals such as carbon tetrachloride (CCl_4), antibiotics and various synthetic drugs (tert-Butyl hydroperoxide), in general, release free radicals, which act on hepatic cells, especially in the liver parenchyma cells [2]. Damage caused by these hepatotoxin, increases the level of liver enzymes. Consequently, this hepatic cell toxicity leads to a range of liver diseases such as ischemia-reperfusion injury, fibrosis and liver failure [3]. Liver damage induced by CCl_4 is commonly used model for the screening of hepatoprotective drugs. Thus, in the present study CCl_4, a hepatotoxin is used to induce acute liver damage.

Many Indian medicinal plants are considered potential sources of hepatoprotective compounds. About 80% of the populations in the third world countries rely on traditional plant-based medicines for their primary health care needs. About 25% of the prescribed drugs in the world are prepared from a variety of plant materials as leaves, stems, roots, bark etc [4]. However, the majority of these plants have not yet undergone DNA damage protection and hepatoprotective potential determination. Triphala is a traditional Ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants, *Terminalia chebula, Terminalia bellirica,* and *Emblica officinalis* in 1:1:1 ratio. *Triphala* is used in the treatment of common cold, sore throat and constipation [5, 6]. Reported studies showed hepatoprotective activity in the extracts from *T. chebula* and *E. officnalis* but there is no study has been done on the activities of the extracts from *T. bellerica* plant parts. *Terminalia bellerica* Roxb is a well-known medicinal plant and used in the Ayurvedic herbal formulation to treat various ailments. It is commonly known as Baheda, found in deciduous forests throughout the greater part of India except the dry region of Western India. Gupta *et al.* reported that *T. bellerica* is the richest source of various bioactive phytochemicals especially polyphenolic compounds [7]. The same group demonstrated that the extracts of *T. bellerica* exhibited strong and effective *in vitro* antioxidant activity by chelating metal ions as well as scavenging free radicals. Therefore, the present study was undertaken to evaluate the *in vitro* oxidative DNA damage protection and hepatoprotective activity of ethanolic extracts of *T. bellerica.*

MATERIALS AND METHODS

Plant materials

Plant materials were collected from the herbal garden of Narendra Dev University of Agriculture and Technology Kumarganj, Faizabad, U. P., India and identified with the help of Dr. M. N. Srivastava, Senior Scientist, Botany Division, CSIR-Central Drug Research Institute, Lucknow, India and the voucher specimens (2322 CSIR-CDRI) were submitted in CDRI herbarium.

Chemicals and reagents

Agarose gel was procured from GE healthcare (USA), calf thymus DNA was procured from Himedia Co. Ltd (India). Culture medium nutrient mixture F-12 Hams, antibiotics/antimycotics, fetal bovine and horse sera were purchased from Gibco BRL, USA. Solutions were prepared in de-ionized ultrapure water (Direct Q5, Millipore, and Bangalore, India). All the other specified chemicals, reagents (fetal bovine serum, horse serum) and diagnostic kit (MTT) were purchased from Sigma, USA, (TOX-7, Sigma St. Louis, MO., USA).

Extraction procedure

Twenty grams of the dried and powdered plant sample of *T. bellerica* leaf (TBL), fruit pulp (TBFP), seed (TBS) and bark (TBB) were extracted with 70% ethanolic solvent (in distilled water) for overnight at room temperature in an orbital shaker. The extracts were sepa-

rated from the residues by filtering through Whatman No. 1 filter paper. The residues were extracted until discolouration with the same fresh solvent and extracts combined. The combined extracts were concentrated and freed of solvent under reduced pressure at 40 °C, using a rotary evaporator and lyophilized till dryness. The dried crude concentrated extracts were stored at-4 °C and used for the *in vitro* DNA damage protective and hepatoprotective activity of *T. bellerica.*

DNA damage assay

The DNA damage assay was performed using supercoiled pBR322 plasmid DNA according to the method of Lee *et al.* [8] with some modifications. Reaction mixture containing 10 µl of plant extract of different concentrations (25 and 50 µg/ml), pBR322 DNA (0.25 µg) and 10 µl Fenton's reagent (30 mmol H_2O_2, 500 µmol ascorbic acid and 800 µmol $FeCl_3$) was incubated for 40 min at 37 °C and analyzed on 0.9% agarose gel by staining with ethidium bromide.

