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The beneficial effects of melinjo (*Gnetum gnemon* L.) seed extracts on vasolidation and cancer cell proliferation

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ABSTRACT

Melinjo (*Gnetum gnemon L.*) seed extracts (MSE) were found to be rich in polyphenols such as dimeric stilbenoids, including resveratrol dimers: gnetin C, gnemonosides A and D and a few amounts of trans-resveratrol. Resveratrol, it's dimers and derivatives have been reported to have several health promoting properties including anti-tumor, anti-cancer and antihypertensive activities. In this study, the beneficial effects of MSE were investigated *in vivo* and *in vitro*. Since maternal dietary fructose ingestion during both gestation and lactation periods caused harmful effects like hyperinsulinemia and changes in glucose metabolism in rat dams and in their offspring. The effects of maternal fructose consumption during pregnancy followed by maternal MSE consumption during lactation period on the offspring were performed *in vivo*. The key findings are (i) the level of renal pAMPK in FM of 17-week female offspring, but not of the male offspring, was increased after maternal MSE intake during lactation; (ii) maternal fructose intake down-regulated renal eNOS expression in FC group while maternal MSE consumption maintained renal eNOS expression in FM group of female offspring but not of the male offspring; and (iii) maternal MSE intake during lactation lowered the systolic blood pressure in FM of 17-week female offspring, but not of the male offspring. These results demonstrate that MSE consumption during lactation improved vasodilation and

attenuated the development of hypertension in the 17-week female offspring of fructose-fed pregnant rats.

Cancer remains to be one of the leading causes of death worldwide. However, there is still no drug that is found to be completely effective and safe. Thus, the search for new anti-cancer drugs that will be more selective and have lesser side effects is still a major challenge. For this reason, most recent research works on cancer drug discovery focus on plants and plant-derived natural products like polyphenols. In the present study, *in vitro* screening of MSE against a panel of human cancer cells revealed its cytotoxicity against HepG2 liver cancer cells, HeLa cervical cancer cells, OSRC-2 renal cancer cells, and H460 lung cancer cells. Among the four tested human cancer cell lines, HepG2 was found to be the most sensitive to MSE with the IC_{50} value of 171.5 μg/mL. Accordingly, the effect of MSE treatment in HepG2 cells was further investigated.

Recent studies reported various pharmacological activities of MSE including its anti-tumor activity and cytotoxicity against a panel of cancer cells. However, there is no study reporting its effect on human hepatocellular carcinoma cells (HepG2). Moreover, there is still no comprehensive investigation on the exact mechanism on how MSE induce apoptosis on any cancer cell line. As such, in this study, we investigated the mechanism on how MSE induces cell death in HepG2 cells. In MTT assay, we found that MSE dose- and time-dependently induced cytotoxicity in HepG2 cells. These results were confirmed by trypan blue exclusion assay. Annexin V-FITC/PI staining analysis by flow cytometry and DNA ladder analysis indicated that MSE induced apoptosis in a dose-dependent manner. This apoptotic effect of MSE was found to be associated with reactive oxygen species (ROS) generation suggested by the dose-dependent depletion of intracellular free-SH levels. Western blot data

showed the modulation of Bcl-2 family of proteins, cytochrome c release and cleavage of caspase 3, implying the induction of mitochondrial apoptotic pathway. Furthermore, MSE inhibited the activation of the prosurvival NF-κB pathway via induction of dephosphorylation and up-regulation of IkB-α, which ultimately led to the inhibition of the translocation of NF-κB p65 to the nucleus. Since NF-κB regulates the transcription of the gene of anti-apoptotic proteins like BCL-2, inhibition of its translocation to the nucleus will lead to cell death and not its survival. These results provide evidence for the first time that MSE has potent anticancer activity against HepG2 cells, thereby, providing basis for future clinical application of MSE in liver cancer cases.

Natural dietary phytochemicals like polyphenols have been widely used in *in vitro, in vivo*, and preclinical studies against various diseases like cancer and hypertension. From these comprehensive data, we can conclude that MSE has a real potential to be an anti-cancer drug substantiated by its inhibition of NF-κB pathway. In addition, MSE consumption during lactation can be beneficial to the offspring of fructose-treated pregnant rats. Finally, the findings obtained from this research contribute to the increasing biological activities and beneficial effects of MSE.