

Chapter 4

Pharmacological Investigations and Toxicity Studies

Abstract Red Silk-Cotton tree has been scrutinized for its pharmacology in various parts of the world. Different parts of *Bombax ceiba* has shown to possess many biological properties predominantly antioxidant, antimicrobial, anti-inflammatory, analgesic, anabolic, hepatoprotective, hypotensive and hypoglycemic activities. It has proved to be safe in various toxicity studies. However, it still needs extensive scientific exploration.

Keywords Antioxidant • Antimicrobial • Antitumor • Anabolic • Hepatoprotective • Hypotensive • Hypoglycemic

4.1 Introduction

In the last decade, *Bombax ceiba* has attracted scientific attention that resulted in exploration of many novel chemical compounds as well as validation of its traditional uses in many diseases of man. Animal experimental studies have shown that *B. ceiba* has important pharmacological activities without much toxicity. Moreover, human studies have further strengthened its therapeutic role as an anabolic, antihyperglycemic, hypolipidemic and fibrinolysis-enhancing agent. The following discussion will restrict to its important pharmacological activities with passing comments on its other effects.

4.2 Antioxidant Activity

Methanolic extract of whole plant material of *B. ceiba* showed DPPH radical scavenging activity with an IC_{50} of 68 $\mu\text{g/ml}$ among 26 folk herbal medicinal plant extracts popularly used in Taiwan. *Bombax ceiba* did show the higher scavenging activity than that of *Ginkgo biloba* leaf extract (IC_{50} 930 $\mu\text{g/ml}$) used as reference. It also exhibited significant protection on $\Psi \times 174$ supercoiled DNA against strand cleavage induced by UV irradiated H_2O_2 . The magnitude of inhibition of open circular DNA formation with *B. ceiba* was 0.35 which is quite comparable to that of catechin 0.38, used as a reference control (Shyur et al. 2005). This effective prevention of hydroxyl radical-induced DNA damage by *B. ceiba* needs further attention.

Dar et al. (2005) studied mangiferin, a xanthone isolated from methanolic extract of fresh leaves of *B. ceiba* and tested it (1) and its acetyl (1a), cinnamoyl (1b) and methyl (2) derivatives along with the methanolic extract (BCL) and the filtrate (BCM) for antioxidant activity in DPPH free radical scavenging assay, Deoxyribose degradation assay and non-enzymatic lipid peroxidation in liposomes. Methanolic extract along with mangiferin showed DPPH scavenging activity where mangiferin has shown IC_{50} value of 5.8 ± 0.96 $\mu\text{g/ml}$ which was quite comparable to IC_{50} of Rutin (5.56 ± 0.33 $\mu\text{g/ml}$). Mangiferin appears to be a better antioxidant compound than 1a and 1b which bears no 6,7-dihydroxylated structure (Catechol moiety). However, methyl derivative of mangiferin (2) was devoid of DPPH radical scavenging activity even at concentration of 200 $\mu\text{g/ml}$ suggesting that the presence of methoxy group abolishes the antioxidant activity. Furthermore, mangiferin could not significantly protect against either deoxyribose damage or lipid peroxidation.

Surveswaran et al. (2007) have studied 133 Indian medicinal plants for their antioxidant activity using three in vitro assays (ABTS, DPPH and FRAP). Eighty percent methanolic extract of gum (*Mochrasa*) of *B. ceiba* was evaluated for its total phenolic contents and antioxidant activity. Total phenolic content of the gum was found to be 5.89 GAE/100 g dry weight. Gum has shown 55.38 mmol TEAC/100 g dry weight in ABTS assay and 80.12 mmol TEAC/100 g DW in DPPH assay, whereas FRAP assay has shown value of 9.06 $\mu\text{mol TEAC/100 g DW}$.

Lately, Vieira et al. (2009) have reported antioxidant activity of defatted methanolic extract of flowers of *B. ceiba*. The EC_{50} ($\mu\text{g/ml}$) obtained for DPPH radical was 87 and for lipid peroxidation of rat liver microsomes and soy bean phosphatidylcholine liposomes induced by ascorbyl radicals were 141 and 105, respectively and by peroxyxynitrite were 115 and 77, respectively. The extract also inhibited myeloperoxidase activity and kinetics of this enzyme inhibition gave $K_{0.5}$ value of 264 $\mu\text{g/ml}$. Cytotoxicity of the extract was also monitored through the mitochondrial activity in the Vero cell line. Extract started to show toxicity toward cells at 750 $\mu\text{g/ml}$ which was a much higher concentration than those which showed antioxidant activity.

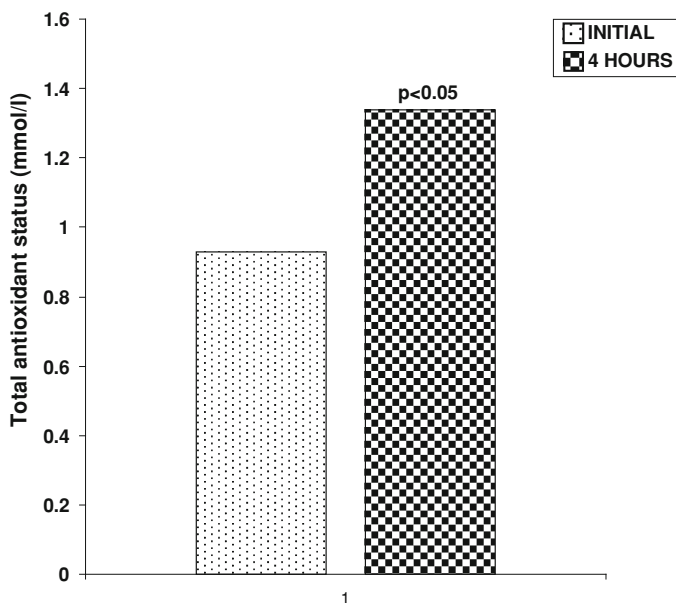


Fig. 4.1 Acute effect of 3 g *Bombax ceiba* root powder administration on total antioxidant status in ten healthy volunteers (Jain et al. 2011a)