Culture of HepG2 cell line

HepG2 cells were procured from National Centre for Cell Sciences, Pune, India, and maintained at *In vitro* Toxicology Laboratory, Indian Institute of Toxicology Research, Lucknow, India, as per the standard protocols described earlier [9]. Briefly, cells were cultured in nutrient mixture (F-12 Hams), supplemented with 2.5% fetal bovine serum (FBS), 15% horse serum (HS), 0.2% sodium bicarbonate ($NaHCO_3$), 100 units/ml penicillin G sodium, 100 mg/ml streptomycin sulphate and 0.25 mg/ml amphotericin B. Cells were maintained at 37 °C in 5% CO_2, 95% atmosphere under high humid conditions. Culture medium was replaced twice weekly and cultures were passaged at a ratio of 1:6 once a week and checked for their viability thereafter using trypan blue dye exclusion assay following the protocols of Pant *et al.* [10]. Batches showing more than 95% viability were only used for in the experimentation.

Identification of biological safe doses of carbon tetrachloride

Non-cytotoxic doses of CCl_4 were identified using standard endpoints of cytotoxicity, i.e., trypan blue assay. Hepatoprotective potential of tested extracts were identified using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide] assay.

Cell viability by trypan blue dye exclusion assay

Cell viability was assessed by measuring the loss of membrane integrity following the method of Pant *et al.* [10]. In brief, HepG2 cells ($4x10^4$/well) were seeded in 48 well culture plates and allowed to grow for 24 h in 5% CO_2-95% atmosphere at 37 °C under high humid conditions. The medium was then replaced and cells were exposed to varying concentrations of CCl_4 (0.1 µmol to 5.0 µmol) for a period of 30-240 min. Following the exposure, the loss of cell viability was assessed. Immediately after the completion of respective time periods, cells suspensions were aspirated and centrifuged at 600 rpm for 5 min, washed twice with sterile phosphate-buffered saline (PBS, pH 7.4), and re-suspended in PBS. The cell suspension was then mixed with trypan blue dye (0.4% solution) at a ratio of 1:5 (dye: cell suspension) and placed on hemocytometer. The live (unstained transparent) and dead (blue stained) cells were counted using countess cell counter (Invitrogen). The untreated control sets were also run in parallel under identical conditions.

Hepatoprotective potential of test compounds

Healthy cultures of HepG2 cells were a pre-exposed to CCl_4 (0.5 µmol) for a period of 60 min. The cells were then washed with PBS and exposed to test compounds at various concentrations (0.001 µg/ml to 100 µg/ml) up to 96 h. The cytoprotective efficacy of the test compounds was observed following the standard endpoint viz., tetrazolium bromide salt MTT assay.

MTT assay

The cells ($1x10^4$ cells/well) were seeded in 96-well tissue culture plates and incubated in the CO_2 incubator for 24 h at 37 ° C. Then the medium was aspirated and cells were exposed to medium containing test compound at 37 °C in 5% CO_2-95% atmosphere under high humid conditions. Tetrazolium salt (10 µl/well, 5 mg/ml of stock in PBS) was added 4 h prior to completion of respective incubation period. At the completion of incubation period, the reaction mixture was carefully aspirated and 200 µl of culture grade DMSO was added to each well. The content was mixed until dissolved completely. Plates were then incubated for 10 min at room temperature and color was read at 550 nm using multiwell microplate reader (Synergy HT, Bio-Tek, USA). The unexposed sets, and sets exposed to $MnCl_2$ (10^{-3} M) were also run parallel under identical conditions that served as a basal and positive control respectively.

