Journal of Medicinal Plants Research Vol. 4(21), pp. 2283-2289, 4 November, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.519
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Rice bran phytic acid (IP_6) induces growth inhibition, cell cycle arrest and apoptosis on human colorectal adenocarcinoma cells

Shafie Nurul-Husna[1], Mohd Esa Norhaizan[1,2]*, Ithnin Hairuszah[1,2], Md Akim Abdah[1], Saad Norazalina[1] and Ismail Norsharina[1]

[1]Institute of Bioscience, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2]Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

Accepted 9 September, 2010

Phytic acid (inositol hexaphosphate or IP_6) is one of the bioactive compound that is present in cereals, nuts and legumes. IP_6 is a naturally occurring polyphosphorylated carbohydrate, recognized to posses various significant health benefits including anticancer effects. Several *in vitro* and *in vivo* studies provide convincing evidence for the anticarcinogenic properties of commercial rice IP_6 whilst the underlying mechanisms by which IP_6 exerts anti-tumorigenic effects are still not fully known. The purpose of this present study is to investigate the growth inhibitory effects of IP_6 extracted from rice bran on human colorectal cancer cell line (HT-29). IP_6 extracted from rice bran induced marked growth inhibition in HT-29 with an IC_{50} value of 12.0 ± 2 µg/ml, in a dose and time dependent manner. Flow cytometry was performed for the analysis of cell cycle and apoptosis. Rice bran IP_6-extract induced cell cycle arrest in HT-29 cell at G_0/G_1 phase. Staining with Annexin V-based assay and propidium iodide confirmed that apoptosis occurred early and late in the HT-29. IP_6 is expected to exert anticarcinogenic activity through disruption of cell cycle progression and induction of apoptosis. Our study further supports the function of rice bran IP_6 as a chemopreventive agent for human colorectal cancer.

Key words: Phytic acid (IP_6), rice bran, colorectal cancer, cell cycle, apoptosis, chemoprevention.

INTRODUCTION

Colon cancer is the malignant neoplasm of the colonic epithelium. It is the third most common cancer and the third leading cause of cancer related deaths for both men and women in United States (American Cancer Society, 2008) and becoming increasingly common in Asian countries. Epidemiological studies have shown that high fiber foods, such as fruits, vegetables, whole grains and cereals may be protective against colon cancer (Howe et al., 1992; Potter, 1993).

Inositol hexaphosphate (IP_6), also known as phytic acid or phytate, is a natural dietary ingredient, which is described as ‘‘natural cancer fighter,’’ being an essential component of nutritional diets. Phytic acid is a major constituent of all plant seeds, occurring at 0.4 to 6.4% (w/w) of most cereals, legumes, nuts, oil seeds and soybean (Shamsuddin et al., 1997) and naturally accounting for 60-90% of the total phosphorus in discrete regions of the seeds, such as the aleurone layer of wheat and rice (Tanaka et al., 1972) and in the germ of corn (O’dell et al., 1972).

Over the years, several studies pioneered by Shamsuddin et al. (1996), and other research groups have shown the potential chemopreventive and anticancer effects of IP_6 in various cancer models (Singh and Agarwal, 2005; Fox and Eberl, 2002). *In vitro* studies proved that IP_6 has been shown to inhibit growth of human breast, colon, and liver cancer cells, and rhabdomyosarcoma and erythroleukemia cells; and cell transformation in mouse epidermal JB6 cells (Shamsuddin et al., 1996; Shamsuddin and Said, 1998; Vucenik et al., 1998; Shamsuddin et al., 1992; Huang,

*Corresponding author. E-mail: nhaizan@medic.upm.edu.my. Tel: +603 89472427. Fax: +603 89426769.

1997).

