Hepatoprotective effects of Tinospora cordifolia extract against bleomycin-induced toxicity in mice

A. N. Tolekova1*, Y. D. Karamalakova2*, G. D. Nikolova2, Tz. K. Georgiev1, V. G. Gadjeva2

1Department of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, 11 Armeiska Str., 6000 Stara Zagora, Bulgaria
2Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, 11 Armeiska Str., 6000 Stara Zagora, Bulgaria

* Both authors have worked equally on the results development and article processing.

Received: February 22, 2020; Accepted: March 13, 2020

Tinospora cordifolia (Willd.) Hook.f.&Thomson extract has previously been reported to alleviate appearance of liver alterations. The current study examined the antioxidant activity, therapeutic potential and action of the T. cordifolia extract to modulate and protect the liver alterations in bleomycin (BLM)-induced toxicity in ICR/w mice models. The hypothesis was that T. cordifolia extract would protect the liver alterations by inhibiting lipid peroxidation, lowering biochemical parameters, decreasing ROS production and reducing oxidative stress levels. Hepatocellular toxicity was induced by intraperitoneal injection of mice once daily with BLM (0.069 U/mL; 0.29 U/kg bw.) for a period of 4 weeks. The T. cordifolia was administered once a day for 4 weeks, 2 h prior at dose (80 mg/mL; 0.295 mg/kg/day). BLM intoxication produced oxidative stress in which the antioxidant system functioned incorrectly and ROS production significantly increased. The T. cordifolia extract provided significant hepatic protection against BLM toxicity by improving SOD, CAT (p < 0.04), MDA and total cholesterol (TC) levels and decreasing ROS in the group receiving BLM (p < 0.05), leading to reduced membrane lipid peroxidation. In conclusion, the T. cordifolia extract facilitated recovery from BLM-induced hepatic injury by suppressing oxidative stress damages. Therefore, the T. cordifolia stimulates antioxidant-scavenging activity and lipid peroxidation reduction in liver. Our results make it appropriate to propose the use of the T. cordifolia extract as a possible addition to the treatment of chronic liver alterations associated with BLM-induced toxicity.

Keywords: T. cordifolia; oxidative-scavenging imbalance; hepatotoxicity, mice.

INTRODUCTION

Tinospora cordifolia (Willd.) Hook.f. & Thomson. (T. cordifolia, Guduchi) belongs to the family Menispermaceae and is used as a protective antioxidant. Ayurveda, India’s traditional health system, recommends that the whole plant be used for therapeutic purposes. The extract from T. cordifolia has various active components in the structure such as alkaloids, steroids, diterpenoid lactones, aliphatics, glycosides, etc [1]. Moreover, T. cordifolia inhibits lipid peroxidation [2], stimulates bile secretion, activates immune effector cells, e.g., differentiation of T cells and B cells [3] and has diuretic properties. Some experimental studies indicate that T. cordifolia extract significantly reduces chemotherapy–induced toxicity, cell membrane oxidation, and has a protective role against neurodegenerative changes in the rat hippocampus [4, 5]. In addition, Sharma and Padney [6] have determined that T. cordifolia extract has strong antioxidant, anti-inflammatory, anti-arthritic, anti-allergic, anti-diabetic, antimalarial, immunomodulatory, antineoplastic and hepatoprotective properties. There is also evidence that T. cordifolia extract reduces damage to cellular oxidative stress and has free radical scavenging activity against reactive oxygen and nitrogen species (ROS/RNS) [7]. In the study of Sangeetha et al. [7] the antioxidant activity of T. cordifolia was attributed to the presence of tannins and phenolic compounds. The presence of alkaloids such as choline, tinosporin, isocolumin, etc. in aqueous or alcoholic extract of T. cordifolia shows detoxification effects and protection against toxin-induced disorders in the mice kidneys [8]. Treatment with T. cordifolia extract effectively increases intestinal absorption, hepatoprotection and the regulated alcohol-induced multivitamin deficiency [9]. Phytochemical analysis has shown that T. cordifolia extract protects carbon tetrachloride hepatotoxicity in animals [10, 11], and alleviates cisplatin –induced nephrotoxicity in vivo [11, 12]. Although T. cordifolia extract has different medicinal properties, its protective role has not yet been evaluated against bleomycin-induced toxicity, if any.

Bleomycin (BLM), is an antitumor antibiotic that has been identified as a medicine that induces...
reactive oxygen species (ROS/ •O2-, H2O2, •OH, NO•); cell membrane instability, lipid and protein peroxidation, inflammatory responses in the lung (fibrosis) [13]; and as a result toxic products are generated [13, 14].

The present study attempts to elucidate the hepatoprotective efficacy of T. cordifolia extract in regulation against experimentally BLM-induced toxicity on free radical production and changes in oxidative stress in the liver cells of male IRC/ w mice.