Statistical analysis

The value obtained for unexposed groups were considered as 100% and compared with the values obtained in the exposed group. Total four triplicates were used for each consideration and mean±standard error (SE) values were taken for comparison. Student t-test was used for analysis and the P value<0.5 was considered as significant.

RESULTS AND DISCUSSION

DNA damage protective activity

Hydroxyl radical scavenging activity of *T. bellerica* extract needed to be further explored by the protection of plasmid pBR322 DNA against damage induced by Fenton reagent. When DNA was exposed to Fenton reaction, H_2O_2 will be generated to hydroxyl radicals, and then the supercoiled form of DNA would cleave to give rise linear form. The DNA damage protective activity of the plant extracts showed a considerable result in retaining the supercoiled from open circular form, gradually with increasing concentration. Addition of Fenton reagent to a mixture containing DNA and *T. bellerica* extracts (25 and 50 µg/ml) showed a significant reduction in the formation of open circular and linear forms and increased supercoiled or native forms of plasmid DNA as showed in the fig. 1.

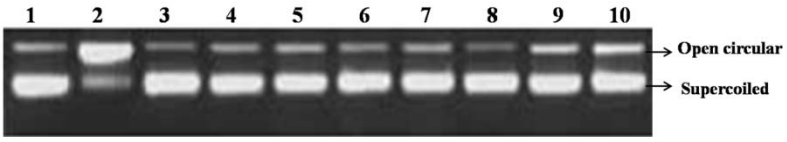

Fig. 1: Concentration dependent DNA damage protection offered by *T. bellerica* leaf (TBL), fruit pulp (TBFP), seed (TBS) and bark (TBB) extracts on native pBR322 DNA nicking caused by hydroxyl radicals. Lane 1: DNA (pBR322 plasmid DNA alone), Lane 2: DNA+Fenton reagent, Lane 3 DNA+Fenton reagent+25 µg/ml TBL, Lane 4: DNA+Fenton reagent+50 µg/ml TBL, Lane 5: DNA+Fenton reagent+25 µg/ml TBFP, Lane 6: DNA+Fenton reagent+50 µg/ml TBFP, Lane 7: DNA+Fenton reagent+25 µg/ml TBS, Lane 8: DNA+Fenton reagent+50 µg/ml TBS, Lane 9: DNA+Fenton reagent+25 µg/ml TBB, Lane 10: DNA+Fenton reagent+50 µg/ml TBB

The results are obtained through gel electrophoresis (fig. 1), showed that the ethanolic extract of *T. bellerica* significantly protects DNA from oxidative damage. As can be observed in the fig. 1 where lane 1 in gel pictures is the positive control with only plasmid (pBR322) DNA in the fully supercoiled form and lane 2 is a negative control contain Fenton's reagent without plant extract and having only the open circular form. The lanes 3-10 in the corresponding gel picture signifies DNA protective activity with different concentrations (25 and 50 µg/ml) of plant extracts. The result suggested that the gradually increasing concentration of the plant extracts is retaining the supercoiled form, thus protecting DNA effectively. The result was significant in comparison to an earlier reported study by Singh *et al.*

2016 [11]. We conclude that a significant contributor to DNA damage prevention is the scavenging of hydroxyl radical by the *T. bellerica* leaf, fruit pulp, seed and bark at 25 and 50 µg/ml, respectively.