With regard to *in vivo* anticancer efficacy of IP_6, it has been shown that 1% (w/v) IP_6 in drinking water 1 week before or 2 weeks after the administration of azoxymethane (AOM) inhibits the development of large intestinal cancer in F344 rats (Shamsuddin et al., 1988). Later, it was reported that in same animal model, treatment with 2% (w/v) IP_6 in drinking water, even after 5 months of carcinogen induction, significantly inhibits both number and size of tumors in large intestines (Shamsuddin and Wah, 1989). Other study by Norazalina et al. (2010), revealed that treatment of 0.2% (w/v) of rice bran IP_6 give the greatest reduction in the formation of aberrant crypt foci (ACF) compared to commercial corn IP_6. Furthermore, administrations of IP_6 in AOM-induced colon carcinogenesis in rat also reduce the incidence and multiplicity of total tumor formation (Norazalina et al., 2010). Various animal studies reported above, have also shown that IP_6 does not cause any adverse side effects or toxicity even at higher doses which are up to 2% (w/v) or 15 mM in drinking water (Shamsuddin and Wah, 1989; Singh et al., 2004; Vucenik et al., 1995).

Because cancer is a major public health issue, the dramatic anticancer effect of IP_6 has resulted in our quest for understanding its mechanism of action. A central pathway of cancer inhibition by IP_6 is via control of cell division; and IP_6 reduces the rate of cellular proliferation both *in vivo* and *in vitro*. Tian and Song (2006), have demonstrated that IP_6 has potent inhibitory effect on proliferation of human colorectal cancer cell line (HT-29) by modulating proliferating cell nuclear antigen (PCNA) and Cip1/p21 expression. Along with this reduction in cell proliferation, IP_6 can regulate the cell cycle to block uncontrolled cell division and force malignant cells either to differentiate or to go into apoptosis (Matejuk and Shamsuddin, 2010).

The laboratory investigation on the antitumor efficacy of IP_6 started in mid 1980s by Shamsuddin et al. (1997), and since then, several studies have shown the anticancer effects of commercial rice and wheat bran IP_6 in various *in vitro* as well as *in vivo* cancer models. To the best of our knowledge, there is no study showing anticarcinogenic effects of rice bran IP_6 on colorectal cancer cells. The study of phytic acid specific from rice bran as anticancer agent is still scarce. Hence, the main purpose of this study was to determine the anticarcinogenic potentials of IP_6 extracted from rice bran on colorectal cancer cells which may critically contribute to its cancer preventive and therapeutic efficacy.

MATERIALS AND METHODS

Chemicals and reagents

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and penicillin–streptomycin were from PAA (Austria). MTT ([4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide, dimethyl sulfoxide (DMSO) and commercial phytic acid were obtained from Sigma (USA). Annexin V-FITC Apoptosis Detection Kit was purchased from BD Biosciences (USA). HT-29 (human colorectal cancer cell line) and BALB/c 3T3 (mouse fibroblast cell line) were bought from American Type Culture Collection (ATCC) (USA). All other chemicals and reagents used were of the highest purity grade available.

Sample preparation

Rice bran (BERNAS, Malaysia) was stabilized according to the method of Ramezanzadeh et al. (1999). Stabilization was performed to prevent oxidative rancidity during storage. After the stabilization process, total lipid was extracted from rice bran samples by using hexane regarding to the modified method of Hu et al. (1996). Phytic acid (IP_6) was extracted from rice bran regarding to the Fruhbeck et al. (1995), with slight modification. The samples were added to hydrochloric acid, HCl (1 g in 20 ml) in pH 1.0. The extraction was carried out at room temperature with constant shaking in an orbital mixer. The obtained creamy mixture was centrifuged at 17300 g for 30 min at 15°C and the supernatants were collected (Norazalina et al., 2010). The modified method of Camire and Clydesdale (1982), was used to neutralize the phytate extract. The neutralized sample was then concentrated by freeze-drying and kept at -20°C.

Growth inhibition assay- 3-(4-5- dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide, MTT)

