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Anti-hepatotoxic activity of in-vitro produced berberine and ethanolic callus and suspension culture extracts of *Tinospora cordifolia* Miers in albino wistar rats

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Tinospora cordifolia (Willd) Miers (Family: Menispermaceae), an edible plant and source of berberine (isoquinoline alkaloid), is employed extensively in South Asian Traditional Systems of Medicines as a tonic for liver and heart, immuno-modulator and antioxidant (Kirtikar & Basu 1991). *T. cordifolia* has rarely been studied empluying plant tissue culture techniques for in-vitro production of berberine, a compound of commercial interest all over world. Plant contains low concentration of berberine. So, the present investigation was aimed to develop standardised protocols for in-vitro enhanced production of berberine and establishment of mechanism based anti-hepatotoxic activity. For callus initiation, excised leaf and stem explants of matured plant were cultured in-vitro on Murashige and Skoog's (MS) medium supplemented with different combination and concentration of plant growth regulators under controlled condition of temperature (25 ± 2°C), light (1600 lux) and humidity (65%) with day/night regime (16/8 h). For suspension culture studies, calli of fourth passage were cultured on agar free medium with same hormonal combinations. For surface sterilisation of explants, sodium hypochlorite solution (2%) with few drops of tween 80 and contact time of five minutes was found most effective. The MS medium with 2,-4 dichloro-phenoxyacetic acid (2,4-D) either alone or in combination with low concentration of Kinetin (Kin) were found favorable for initiation and development of leaf and stem callus. Medium with 2,4-D (2 ppm) and Kin. (0.5 ppm) showed best callus initiation while medium with 2,4-D (3 ppm) and Kin (1 ppm) along with ascorbic acid (1 gl^{-1}) showed best development of calli. For suspension culture same hormonal combinations devoid of agar were found suitable. Berberine was isolated, purified, characterized and quantitatively estimated in cultures by HPLC analysis (Chang et al 1991). To induce hepatotoxicity, carbon–tetrachloride (CCl_4) was administered orally/intraperitonealy in albino wistar rats. Purified berberine and extracts were administered orally and intraperitonealy at different doses for one week. CCl_4 (1.5 ml/kg/day p.o. and 0.5 ml/kg/day i.p.) produced severe liver damage marked by increase in serum liver transaminases (Reitman & Frankel 1957), albumin, total protein (Gornal & Bardawill 1949) billirubin and alkaline phosphatase (Lowry & Brock 1946). Assessed biochemical parameters were correlated with hepatic lesions produced which include hepatic necrosis, degeneration, broad infiltration of lymphocytes and kupffer cells. One week treatment with extracts at different doses (100 mg/kg/day p.o.; 250 mg/kg/day p.o.) and berberine (10 mg/kg/day p.o.; 5 mg/kg/day i.p.) significantly alleviated serum enzyme activity and liver body weight ratio. Histopathology showed reversal effects. Effects were compared with commercially procured berberine (Sigma; 10 mg/kg/day p.o.; 5 mg/kg i.p.) and standard drug (i.e. silymarin 100 mg/kg/day p.o.). Purified berberine as well as extracts showed remarkable anti-hepatotoxic activity (85% and 60–80%, respectively). Moreover, suspension culture extracts produced better than callus culture extracts due to high berberine content. Berberine possesses significant anti-hepatotoxic property. Its pharmacodynamics include inhibition of phopholipase A2, alleviation of lipid (LDL), cholesterol, and triglycerides, attenuation of CCl_4 induced depletion of glutathione, increased DNA repair synthesis and anti-oxidant property by quenching of free radicals.

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