Safe doses of CCl_4

The screening of hepatoprotective activity of *T. bellerica* was based on the protection of human liver-derived HepG2 cells against CCl_4 induced oxidative damage, determined by estimating mitochondrial membrane integrity trypan blue exclusion assay and tetrazolium bromide salt MTT assay. Administration of CCl_4 resulted in a significant ($p<0.05$) reduction in cell viability and loss of membrane integrity as compared with the normal control group in a dose and time-dependent manner (table 1). CCl_4 treatment showed 75.52% cell viability at 0.5 µmol/ml concentration at 60 min, so this concentration and time period is selected for pretreatment of HepG2 cells for determining the hepatoprotective potential of tested extracts of *T. bellerica.*

Table 1: **CCl_4 treatment on HepG2 cell line**

Concentration of CCl_4	Percentage cell viability at different time period (min)			
	30 min	60 min	120 min	240 min
Control	100±1.8	100±1.2	100±1.4	100±1.0
0.1 µmol/ml	92.98±3.3	83.35±3.1	76.07±5.7	69.81±3.1
0.5 µmol/ml	79.27±5.0	75.52±3.3	48.10±2.3	19.96±1.7
1.0 µmol/ml	52.09±3.3	41.80±3.8	20.23±1.5	1.724±0.7
2.0 µmol/ml	26.92±3.4	17.40±4.6	1.875±1.5	0.380±1.1
5.0 µmol/ml	2.617±3.1	2.115±3.1	0.254±1.9	0±0.0

Values are mean±SD of three replications, CCl_4: Carbon tetrachloride

Hepatoprotective potential of tested extracts of *T. bellerica*

Plant extracts were tested for a possible restoration of the reduced viability of CCl_4 exposed HepG2 cells at different concentration (0.001 to 100 µg/ml) and time (24 to 96 h). All the extracts of *T. bellerica* showed dose-dependent hepatoprotective activity. Results were presented as the percentage (%) cell viability. The viability of HepG2 cells was found to be maintained at low doses of leaf and bark from 0.001-0.1 µg/ml for 48 h. Further increasing doses of both extracts from 1.00-100 µg/ml proved to be toxic for 96 h and resulted in a decrease of HepG2 cell viability. As per results, 0.1 µg/ml dose of leaf and bark was chosen as the protective dose and results were significant ($p<0.05$) in comparison with the unexposed group (fig. 2 and fig. 5). Fruit pulp and seed improved viability of HepG2 cells at lowest concentration, i.e., 0.001 µg/ml for 24 h. On increasing the concentration from 0.01 to 100 µg/ml, it proved to be toxic to cells. The concentration of 0.01 µg/ml was defined as the protective dose (fig. 3 and fig. 4). Hiraganahalli *et al.* [12] showed that a significant decrease in cell viability was observed upon treatment of HepG2 cells with tertiary butyl hydroperoxide (t-BH, 1 mmol). Treatment with *Phyllanthus emblica, Camellia sinensis, Mangifera indica, Punica granatum* and *Acacia catechu* extracts demonstrated a significant dose-dependent protection toward cell toxicity resulting from t-BH exposure. According to Priscilla and Jasmine [13] 95% of ethanolic fruit extracts of *T. chebula* showed 71.96% cell viability at 24 h which was lower than our 70% ethanolic fruit pulp and seed extracts.

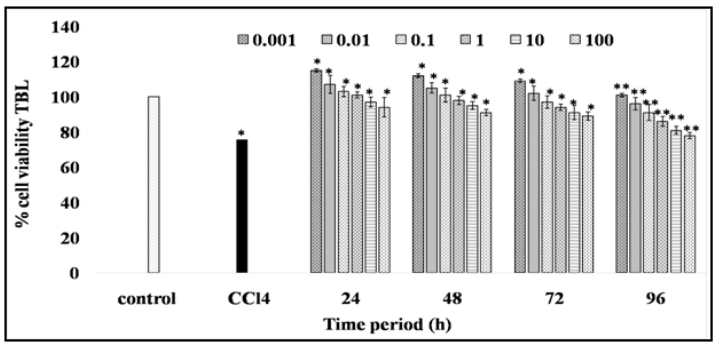

Fig. 2: Cell viability of HepG2 cells following the exposure of *T. bellerica* leaf (TBL) for different time periods (24-96 h) and concentration (0.001-100 µg/ml). Exposure of 0.5 µmol CCl_4 for 60 min was used as the positive control. The values obtained for control samples for each day was considered basal and compared with the values of corresponding treated samples. The data are expressed as mean percent of the unexposed control±SEM, n = 3. * =p<0.05, ** = p<0. 001