HT-29 and 3T3 cell lines were grown in DMEM supplemented with 10% FBS and 100 IU/ml Penicillin and 100 µg/ml Streptomycin, and incubated at 37°C under 5% CO_2 in a humidified atmosphere. To evaluate the effect of IP_6 on the proliferation of HT-29 cells, a colorimetric MTT assay was used according to Shamsuddin et al. (1996), and Vucenik et al. (1998). This assay measures the reduction of tetrazolium salt, MTT to a purple-colored formazan product. HT-29 cells were preincubated at density of 1 $x10^5$ cells/well on 96-well microtitre plates for 24 h. The old medium was tapped out and IP_6 (diluted in medium) in the concentration range of 0-20 µg/ml were added into the plate. The plate was incubated for a further 72 h. Then, 20 µl of MTT reagent (5.0 mg/ml) was added into each well and the plate was incubated for four more hours at 37°C. Subsequently, 100 µl of solubilisation solution (DMSO) was added into each well and the absorbance was read at 570 nm using the microplate reader (Tecan, Switzerland). In this study, the effect of commercial rice phytic acid on cell proliferation was also determined as a comparison and the toxicology study by using normal cell (3T3 cell line). Therefore, we selected 50% growth inhibition concentration (IC_{50}) for the analysis of cell cycle and apoptosis.

Cell cycle distribution analysis

HT-29 cells were pre-incubated at a density of $1x10^5$ cells in a culture flask for 24 h. The culture medium was replaced with fresh aliquots containing IP_6 compounds at three different concentrations (9.5, 12 and 14.5 µg/ml). After 24, 48 and 72 h exposure, the cells were trypsinized, washed three times with ice-cold phosphate-buffered saline (PBS) (10 mM sodium phosphate pH 7.2, 150 mM sodium chloride), re-suspended in 70% ethanol and further incubated at -20°C for 2 h. Then, the cells were washed with PBS and re-suspended in 50 µl of RNase solution (10 mg/ml) and stained with 40 µl of propidium iodide (1 mg/ml). The cell cycle was analyzed with flow cytometry (Beckman Coulter, USA).

Detection of apoptotic cell death

This assay was carried out using Annexin V-FITC Apoptosis

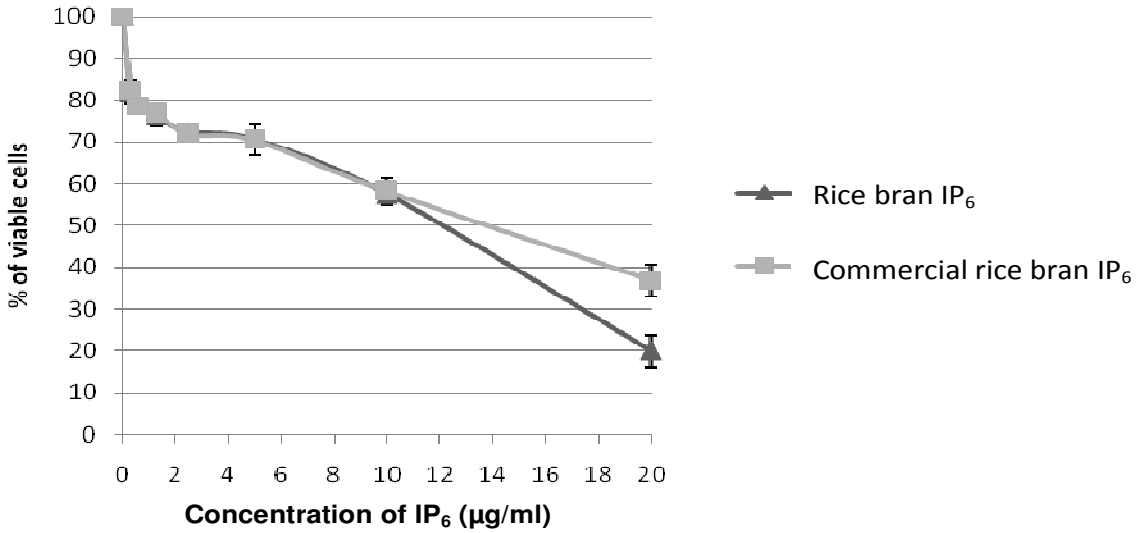

Figure 1. Treatment of rice bran IP_6 extracts and commercial rice IP_6 on HT-29 cells. The cell viability was measured by MTT assay after 72 h exposure. The concentration was expressed as a percentage compared to control cells.

