collagenase solution and were centrifuged at 100 x g for 3 min at 4 °C. The supernatant was aspirated, then cells were resuspended in 40 ml cold William's complete medium, washed and centrifugation was repeated. The supernatant was aspirated again; cells were resuspended in 40 ml cold William's complete medium then were centrifuged at 200 x g for 10 min at 4 °C. Finally, cells in the suspension were counted using a hemocytometer.

2.2.9.4 Hepatocyte culture

Hepatocytes were maintained in tissue culture flasks or plastic dishes in a humidified atmosphere of 5% CO_2 at 37°C. Hepatocytes were seeded in the required format for experiments to reach 70-80% confluence by the end of the treatment. Hepatocytes were diluted with 30 ml warm William's complete medium to the desired concentration. Upon plating, the plates were left inside the cell culture hood for 30 minutes before being placed into the incubator to form an even monolayer of hepatocytes. Cells were allowed to recover and grow at least overnight prior to the day of experimentation (Shen *et al.* 2012).

2.2.9.5 Treatment of the cultured cells

After 20 h incubation, rat hepatocytes were pre-treated (200, 40 and 4 mM) GlcN, (5 μM) cimetidine and (50 μM) rifampin which were dissolved in the incubation medium to obtain a final concentrations of (2.87, 28.6 and 143.4 mg/ml) GlcN, (0.005 mg/ml) cimetidine, and (0.164 mg/ml) rifampin dissolved in the incubation medium. After 30 min, 0.0236 mg/ml PRN was added. Samples were pooled after 15, 30, 60 and 120 min of incubation and placed in Eppendorf tubes then kept in freezer at -20°C till HPLC analysis.