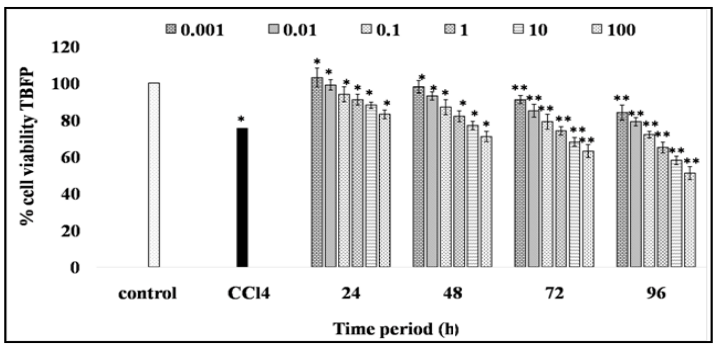

Fig. 3: Cell viability of HepG2 cells following the exposure of *T. bellerica* fruit pulp (TBFP) for different time periods (24-96 h) and concentration (0.001-100 µg/ml). Exposure of 0.5 µmol CCl_4 for 60 min was used as the positive control. The values obtained for control samples for each day was considered basal and compared with the values of corresponding treated samples. The data are expressed as mean percent of the unexposed control±SEM, n = 3. * = p<0.05, ** = p<0. 001

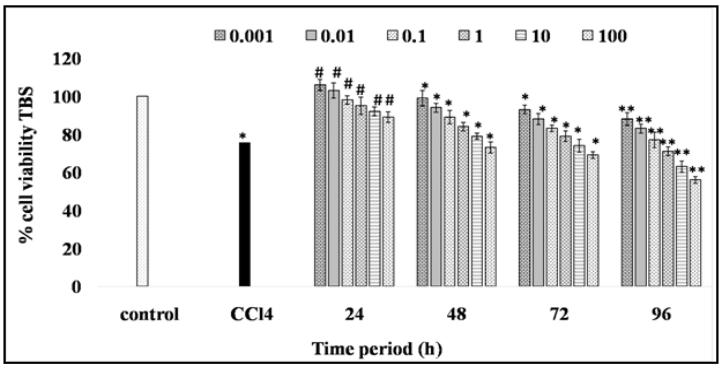

Fig. 4: Cell viability of HepG2 cells following the exposure of *T. bellerica* seed (TBS) for different time periods (24-96 h) and concentration (0.001-100 µg/ml). Exposure of 0.5 µmol CCl_4 for 60 min was used as the positive control. The values obtained for control samples for each day was considered basal and compared with the values of corresponding treated samples. The data are expressed as mean percent of the unexposed control±SEM, n = 3. * = p<0.05, ** = p<0. 001, # = not significant

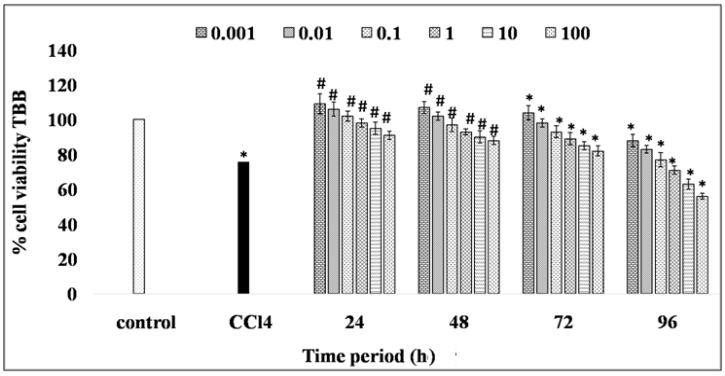

Fig. 5: Cell viability in HepG2 cells following the exposure of *T. bellerica* bark (TBB) for different time periods (24-96 h) and concentration (0.001-100 µg/ml). Exposure of 0.5 µmol CCl_4 for 60 min was used as the positive control. The values obtained for control samples for each day was considered basal and compared with the values of corresponding treated samples. The data are expressed as mean percent of the unexposed control±SEM, n = 3. * = p<0.05, ** = p<0. 001, # = not significant