Detection Kit I according to manufacturer's protocols. HT-29 cells at a density of $1x10^5$ cells in culture flask were pre-incubated for 24 h. The culture medium was replaced with fresh aliquots containing IP_6 extract at three different concentrations (9.5, 12 and 14.5 µg/ml) for 24, 48 and 72 h. Then, the cells were trypsinized, washed twice with ice-cold PBS, and re-suspended in 100 µl of 1x binding buffer (0.1 M Hepes/NaOH, pH 7.4 and 1.4 M NaCl, 25 mM $CaCl_2$). The cells were added with 5 µl of Annexin V-FITC and 5 µl of propidium iodide for staining and were gently vortexed and incubated for 15 min at room temperature in the dark. Another 400 µl of 1x binding buffer was added and the fluorescence of the cells was immediately analyzed by flow cytometry (Beckman Coulter, USA).

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and statistically analysed by one-way ANOVA using Turkey's test and applying a significance level of $p < 0.05$.

RESULTS

Growth inhibition effect of IP_6 on human colorectal cancer cells

Different dose of IP_6 ranging from 0-20 µg/ml were applied on HT-29, human colorectal cancer cell line and the effect of their growth was determined by MTT assay. There was a dose-related decrease in cell number upon exposure with IP_6 after 72 h of treatments. From the data, we determined that the IC_{50} value of rice bran IP_6 and commercial rice IP_6 were 12.0 ± 2 and 14.2 ± 5.3 µg/ml, respectively as shown in Figure 1. The results showed that IP_6 extracted from rice bran has higher sensitivity towards human colorectal cancer cell line (HT-29) compared with commercial rice IP_6. Rice bran IP_6 also did not cause any toxicity towards normal cells, 3T3 with <10% of cells were died (Data not shown).

Effect of IP_6 on cell cycle kinetics

Based on the growth inhibitory response of rice bran IP_6 in HT-29 cells, we next examine its effect on cell cycle progression. After 24, 48, and 72 h exposure with IP_6, cell cycle kinetics of HT-29 cells were analyzed. As shown in Figure 2, IP_6 increased the G_0/G_1 phase cells due to the increase in IP_6 dosage (Figure 2a) and IP_6 also increased the G_0/G_1 phase cells due to the increase in exposure times (Figure 2b). Consistence with its effect on cell growth inhibition, IP_6 induced significantly G_0/G_1 arrest in HT-29. IP_6 treatment (9.5, 12 and 14.5 µg/ml IP_6) for 24, 48 and 72 h resulted in accumulation of 63-65% ± 0.6 cells in G_0/G_1 phase compared to control showing 50% ± 3.5 ($p < 0.05$).

Apoptosis induction analysis of IP_6 treated HT-29 cells

The Annexin assay revealed that rice bran IP_6 significantly increased total apoptosis of HT-29 cells. IP_6 also increased the early and late apoptotic HT-29 cells in a dose- and time dependent manner. As shown in Figure 3, the total apoptotic cell death was significantly increased after 24 h of IP_6 treatment (9.5 µg/ml) compared to the control ($p<0.05$). IP_6 significantly increased the number of early (30% ± 1.4) and late apoptotic (41% ± 2.9) HT-29 cells in dose dependent manner (Figure 3a) compared to control only <1% of cell

