ABSTRACT

One of the most prevalent malignancies, hepatocellular carcinoma (HCC), is linked to high rates of morbidity and mortality and has no effective mechanism-based treatments. A novel therapeutic approach for the treatment of cancer is to target abnormal tumor metabolism. Cancer metabolism has received much attention, particularly in relation to glucose metabolism. A major part of glucose metabolism is the pentose phosphate pathway (PPP) and Glucose-6-phosphate dehydrogenase (G6PD) is the basic rate-limiting enzyme in the pentose phosphate pathway. Glucose-6-phosphate dehydrogenase has recently been reported to be over expressed in human cancers and has been linked to apoptosis and angiogenesis, making it a promising target for cancer therapy. Therefore, the current study was aimed to screen the plants extracts library to find potent hits against G6PD and to identify potentially active entities against HCC. The target protein based screening of plants extracts library was done by enzymatic activity assay. The recombinant plasmid pET-24a-HmG6PD was expressed in *E. coli* BL21-DE3 strain followed by its purification and evaluation using metal affinity chromatography with Ni-NTA columns and SDS-PAGE. The cell based screening of the plants extracts to evaluate their cytotoxicity against HepG2 cells of the liver cancer was done by MTT assay. To identify the phytochemical constituents present in the methanolic extract of a plant, GC-MS analysis was performed, followed by a molecular docking study to determine their binding affinity with G6PD. Based on the cytotoxicity against cancer cells, three plants extracts were further selected for toxicity profiling in mice. The extracts were given orally to Swiss albino mice in various concentrations (50 mg/kg b.w., 100 mg/kg b.w., 200 mg/kg body weight) in subacute toxicity study for 28 days and a single dose of (2000 mg/kg body weight) in acute toxicity trial for 3 days. At the end of experimental period different biochemical (ALT, AST, ALP, Creatinine, Urea, Uric acid) and hematological (RBCs, WBCs, PLT, Hb, Hematocrit, MCV) parameters were analysed.

Vital organs (Liver, Kidney, Spleen) were also examined for histopathology.Out of 51 screened plants extracts, the twenty plants extracts have shown inhibitory activity against the G6PD protein. Out of 20 plants extracts that were found active against G6PD, 15 extracts, exhibited the highest inhibitory activity (> 80% inhibition). Three extracts such as *Cassia fistula* (leaves), *Lowsonia inermis* (leaves) and *Mangifera indica* (pulp) were found moderately active (60% -80% inhibition), whereas, two plants extracts *Ageratum conyzoides* (whole plant) and *Asphodelus tenuifolius* (whole palnt) were slightly active (< 60% inhibition) against G6PD. The highly active plants extracts were further tested at different concentrations and their IC_{50} values were calculated. The eleven plants extracts, *Aloe barbadensis* (whole plant, IC_{50}: 0.857 µg/ml), *Bombax ceiba* (bark, IC_{50}:0.457 µg/ml), *Smilax china* (root, IC_{50}: 1.397 µg/ml), *Eucalyptus camaldulensis* (bark, IC_{50}: 0.059 µg/ml), *Litchi chinensis* (seeds, IC_{50}: 1.238 µg/ml; bark, IC_{50}: 2.350 µg/ml; leaves, IC_{50}: 1.199 µg/ml), *Mangifera indica* (Peel, IC_{50}: 0.825 µg/ml), *Helianthus annuus* (Seeds, IC_{50}: 4.644 µg/ml), *Cyprus esculentus* (flowers, IC_{50}: 3.270 µg/ml) and *Punica granatum* (seed coat, IC_{50}: 3.573 µg/ml), inhibited the G6PD activity significantly at lower concentrations.Out of 11 plants extracts screened against HepG2 cells, four plants extracts (*Smilax china* root extract > 80%, *Litchi chinensis* leaves, *Punica granatum* seed coat extracts > 40% and *Mangifera indica* peel extract > 35%) were found cytotoxic against these cells at a final concentration of 200 µg/ml. Out of 4 cytotoxic plants extracts, *Smilax china* root extract was found most effective inhibitor of HepG2 cells. Therefore, *Smilax china* root extract was further evaluated against HepG2 cells in a dose dependent manner and was found as potent inhibitor of cancer cell advancement with an IC_{50} value of 16.017 µg/ml. It was revealed from GC-MS analysis that methanolic extract of *Smilax china* root has thirty high bioactive compounds. The results of docking study showed that the topmost three compounds were Scirpusin A, Smilachinin and Daucosterol with MolDock Score of -156.832, -148.215, and -145.733 respectively, against NADP+ binding site of G6PD.

After toxicity profiling in mice, three plants extracts (Punica granatrum seed coat (PG), Litchi chinensis leaves (LC) and Smilax china root (SC) were found non toxic. The level of all parameters showed no significant differences as compared to control mice even at the highest dose of 2000 mg/kg of body weight as ALT (control: 64.2 ± 3.0 U/L; PG: 68.4 ± 2.8 U/L; LC: 68.8 ± 3.9 U/L; SC: 60.6 ± 3.3 U/L), ALP (control: 167.4 ± 12.2.15 U/L; PG: 175.6 ± 11.2 U/L; LC: 186.6 ± 7.6 U/L; SC: 179.2 ± 7.0 U/L), AST (control: 51.2 ± 2.7 U/L; PG: 56.6 ± 4.1 U/L; LC: 50.4 ± 3.2 U/L; SC: 55.6 ± 4.1), Creatinine (control: 0.6 ± 0.1 mg/dl; PG: 0.7 ± 0.0 mg/dl; LC: 0.8 ± 0.0 mg/dl; SC: 0.8 ± 0.1 mg/dl), Urea (control: 27.4 ± 1.8 mg/dl; PG: 29.2 ± 0.9 mg/dl; LC: 32.2 ± 1.8 mg/dl; SC: 31.2 ± 0.6 mg/dl), Uric acid (control: 19.8 ± 0.9 mg/dl; PG: 20.2 ± 0.8 mg/dl; LC: 21.4 ± 1.1 mg/dl; SC: 20.2 ± 0.8 mg/dl), HDL (control: 57.4 ± 3.9 mg/dl; PG: 67.4 ± 5.0 mg/dl; LC: 70.6 ± 3.6 mg/dl; SC: 71.2 ± 2.3 mg/dl), LDL (control: 5.4 ± 0.7 mg/dl; PG: 6.1 ± 0.5 mg/dl; LC extract: 7.0 ± 0.3 mg/dl; SC: 7.5 ± 0.5 mg/dl). Histopathological study of liver, kidney and spleen also showed the non toxic effect of the plants extracts. It is investigated that, Punica granatrum seed coat, Litchi chinensis leaves and Smilax china root extracts could be safer drug candidates for the treatment of hepatocellular carcinoma.