Abstract

This research work is carried out in partial fulfillment of Doctor of Philosophy (Pharmacy). The current research work entitled "Natural Remedies for Hepatic Diseases" was used as a hepatoprotective natural product.

Liver disease is one of the most common lifestyle diseases these days, affecting one in every three people. It is one of the greatest health challenges of the 21st century. Although this condition is common, easily recognised, yet no proper comprehensive treatment unveiled till now. It is usually asymptomatic and often leads to serious complications such as fatty liver, jaundice, organ failure, fibrosis, acute liver failure, chronic liver damage, and metabolic imbalance syndrome if left unattended. In addition, excessive use of medications eg NSAIDs, exposure to industrial chemicals, and alcohol abuse can cause complete damage of liver. Here earthworm crude extract and 50 kDa MWCO of earthworm extract proteins were isolated. Then different concentrations were made especially at 25, 50, 100, and 200 mg/ml, and used in the in-vivo model. Moreover, it was found to significantly increase its expression in primary hepatocytes with 25, 50, 75, 100 ug/ml in an in vitro model. Crude protein is first extracted from *Metaphyre Posthuma* in protein extraction. Additionally, for 50 MWCO we used Sephadex G 75, Viva spin column, 50-kDa nominal molecular weight cut-off (MWCO), 3-kDa MWCO column, Centriprep® Centrifugal Filter 10 kDa, and for fast protein purification after dialysis. FPLC is performed. For the protein test, total protein assay, bradford assay, and lowry protein assay are done. In addition, crude protein, protein below 50 MWCO, and 6.023 kDa were analyzed by SDS PAGE then trypsin digestion and MALDI TOF MS was performed, and some known and also some unknown sequence of the peptide were found. It could be hypothesized that this protein or peptide may have hepatoprotective activity.

High doses of paracetamol, carbon tetrachloride, and ethanol were applied in an in-vivo model to evaluate the hepatoprotective activity of *Metaphire Posthuma* protein powder (MP). Acute and chronic model of toxicity, especially the liver fibrosis models were used in this study. Standard medicine especially silymarin, ursodeoxycholic acid, and goat milk as vehicle were used accompanying with earthworm protein.

During the study, wistar albino rats were divided into four groups: control, paracetamol-treated, paracetamol alone with EP(earthworm extract protein powder), and paracetamol

along with silymarin. The animals showed significant elevations in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, LPO or MDA, total bilirubin in plasma, mRNA expressions of TNF-α and IL-6 in liver tissue while the levels of CAT, SOD, and GSH in plasma was found decreased in the paracetamol group. The separated MWCO 50 kDa protein from EP showed significant reduction of AST and ALT, ALP, and bilirubin in primary hepatocyte culture and increased percentage viability of cells. The histological examination showed disruption of liver histoarchitecture in the paracetamol group, but restoration was found in the paracetamol-EP group. The in-vivo study, restoration of biochemical parameters, oxidative stress markers modulation, changes in mRNA expressions, and histopathological findings in the paracetamol-EP and paracetamol-silymarin groups. The acute liver damage due to paracetamol is attributed to oxidative stress in the animal model. EP decreased oxidative stress and inflammation, slowing down the liver damage progression caused by paracetamol. EP activities involving cytokine expressions using lower molecular weight protein fractions are of scientific importance with correlation to real-time use.

Another study aimed to evaluate the effectiveness of pre-treating primary hepatocyte cells with earthworm extract to prevent damage from carbon tetrachloride (CCl_4). Cell viability, AST, ALT, MDA levels, TNF-α activities, and hepatoprotective activity of Earthworm extract protein powder (EEP), goat milk powder (GP), and Silymarin (SLM) were assessed in-vitro on rat primary hepatocyte culture. The study found that CCl4-induced cytotoxicity is influenced by oxidative stress. The hepatoprotective activity of EEP and EEP+GP was identified, and lipid peroxidation assays showed that MDA levels were inhibited by EEP and GP treatment. The study concluded that EEP and GP function as efficient hepatoprotective agents, preventing CCl_4 treatment-induced primary hepatocyte toxicity.

Ethanol consumption is responsible for 60-80% of liver disease deaths, including Alcoholic liver disease (ALD). Et-OH (ethanol) causes oxidative stress and inflammation, leading to immunological dysfunction and cytokine imbalance. Addressing Et-OH-induced liver damage can be achieved using GSH and CAT enhancers. ALD disrupts bile acid metabolism and increases chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA). Goat milk (GM) is a suitable delivery medium for MP, which can produce synergistic effects against hepatic damage. Acute toxicity studies show a maximum tolerated dose of MP (*Metaphire Posthuma* protein powder) is over 2 g/kg in rats, while sub-acute toxicity evaluation shows no toxic effects. MP+GM reduces pro-inflammatory markers and protects against Et-OH-induced

hepatotoxicity by increasing CAT, GSH, Albumin, total bilirubin, cholesterol, and suppressing triglyceride activity.

Chronic liver disease, a major cause of severe hepatotoxicity, is primarily mediated by liver fibrosis. In our study the potential of Earthworm Extract Protein (EEP) from *Metaphire posthuman* protein *(MP)*, was investigated against CCl_4-induced liver fibrosis. The rats were exposed to CCl_4 for 12 weeks, and MP was administered twice weekly. The effects of MP were evaluated by examining parameters associated with liver fibrosis, such as body weight, histological changes, biochemical indicators, and antioxidant activity. The study found that MP modulate the expression of AKT, BAX, α-SMA, Nrf-2, IL-6, STAT-3, TNF-α, and IL-4 pathways, inhibited liver function testing, activated antioxidants, and prevented an increase in cholic acid in blood plasma. The findings suggest that MP could be a candidate for natural remedy of chronic liver damage.