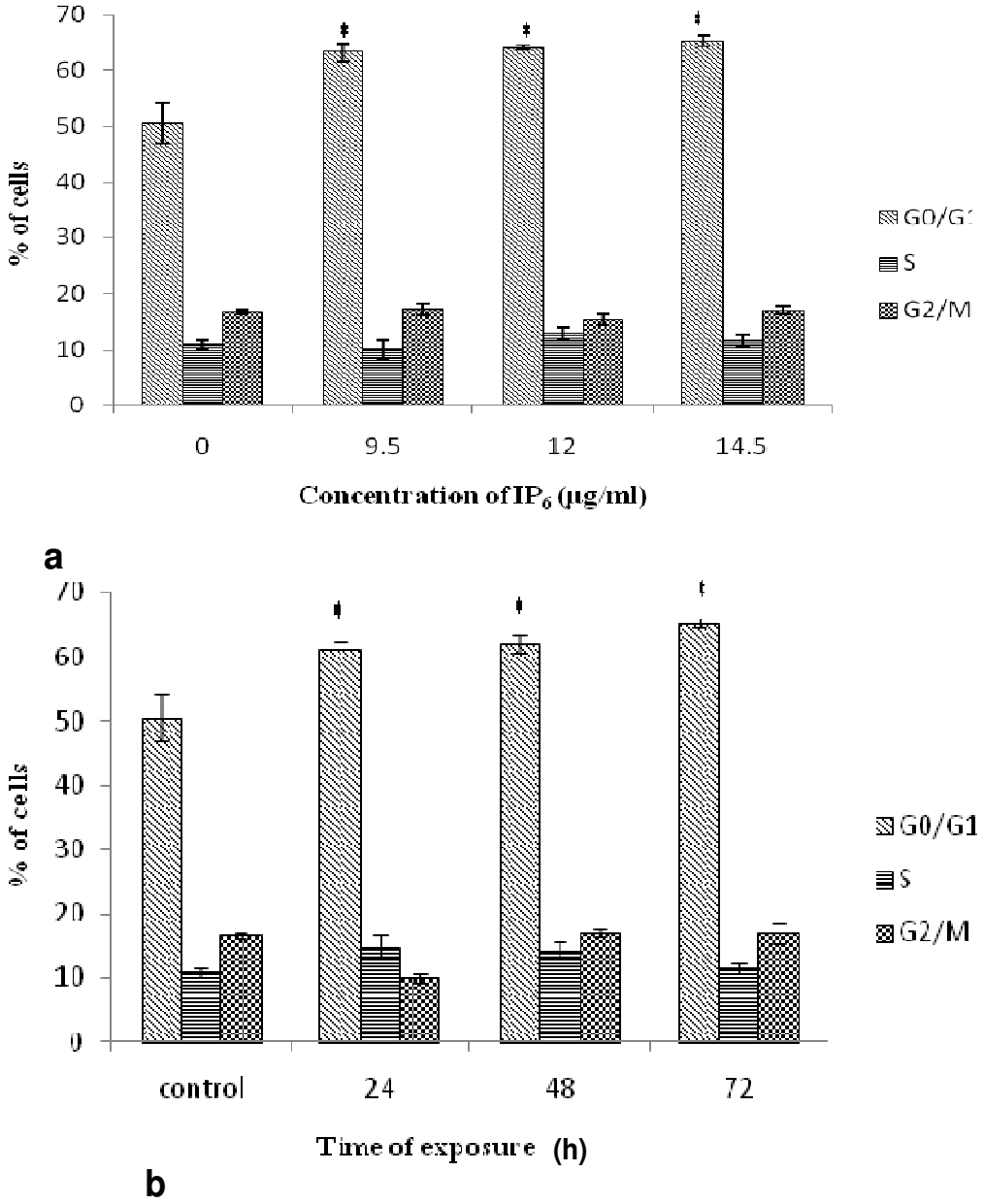

Figure 2. Cell cycle kinetics of rice bran IP_6 treated HT-29 cells in different dosage (a) and different exposure times (b). $1x10^5$ cells were seeded in culture flasks. After 24, 48 and 72 h exposure to IP_6, the cell cycle kinetics was analyzed by flow cytometry. The values are presented as mean ± standard error of mean of three determinations, and, where indicated by *, showed a significant difference ($P < 0.05$) relative to the respective control.

death ($p<0.05$). Furthermore, IP_6 also significantly increased the number of early apoptotic (29% ± 0.8) HT-29 cells in a time dependent manner compared to control <1% of cell death ($p < 0.05$) (Figure 3b).

DISCUSSION

IP_6 has been demonstrated to be instantaneously absorbed by variety of cancer cell lines (Shamsuddin, 1999). The rate and pattern by which IP_6 is metabolized by cancer cells varies depending on the cell type (Shamsuddin, 1999). Cells from different origin have different sensitivity to IP_6 suggesting that IP_6 may affect different cell types through different mechanisms of action (Vucenik and Shamsuddin, 2003).

