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INTRODUCTION

Tight junction (TJs) structure is essential for the ''tight'' bonding between adjacent cells of the epithelial/endothelial protective barriers and regulating paracellular transport across cell layers. An important structural correlate of intestinal barrier function is represented by epithelial TJs (Mitic et al., 1998). Oxidative stress characterized by an excess of reactive oxygen species (ROS) also leads to the development of the intestinal pathological conditions such as inflammatory bowel disease. Hydrogen peroxide (H_2O_2) has been reported to decrease trans-epithelial/endothelial electrical resistance values by disrupting paracellular junctional complexes in vitro (Kevil et al., 2002). This effect is paralleled by decrease in tight junction proteins including claudin family and occludin (Fedwick et al., 2005).

Cajanus cajan (L.) Millsp. (pigeon pea) is a famous and multi-use grain legume crop in semi-tropical and tropical developing countries. In north and north-east of Thailand used young pods as indigenous vegetable. Recently, the use of natural protein extracts or purified proteins as antioxidants has been attracted particular interest. This study, therefore, investigate the protective effects of protein isolated from pigeon pea seeds against H_2O_2-induced oxidative stress in human intestinal epithelial cells (Caco-2 cells).

MATERIALS AND METHODS

Cajanus cajan (pigeon pea) seeds were harvested from Khonkaen Field Crop Research Center (KFCRC), Thailand. The powder of pigeon pea was suspended and stirred with 0.1M PBS solution at 4°C for 3 h with a ratio of 1:5 (w/v) and 1% protease inhibitor. The crude protein was precipitate by ammonium sulfate 30-60% saturation. The precipitate was desalted by HiTrap™ Desalting Column (Sephadex™ G-25 Superfine, 1.6×2.5 cm). The proteins isolated from pigeon pea (PPP) was lyophilized and stored at -20°C.

The Caco-2 cell lines were grown for 2-3 days to confluency of monolayers. Cytotoxicity was analyzed by using cell count reagent kit (Nacalai Tesque, Japan). Tight junction proteins expression were analyzed by western blot assay by pre-incubated of culture cells with PPP for 24 and 48 h and followed by an addition of 0.1 mM H_2O_2 in FBS-free DMEM for 30 min. Transepithelial electrical resistance (TER) assay was used to evaluate tight junction function by incubating culture cells with PPP for 24 h prior to H_2O_2 exposure. The powerful antioxidant; glutathione (GSH) was used as a positive control.

RESULTS

Cell Viability

Regarding our study, both H_2O_2 (0.1mM) and PPP (1 mg/mL) had no significant effects on the viability of Caco-2 cells (Figs. 1-2). Following treatments with PPP and H_2O_2, the viability rate of Caco-2 cells was greater than 90%.

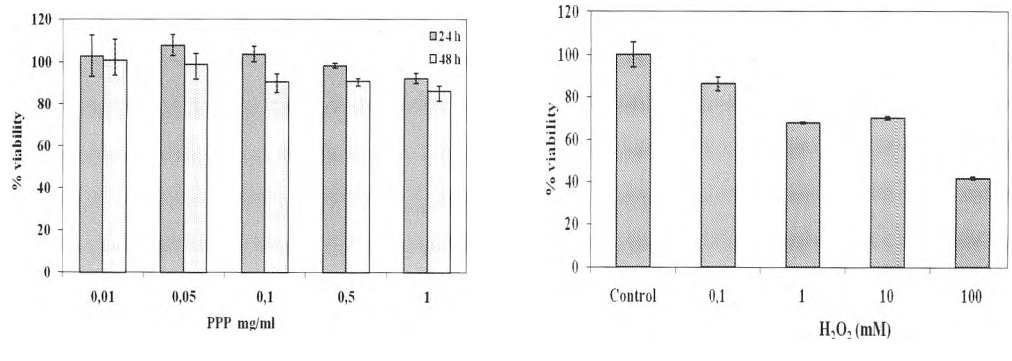

Fig 1-2. Effect of PPP and H_2O_2 on viability of Caco-2 cells after 24 and 48 h

Western blot analysis

TJs proteins; claudin-4 (CL4) and occludin (OCL) were analyzed by immunoblotting and immunostaining. Expression (%) of the TJs proteins revealed an increase in CL4 and OCL levels in pre-treated Caco-2 cultures with 1 mg/mL PPP prior to H_2O_2 exposure (Fig. 3). As demonstrated in Fig 3, incubation of cells with 0.1mM H_2O_2 for 30 min resulted in significant suppression on CL4 and OCL levels by approximately 80 and 60%, respectively in comparison to untreated group (control). Pretreatment of the Caco-2 cells with PPP for 48 h prior to exposure of H_2O_2 was able to preserve CL4 and OCL expression by about 30-40%. The GSH (used as positive control) exhibits the strongest protective effect against H_2O_2 by approximately 60%.

