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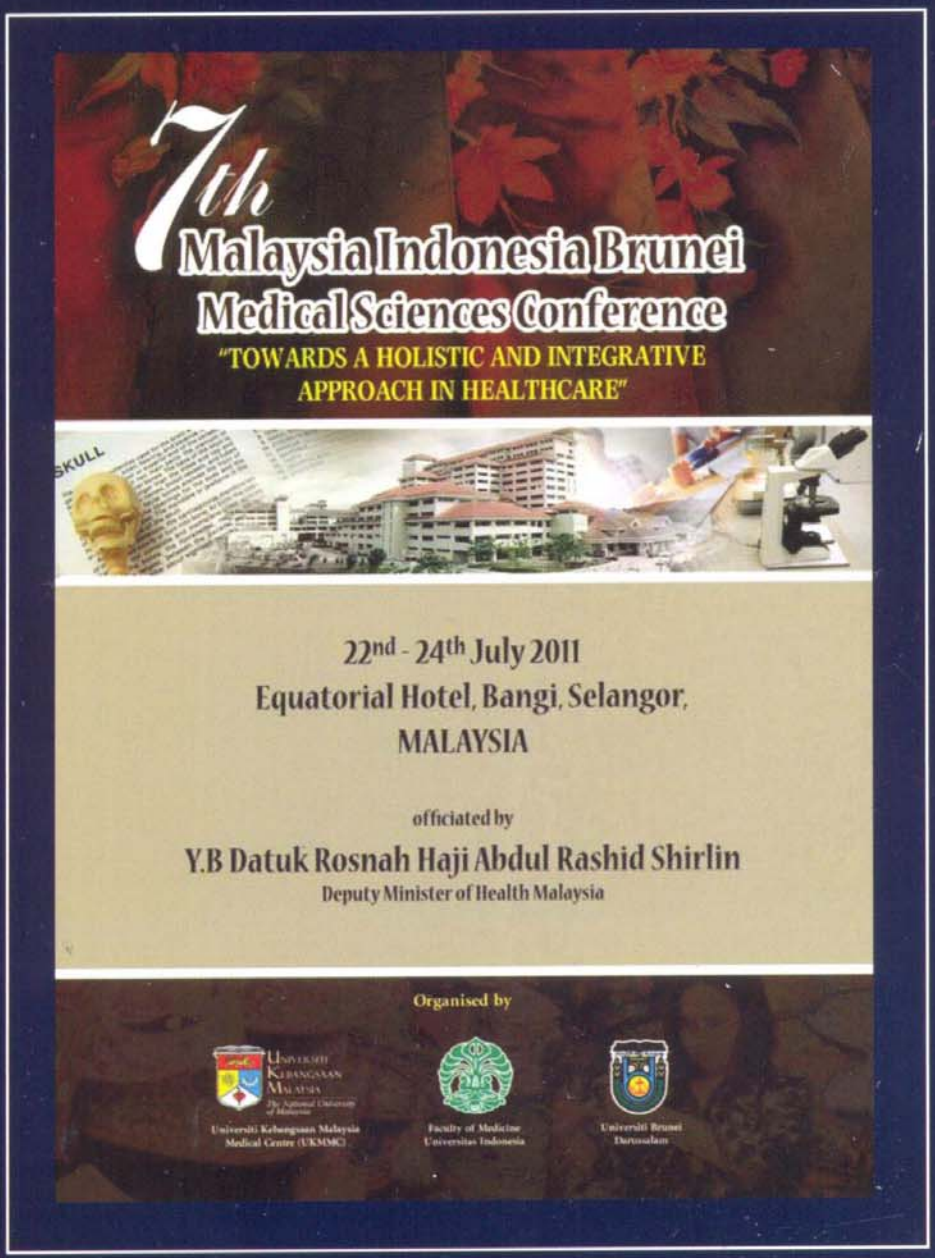

PALM OIL VITAMIN E (γ-TOCOTRIENOL) AND HYDROGEN PEROXIDE TRIGGER APOPTOSIS ON HEPG2 LIVER CANCER CELLS BY ACTIVATING THE SAME SIGNALING TRANSDUCTION CASCADE

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Background:
Tocotrienols has been reported as antitumour agents and widely commercialized as an antioxidant dietary supplement. Our findings showed that, inhibition of proliferation and apoptosis effects induced by γ-tocotrienol (GTT) were comparable to the oxidative injury demonstrated by hydrogen proxide (H_2O_2) and pretreatment with N-acetylcysteine (NAC), a direct antioxidant reduced the antiproliferation and apoptosis effects of GTT. This study suggested that antiproliferation effect, induction of apoptosis and modulation of cell cycle are involved in anticarcinogenesis mechanism of palm oil tocotrienols on HepG2 liver cancer cells.

Materials and Methods:
HepG2 cancer cells were treated with GTT, NAC-GTT and H_2O_2. Apoptosis assays were conducted to determine the IC_{50} for GTT and H_2O_2 after treatments. DNA laddering assays and morphological evaluation were performed to confirm the apoptosis of cancer cells after treatments. Proteins were extracted from treated cells for western blotting technique to determine the changes of protein expression involved in signals transduction.

Results:
Apoptosis rate was increased 7.4-fold and 6.8-fold ($p<0.05$) after treatment with 170μM GTT and 5.5mM H_2O_2 respectively. Morphological evaluation of propidium iodide stained cells showed that the cells undergoing apoptosis with GTT and H_2O_2 treatment exhibited typical apoptotic features such as reduction in cell volume, nucleus fragmentation, chromatin condensation and formation of apoptotic bodies. Despite the potent apoptotic effect of GTT, pretreatment of cells with NAC, a direct antioxidant, reduced the apoptotic effects of GTT. Apoptosis rate was only enhanced 2.5 fold with NAC-pretreatment which was dramatically reduced compared to treatment with GTT alone while DNA fragments were not detected by DNA laddering assays. These findings suggest that oxidant/antioxidant equilibrium may be involved in GTT action. To elucidate the GTT and H_2O_2 mechanism of action, changes in apoptosis and signal transduction proteins were examined. Both treatments showed similar changes of proteins expression. Cell cycle protein expression (CDK2, CDK 4 and CDK 6) and Bcl-2 were decreased. MAP Kinase proteins expression (ERK-1, ERK-2, MEK-2, JKN46 & p38) were also decreased The active form of caspase-3 and caspase-8 were detected after 1 hour treatment and p53 protein expression were increased ($p<0.05$).

Conclusion:
These findings suggest that the antiproliferative effect of GTT is comparable to H_2O_2 and apoptosis is induced by activating the same signaling transduction cascade.

Keywords:
apoptosis, proliferation, GTT, cancer, signaling