Liver injuries induced by CCl_4 are the best-characterized system of xenobiotic-induced hepatotoxicity and commonly used models for the screening of hepatoprotective activities of drugs [14]. It has been established that CCl_4 is accumulated in hepatic parenchyma cells and metabolically activated by cytochrome P450 dependent monooxygenases to form a trichloromethyl radical (CCl_3•). The CCl_3•alkyls cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen to produce lipid peroxides, leading liver damage [15] which results in hepatocellular damage and enhances the formation of inflamed tissues. A single dose of CCl_4 leads to necrosis and steatosis while high administration leads to liver fibrosis, cirrhosis and hepatic carcinoma within few minutes. Thus antioxidant or inhibition of free radical scavengers is important in protection against CCl_4 induce liver damage [16, 17]. CCl_4 also induced the excessive release of serum enzymes and increase bilirubin levels, which cause a marked decrease in proteins levels.

T. bellerica contains a diverse set of vital phytoconstituents like gallic acid, ethyl galate, quinic acid, octadecanoic acid, glucopyranose, sitosterols etc [7]. Polyphenolic compounds in *T. bellerica* had dual effects on cells and modulated cell proliferation in a dose-dependent manner. In fact, at high dose, they were attributed to direct toxic effect and induced cells to death. It is evident from the extent of activity that *T. bellerica* contains hepatoprotective principles not only in fruits as reported earlier [18, 19], but the leaves and bark also contain various bioactive principles which may responsible for the hepatoprotective potential of *T. bellerica* [7]. Thus, determination of percentage cell viability of plant extracts is a biochemical parameter which indicates towards the potential of medicinal plants for their antioxidant, anti-inflammatory, anticancer and hepatoprotective activities. Thus, this study has generated leads for the future exploration of the medicinal value of *T. bellerica*.

In conclusion, it may be mentioned that the altered hepatic cell viability due to CCl_4 exposure is reversed towards normalization by four different extracts (leaf, fruit pulp, seed and bark) of *T. bellerica*. The effect was more pronounced with leaf and bark extracts in comparison to fruit pulp and seed. The contents of the extract protect the integrity of plasma membrane and increase the regenerative power of the liver at the same time period. Beneficial effect of the *T. bellerica* extracts may be due to the presence of phenolic compounds that have membrane stabilizing effects. Phenolic and other bioactive principles of *T. bellerica* may directly combine with free radicals and lead to inactivate them, which may suppress the intracellular concentration of free radicals. Thus results suggest that the compound present in the plant extract efficiently works on the liver to keep its normally functioning and minimizing cell membrane disturbances.

CONCLUSION

The present study first time demonstrated the protective activity of *T. bellerica* against hydroxyl radical-induced DNA damage and CCl_4 induced hepatotoxicity. In our earlier studies, *T. bellerica* showed the presence of many polyphenolics compounds which may contribute to the antioxidant, DNA damage protective and hepatoprotective activities. Phenolics and flavonoids normally scavenge free radicals and play an essential role in prevention and therapy of cardiovascular diseases and cancer. This study deals with the percent cell viability of different extracts in a concentration-dependent manner at the different time period. Leaf and bark showed significant hepatoprotective activity at higher concentrations than fruit pulp and seed. Overall, *T. bellerica* could be a potential source of natural hepatoprotective agent for many Ayurvedic remedies.

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AUTHOR CONTRIBUTIONS

Rasna Gupta: Design of the work, data collection and analysis, drafting article, writing manuscript, critical revision of the article.

