Purpose
To develop a hepatocarcinoma targeted intracellular delivery carrier for protein drug delivery. Asialoglycoprotein receptors (ASGPR) express on human hepatocellular carcinoma cells bind and internalize galactosamine modified substance. In this study, galactosamine modified bovine serum albumin (BSA-GaSM) was used as a carrier for intracellular delivery of cytochrome c (Cyt c).

Methods
Galactosamines (GaSM) were chemically conjugated on BSAs through direct amidation in the present of coupling reagent, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). Cyt c was conjugated on BSA or BSA-GaSM via chemical linker, N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP). Number of galactose conjugated was evaluated by MALDI-TOF MS. Molecular weight and particle size change of Cyt c conjugates were analyzed using electrophoresis and zetasizer. Intracellular uptake of BSA-GaSM and Cyt c conjugates were tested on three hepatocarcinoma cell lines, HepG2, Hep3B and Mahlavu, using flow cytometry. Cell viability respond to Cyt c conjugates were tested by MTT assay.

Results
There were average of 16 GaSM per BSA conjugated after the reaction. BSA-GaSM showed a dose-dependent increase of cellular uptake in all three hepatocarcinoma cells. For the highest does, uptake of BSA-GaSM was 39-fold to BSA in HepG2, 53-fold in Hep3B, and 74-fold in Mahlavu. When Cyt c conjugated on BSA-GaSM, it showed increased cellular uptake of Cyt C at levels of 29%, 19%, and 30% in HepG2, Hep3B and Mahlavu, respectively, comparing to BSA-Cyt c conjugates. Lastly, BSA-GaSM-Cyt c conjugates statistically significantly decreased cell viability compared to BSA-Cyt c conjugates in HepG2 (15%), Hep3B (18%) and Mahlavu (16%), respectively.

Conclusion
BSA-GaSM shows the potential as a carrier for intracellular delivery of therapeutic protein drug to hepatocarcinoma in vitro. Its ability of hepatocarcinoma targeted delivery in vivo would be further investigated in the future.