Yu et al. (2011) assessed the antioxidant activities of water, 50% ethanol, 80% acetone extracts from flowers of *Bombax malabaricum* on DPPH radical scavenging, ORAC, reducing power and inhibition on phosphatidylcholine liposome peroxidation. All extracts showed remarkable antioxidant capacity compared with ascorbic/gallic acid.

Recently, Jain et al. (2011a) have assessed antioxidant activity of *B. ceiba* root using DPPH radical scavenging and reducing power assay. Methanolic extract of root showed high amounts of phenolics (30.9% w/w) and tannins (15.45% w/w) and a very good DPPH scavenging activity (EC_{50} 15.07 $\mu\text{g/ml}$) in a dose-dependent manner as well as dose-dependent reduction ability (Fe^{3+} to Fe^{2+} transformation) with a maximum absorbance of 1.11 at a concentration of 500 μg of extract. Furthermore, acute study in human healthy volunteers showed a significant ($p < 0.05$) rise in total antioxidant status at the end of 4 h after administration of 3 g root powder (Fig. 4.1).

4.3 Antimicrobial Activity

The plant is endowed with strong capacity to fight against microorganisms such as bacteria, fungi and viral attacks. Each part possesses antimicrobial activity as has been demonstrated in various in vitro experimental studies. The research can be

traced back to 1968 when Dhar et al. demonstrated antiviral activity of 50% ethanolic extract of *B. ceiba* flowers at a maximum tolerated dose of 250 mg/kg body weight against Ranikhet disease virus.

Antimicrobial research work then took a pause of 20 years when in 1991 Mishra et al. reported the effect of water extract of leaves of *B. ceiba* against fungi. They observed that water extract of leaves of *B. ceiba* demonstrated 90.4% mycelial inhibition against *Epidermophyton floccosum*, 80.4% against *Tricophyton mentagrophytes* and 75.25% against *Microsporium gypseum*.

In 1999, Faizi and Ali reported antibacterial and antifungal activity of this plant. Shamimin, isolated from ethanolic extract of its fresh leaves at a concentration of 100 µg, showed zone of inhibition (8–12 mm) against three Gram-positive (*Listeria monocytogenes*, *Bacillus subtilis* and *Streptococcus pyogenes*) and five Gram-negative (*Shigella sonnei*, *Salmonella typhi*, *Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Shigella flexneri*) bacteria. Furthermore, at a similar concentration, it inhibited growth of *Candida albicans* among the five fungi in an in vitro antifungal assay.

Interestingly, leaves and bark of *B. ceiba* were also tested against multi-drug resistant *S. typhi* strains MTCC 531 and B 330. Methanolic and aqueous extract of bark at a concentration of 50 mg/ml showed strong antimicrobial activity (≥ 5 –9 mm diameter of zone of inhibition) against *S. typhi* strain MTCC 531. Methanolic extract of bark showed ≥ 9 –15 mm diameter of zone of inhibition while aqueous extract showed ≥ 5 –9 mm diameter of zone of inhibition against *S. typhi* strain B 330. Minimum inhibitory concentration (MIC) of methanolic bark extract assessed was found to be 256 µg/ml against both the strains used. Methanolic extract of leaves, on the other hand, showed ≥ 5 –9 mm diameter of zone of inhibition against only strain MTCC 530 and aqueous extract was completely ineffective (Rani and Khullar 2004).

Wang and Huang (2005) while screening 50 Taiwanese folk medicinal plants against ten strains of *Helicobacter pylori* observed that ethanolic extract (95%) of *B. ceiba* root has shown anti-*H. pylori* activity against all the strains. MIC values were ranging from 1.28 to 5.12 mg/ml.

Not only the plant part, such as flower, leaves, stem bark and root showed antimicrobial activity, but also the fungal endophytes isolated from leaves and stem have shown anti-*Mycobacterium tuberculosis* activity using microplate Alamar blue assay and anti-Herpes Simplex Virus activity in Vero cell assay. However, no antimalarial activity was observed (Wiyakrutta et al. 2004).

It is interesting to note that as far as antimicrobial activity is concerned, brunt of work is on flower, leaves and stem bark. However, there are very few studies on root. Recently, the methanolic extract of root in five concentrations (50, 25, 12.5, 6.25 and 3.12 mg/ml) has shown a significant dose-dependent antibacterial potential in in vitro agar well diffusion assay. Zone of inhibition (mm diameter) obtained at the highest concentration of methanolic extract (50 mg/ml) for Gram-positive bacteria *Staphylococcus aureus*, *B. subtilis* and Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* was 17.0, 16.0, 17.16 and 17.10, respectively (Jain et al. 2011b).



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