MATERIALS AND METHODS

Chemicals and preparation of T. cordifolia extract

Bleomycin sulfate (EP 9041-93-4), Carboxy-Ptoio.K, and other chemicals were purchased from Sigma Aldrich Co., USA, and were of analytical grade. T. cordifolia fine powder (ABC Limited, India; identified by a plant taxonomist) was kinetically extracted (for 48 h in 100% ethanol, v:v). The total filtrate was dried using a rotary evaporator (Buchi B-480, India) at 400c and was lyophilized (Iishin Lab Co. Ltd, USA) to crude extract. The T. cordifolia extract was stored in air-tight glass bottle at 8°C, and it was used as a practical approach to protect against BLM-intoxication.

Maintenance of animals

Male ICR/w mice weighing approximately 45-50 ± 3.0 g were obtained from the Medical Faculty, Trakia University, (Suppliers of Laboratory Animals), Stara Zagora, Bulgaria. The animal procedures were in accordance with Directive 2010/63/EU on the protection of animals used for experimental and other scientific work, and approved by the Ethical Committee for Animals of BFSA and Trakia University, Stara Zagora, Bulgaria. The animal procedures were in accordance with Directive 2010/63/EU on the protection of animals used for experimental and other scientific work, and approved by the Ethical Committee for Animals of BFSA and Trakia University, Stara Zagora, Bulgaria (131/ 6000-0333/ 09.12.2016). The mice were housed in polypropylene cages at a temperature of 18–20°C and under a light/dark period of12/12 h. They were fed on a standard commercial feed and the activities of superoxide dismutase (SOD) estimated by the method of Plaser et al., 1966 [16], and the activities of superoxide dismutase (SOD) and catalase (CAT) were analysed using the method described by Sun et al., 1988 [17] and by Aebi, 1984 [18], respectively. The TC in blood was estimated using a commercially available diagnostic kit (AM-2035-KA, 2017). The biochemical analyses were performed on a UV–VIS spectrophotometer-400 (TERMO Sci., RS232C, Stratagene, USA).

Electron paramagnetic resonance (EPR) in vivo evaluation of ROS production

ROS production in the liver samples was investigated by in vivo EPR (X-Band, Emxmicro spectrometer, Bruker) method according to Shi et al. (2005) [19]. Briefly, to 100 μl plasma and 100 mg of spleen were added 900 μl of 50 mM N-t-butyl-alpha-phenyl nitron (PBN) dissolved in dimethyl sulfoxide (DMSO) and centrifuged at 4000 rpm/ 10 min at 4°C, with settings: 3505 g centerfield, 6.42 mw microwave power, 5 g modulated amplitude, 1-5 scans. All experiments were made in triplicate.
A. N. Tolekova et al.: Hepatoprotective effects of Tinospora cordifolia extract against bleomycin-induced toxicity

Statistical analysis

The processing of the spectra was performed using Bruker Win-EPR and Sim-sonia software. Statistical analysis was performed with Statistica 8.0, Stasoft, Inc., one-way ANOVA, Student-t test to determine significant difference among data groups. The data were expressed as means ± standard error (SE). A value of p < 0.05 was considered significant.

RESULTS AND DISCUSSION

Changes in biochemical enzymes after exposure to BLM, both alone and in combination with T. cordifolia extract, showed a significant change in oxidative / pro-oxidative activity. BLM exposure (Fig. 1) produced statistically significant decrease in SOD (6.88±0.46 IU/gHb, p<0.03) and CAT (5.433±0.91IU/gHb, p<0.05) activities, compared to CG (16.28±1.35 IU/gHb). In addition, T. cordifolia extract showed a statistically significant increase in the levels of both antioxidant enzymes (SOD: 18.49±3.16 IU/gHb; CAT: 14.83±1.21 IU/gHb), compared to untreated CG (p<0.05) and to BLM treated (p<0.05) group. Moreover, administration of the T. cordifolia extract 2 h before BLM treatment showed a protective effect on the hepatic cells in SOD (p<0.05) and CAT (p<0.003) activities.

The BLM induced toxicity increased oxidative stress disorders and inflammatory responses, due to the destructive free-oxygen production and leading to highly lipid peroxidation. Experimentally, BLM has been used to induce chronic toxicity in mice models at a dose of 0.069 U/mL; 0.29 U/kg bw dissolved in saline and to produce oxidative hepatocellular changes [21].

A number of studies have reported the isolation and protection of active biomolecules from plant antioxidants against chemotherapy-induced damage and toxicity [6, 20, 22] as effective inhibitors of ROS. There is evidence that plant extracts containing alkaloids and glycosides are potent inhibitors of various oxidative processes, exhibit significant antioxidant activity, prevent lipid peroxidation and restore SOD and CAT activity [23].

Superoxide dismutase (SOD) catalyses the dismutation of superoxide anion (•O₂⁻) to H₂O₂ and O₂, while catalase (CAT) reduces the H₂O₂ levels into H₂O molecules [24]. In our experiment, it was shown that SOD activity and CAT activity were statistically significantly decreased in the BLM group compared to controls (p <0.05 vs. CG).