Prof. Ram Lakhan Singh: Design of the work, data analysis, critical revision of the article, final approval of the version to be published.

Dr. AB Pant: Data analysis, critical revision of the article.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Subramaniam S, Khan HBH, Elumalai N, Lakshmi SYS. Hepatoprotective effect of ethanolic extract of the whole plant of *Andrographis paniculata* against CCl_4 induced hepatotoxicity in rats. Comp Clin Path 2015;24:1-7.
2. Gu X, Manautou JF. Molecular mechanisms underlying chemical liver injury. Expert Rev Mol Med 2012;14:e4.
3. Cichoż Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 2014;20:8082-91.
4. Lahlou M. The success of natural products in drug discovery. Pharmacol Pharm 2013;4:17-31.
5. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A handbook of medicinal plants. 1st ed. India: Agrobios Publishers; 2003.
6. Singh R, Singh SK, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn Food Chem Toxicol 2007;45:1216-23.
7. Gupta R, Singh RL, Dwivedi N. *In vitro* antioxidant activity and GC-MS analysis of the ethanolic extracts of *Terminalia bellerica* ROXB (Baheda). Int J Pharm Pharm Sci 2016;8:275-82.
8. Lee JC, Kim HR, Kim J, Yang YS. Antioxidant property of an ethanol extract *of Optunia ficus-indica Saboten*. J Agric Food Chem 2002;50:6490-6.
9. Kashyap MP, Singh AK, Siddiqui MA. Caspase cascade regulated mitochondria-mediated apoptosis in monocrotophos exposed PC12 cells. Chem Res Toxicol 2010;23:1663-72.
10. Pant AB, Agarwal AK, Sharma VP, Seth PK. *In vitro* cytotoxicity evaluation of plastic biomedical devices. Hum Exp Toxicol 2001;20:412-7.
11. Singh P, Singh RL, Kakkar P. Antioxidant, DNA damage protective and hepatoprotective activities of *Amorphophallus campanulatus*. Int J Pharm Pharm Sci 2016;8:330-8.
12. Hiraganahalli BD, Chinampudur VC, Dethe S, Mundkinajeddu D, Pandre MK, Balachandran J, *et al*. Hepatoprotective and antioxidant activity of standardized herbal extracts. Pharmacogn Mag 2012;8:116-23.
13. Deena Priscilla H, Jasmine R. Evaluation of *in vitro* anticancer activity of *Terminalia chebula* and identification of phytocompounds by GC MS analysis. J Chem Pharm Res 2016;8: 683-8.
14. Vasanth PR, Chandrasekhar RH, Vijayan P, Dhanaraj SA, Rao MC, Rao VJ, *et al. In vitro* and *in vivo* hepatoprotective effects of the total alkaloid fraction of *Hygrophila auriculata* leaves. Indian J Pharmacol 2010;42:99-104.
15. Knockaert L, Berson A, Ribault C. Carbon tetrachloride-mediated lipid peroxidation induces early mitochondrial alterations in mouse liver. Lab Invest 2012;92:396-410.
16. Talwar S, Jagani HV, Nayak PG. Toxicological evaluation of *Terminalia paniculata* barks extract and its protective effect against CCl_4-induced liver injury in rodents. BMC Complementary Altern Med 2013;13:127-33.
17. Haidry MT, Malik A. Hepatoprotective and antioxidative effects of *Terminalia Arjuna* against cadmium provoked toxicity in albino rats (*Ratus Norvigicus*). Biochem Pharmacol 2014;3:130.
18. Saroya AS. Herbalism phytochemistry and Ethnopharmacology. CRC Press: Taylor and Francis Group; 2011. p. 357-61.
19. Devi PN, Kaleeswari S, Poonkotha M. Antimicrobial activity and phytochemical analysis of fruit extracts of *Terminalia bellerica*. Int J Pharm Pharm Sci 2014;6:639-42.