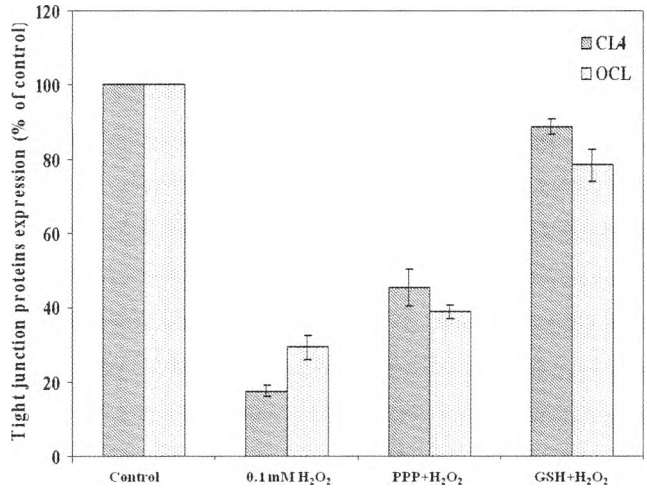

Fig 3. Effects Caco-2 cells monolayer pretreatment with PPP prior exposed to H_2O_2 on claudin-4 (CL4) and occludin (OCL) expression

Transepithelial electrical resistance (TER) analysis

The control group showed an increase in TER values with a time-dependent response (Fig 4). The effect of H_2O_2 on TER was measured in Caco-2 monolayers for over 24 h. It was revealed that H_2O_2 treatment of epithelial monolayer caused a significant decrease in TER at both 2 and 24 h. Pretreatment of the cells with PPP (1mg/mL) for 24 h prior to exposure to 0.1 mM H_2O_2 attenuated the effect of H_2O_2 on TER reduction.

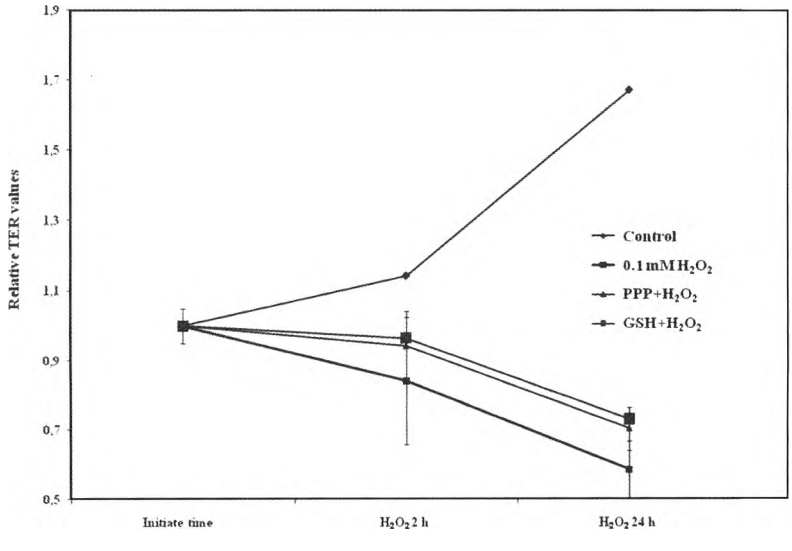

Fig 4. Effects Caco-2 cells monolayer pretreatment with PPP prior exposed to H_2O_2 on the transepithelial electrical resistance (TER) values

DISCUSSION

In our studies, H_2O_2 induced TJ disruption evidenced by suppression of TJ proteins including claudin-4 and occludin. Exposure of Caco-2 cells to H_2O_2 resulted in increased paracellular permeability along with decreased electrical resistance across barriers (Fischer et al., 2005). Down-regulation of claudin-4 and cludin-7, and up-regulation of cludin-2, might lead to altered TJ structure and be related to the impaired epithelial function in active UC. Therefore, overexpression of claudin-4 increases the barrier function and also improves for epithelial function in active UC (Oshima et al., 2008). Our data demonstrated the protective effects of protein fractions isolated from pigeon pea by increasing of claudin-4 expression and also in TER values. Overexpression of claudin-4 in epithelial cells by means of stable cDNA transfection increased TER and decreased Na^+ permeability (Van et al., 2001). The loss of occludin expression upon H_2O_2 exposure was possibly due to an organization of junctional proteins and had been well collaborated with protein kinase activation (Jepson et al., 2003). The mechanism of action of PPP found in our study was similarly with action of quercetin to suppress a significant loss of occludin expression upon challenging with H_2O_2 (Chuenkityanon et al., 2010). It could be said that PPP possessed capability to preserve tightness of the junction by increasing the TJs proteins and TER during H_2O_2 exposure.

CONCLUSION

H_2O_2 radicals were proved to be a good inducer of barrier dysfunction of cells. Pretreatment of the Caco-2 cells with natural protein (PPP) isolated from pigeon pea could preserve the expression of claudin-4 and occludin tight junction proteins followed by H_2O_2 treatment. The protective effect of pigeon pea protein against H_2O_2-induce-oxidative stress was evident by an improvement of transelectrical resistance (TER). These results suggest that pigeon pea protein exhibits tight junction protective effect against H_2O_2 by an improvement of the transelectrical resistance (TER) and that supports maintenance of intracellular tight junction integrity of Caco-2 cells.

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