The decrease in endogenous antioxidant enzymes is likely to be associated with increased oxidative damage that contributes to the inflammatory response of BLM administration. In addition, T. cordifolia extract contains alkaloids and glycosides and has the potential to reduce oxidative stress damage by inactivating H₂O₂ and by inhibiting inflammatory responses.

These results simultaneously support the claim that treatment with 80 mg / mL T. cordifolia provides protection against the effects of BLM-induced stress; and indicate the protective role of T. cordifolia in liver tissues [6, 25].

Oxidative stress is associated with an imbalance between the production and purification of ROS products. ROS overproduction and BLM-induced oxidative damages contribute to hepatocyte injuries and these processes increase cell lipid damage and induce hepatic cell malformation [26, 27]. To investigate the effects of T. cordifolia extract on
hepatic lipid accumulation, we measured MDA levels in liver homogenates and total plasma cholesterol (TC) in all tested groups (Fig. 2).

However, MDA (11.6±4.16 μM/ng Pr vs. 6.16±1.03 μM/ng Pr; p<0.003, t-test) and TC (80.56±11.12 μM/ng Pr vs. 45.7±7.03 μM/ng Pr; p<0.05, t-test) levels all significantly increased in the BLM model, compared with the CG. The combination of T. cordifolia and BLM (80.56±11.12 μM/ng Pr vs. 45.7±7.03 μM/ng Pr; p<0.05, t-test) correspondingly reduced the increased plasma lipid concentrations, in MDA (9.253±0.91 μM/ng Pr vs. 11.6±4.16 μM/ng Pr; p<0.05, t-test) and TC (47.56±11.12 μM/ng Pr vs. 80.56±11.12 μM/ng Pr; p<0.05, t-test), compared to BLM treatment.

Consistent with these findings, we found comparable values in the plasmatic lipid peroxidation between the T. cordifolia extract and controls. In accordance with our results, other investigations report inhibition in the lipid peroxidation process, prevention of tissue damages thereby maintaining the membrane integrity and free-radicals reduction in chemo-induced toxicity, after T. cordifolia extract application [28-30].

**Figure 3.** In vivo ROS radical production. Liver samples were collected from all sacrificed animals. Results were calculated by double integration of the corresponding EPR spectrum immediately registered in liver homogenates (expressed in arbitrary units/arb. units). The experiments were repeated three times. *p<0.05 vs. the CG group; **p<0.004 vs. the BLM group (n=6).

Banerjee et al. [31] commented that intracellular, endogenous ROS expression in peripheral blood of patients suffering from persisting polyarthralgia post CHIK infection was significantly scavenged by ex vivo treatment with T. cordifolia leaf extract. To confirm the efficacy of T. cordifolia extract containing alkaloids and glycosides, reduction of the BLM-induced toxicity in liver homogenates was evaluated. Figure 3 shows the EPR spectra of ROS products in liver homogenate measured in arbitrary units.

The results demonstrate the highly toxic effects of BLM administration, and showed a statistically significant increase of ROS production in hepatic cells (1.72 ± 0.901 vs. 0.858 ± 0.21 a.u., p<0.05, t-test), relative to the CG. However, the ROS products levels were close to that in CG in the group treated with T. cordifolia (0.739 ± 0.14 vs. 0.858 ± 0.21 a.u., t-test), or with a combination of T. cordifolia + BLM (0.997 ± 0.33 vs. 0.858 ± 0.21 a.u., t-test). The EPR method indicated an increased ROS concentration in hepatocytes. The statistically significant decrease in ROS production in hepatic cells was observed in T. cordifolia + BLM combination (0.923 ± 0.5 a.u. vs 1.72 ± 0.901, p<0.004, t-test), in comparison to the BLM administration. However, T. cordifolia extract administration completely ameliorated the ROS production and hepatic pro-oxidative effect in BLM-intoxicated mice (p<0.05). Different investigations have suggested that the plant extract has a protective effect against damages in hepatic function due to direct antioxidant [32] and free radical scavenging mechanisms and regulation of ROS production [31-33]. Moreover, Baskaran et al. [25] reported that T. cordifolia extract regulates free radicals levels and lipid peroxidation by countering Cd-induced oxidative stress and by controlling enhanced ROS production effected over tissue glycoproteins in liver cells and hepatotoxicity.

**CONCLUSION**

Finally, our results indicated that T. cordifolia extract treatment stimulated endogenous antioxidant activity, reduced lipid peroxidation and scavenged ROS products. These results make it appropriate to propose the use of the T. cordifolia extract as a possible addition to the treatment of chronic hepatotoxicity associate with chemo-induced oxidative damages.

**Acknowledgement:** This study was supported by scientific projects 6/2016 and 5/2017 of Medical Faculty, Trakia University, Stara Zagora, Bulgaria.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**REFERENCES**