The major finding of this present study is that rice bran IP_6 strongly induced growth inhibition, disruption of cell

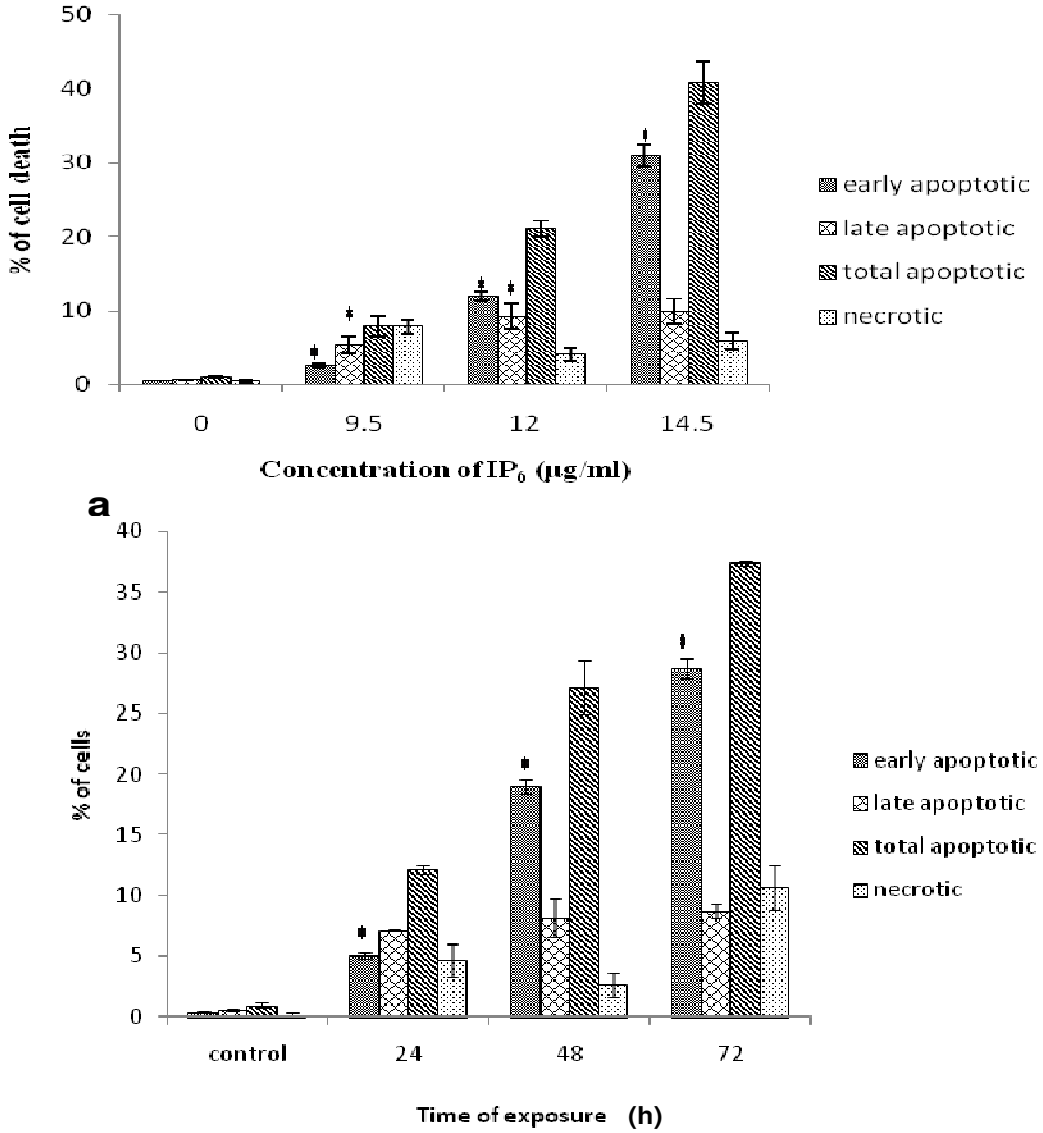

Figure 3. Apoptotic cell death of rice bran IP_6 treated HT-29 cells in different dosage (a) and different exposure times (b). After 24, 48 and 72 h exposure to rice bran IP_6, apoptosis was evaluated by means of Annexin assays. The values are presented as mean ± standard error of mean of three determinations, and, where indicated by *, showed a significant difference ($P < 0.05$) relative to the respective control.

cycle progression and apoptosis on human colorectal cancer cells (HT-29). These molecular effects of IP_6 could be one of the possible underlying mechanisms that resulted in inhibition of cell growth and G_0/G_1 arrest in HT-29 cell cycle progression. Moreover, we can reveal that IP_6 induces apoptotic cell death on human colorectal cancer cells.

