

treatment (Fig. 3). Because of the higher levels of cytosolic protein measured, HepG2 cells showed lower total GST-specific activity than did Hepa-1 cells.

### Effects of ICZ on the Cytotoxicity in Hepa-1 Cells

To evaluate whether ICZ can cause cytotoxic effects in Hepa-1 cells, an MTS assay was carried out.

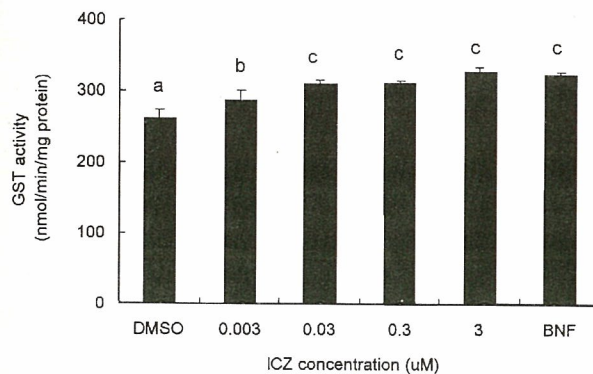


Fig. 2. Effects of ICZ on the induction of total GST activities in Hepa-1 cells. Cells were treated with different concentrations of ICZ as indicated for 24 h. The cytosol was then isolated, and the total GST activity was determined. BNF (10  $\mu$ M) served as a positive control. Columns represent the mean  $\pm$  SD of 3 measurements. Columns with different superscripts significantly differ ( $p < 0.05$ ).

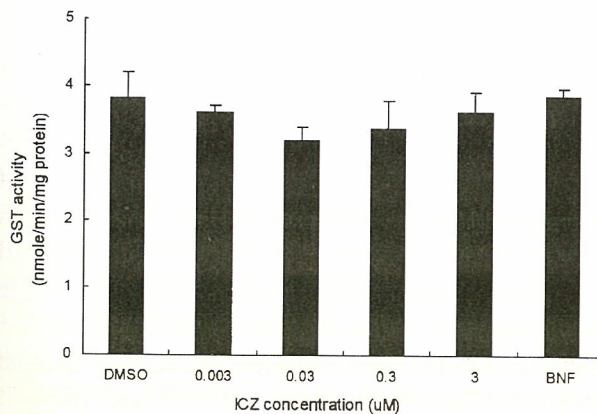


Fig. 3. Effects of ICZ on the induction of total GST activities in HepG2 cells. Cells were treated with different concentrations of ICZ as indicated for 24 h. The cytosol was then isolated, and the total GST activity was determined. BNF (10  $\mu$ M) served as a positive control. Columns represent the mean  $\pm$  SD of 3 measurements ( $p > 0.05$ ).

As shown in Fig. 4, no cytotoxic effect was observed after treatment with various concentrations of ICZ for 24 h ( $p > 0.05$ ).

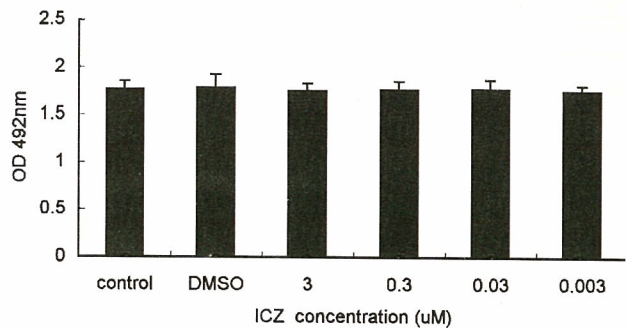


Fig. 4. Effects of ICZ on cytotoxicity in Hepa-1 cells. Cells were treated with various concentrations of ICZ for 24 h, and the cytotoxicity was determined with an MTS assay kit. Columns represent the mean  $\pm$  SD of 8 measurements.

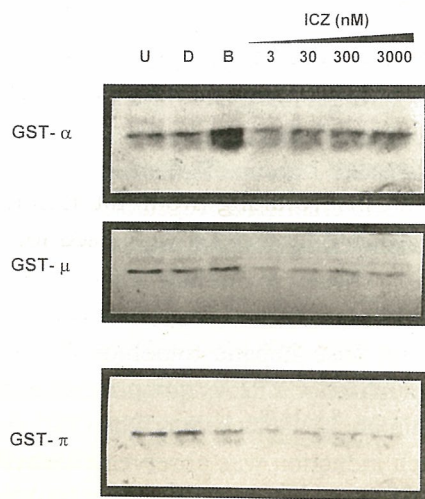


Fig. 5. Western blot analyses of GST isoenzymes in Hepa-1 cells. Cells were treated with various concentrations of ICZ, and the cytosol was isolated after 24 h. Twenty micrograms of cytosolic protein was separated by SDS-PAGE, and was transferred to a nitrocellulose membrane. The isoenzymes, GST- $\alpha$ , GST- $\mu$ , and GST- $\pi$ , were detected separately. U, untreated; D, DMSO treated; B, BNF treated; treated with 3, 30, 300, and 3000 nM ICZ. This experiment was repeated twice and similar results were obtained.