As stated earlier, one of the mechanisms for cancer inhibition is through the reduction of cell proliferation rate. According to National Cancer Institute Guidelines, extracts with IC_{50} < 30 µg/ml is considered active as antiproliferative agent (Suffness and Pezzuto, 1990). Previous study by Yang and Shamsuddin (1995) observed that commercial rice IP_6 have been shown to inhibit the growth of HT-29 cells in a dose- and time-dependent manner. Results from our study showed that IP_6 extracted from rice bran was sensitive towards human colorectal cancer cell line (HT-29) and no sensitivity towards normal cell line, 3T3 (IC_{50} cannot be determined). The confirmation of activity with exposure time of 72 h found that IP_6 extracted from rice bran showed higher sensitivity towards colorectal cancer cell line (IC_{50} = 12.0 ± 2 µg/ml) compared to commercial rice IP_6 (IC_{50} = 14.2 ± 5.3 µg/ml). In order to identify the least cytotoxicity towards non-tumorigenic cells, the inhibitory effect of the rice bran IP_6 was evaluated on 3T3 cells as

mentioned above. This cell line is recommended by US National Institute of Environmental Health Sciences (NIEHS), Interagency Coordinating Committee in the Validation of Alternative Methods (ICCAM) to access basal cytotoxicity (NIEHS, 2001). It is important for an anticancer agent to exhibit cytotoxicity but such activities should be specific for cancer cells only. Moreover, IP_6 selectively inhibits cancer cells without affecting the normal and acts synergistically with standard therapeutics (Vucenik et al., 2005; Tantivejkul et al., 2003).

Our study revealed that rice bran IP_6 extract showed a significant growth inhibitory effect at all doses (9.5, 12 and 14.5 µg/ml IP_6) and time points (24, 48 and 72 h) employed in this *in vitro* assay. Based on the parameter of flow cytometry, this present study demonstrated that IP_6 controls the progression of human colon cancer cell lines through the cell cycle arrest in the G_0/G_1 phase. After only 24 h treatment, IP_6 prevented cells from entering the S phase of the cell cycle, resulting in the accumulation of cells in the G_0/G_1 phase. This finding is consistent with earlier reports in which commercial rice IP_6 shown to induce G_0/G_1 arrest in colon cancer cells (El-Sherbiny et al., 2001). G_1 arrest can prevent the replication of damaged DNA and therefore, is helpful in checking the uncontrolled proliferation of cancer cells (Andreeff et al., 2000).

Apoptosis is an active physiological process resulting in cellular self-destruction that involves specific morphological and biochemical changes in the nucleus and cytoplasm (Mans et al., 2000). Agents that suppress the proliferation of malignant cells by inducing apoptosis may represent a useful mechanistic approach to both cancer chemoprevention and chemotherapy. In this study, we demonstrated the influence of IP_6 extracts on apoptosis of human colorectal cancer cells. Our results showed that large amount of apoptosis could be detected after only 24 h of IP_6 treatment by flow cytometry using an Annexin V-based staining assay, indicating that it may be used as a therapeutic agent for human colorectal cancer. Our results included the induction of HT-29 apoptotic cell death by IP_6 extracted from rice bran in a dose- and time-dependent manner. In addition, several previous colon studies have supported its ability to favourably influence colon morphology by increasing both cell apoptosis and differentiation (Jenab and Thompson, 2000). In the colon, enhancement of cell proliferation, expansion of the cell proliferation zone and inhibition of apoptosis are considered risk factors for tumor development (Deschner and Lipkin, 1963; Deschner and Lipkin, 1975; Scalmati and Lipkin, 1993; Kelloff et al., 1994; Thompson, 1995). Among the reported mechanisms by which IP_6 exerts its anti-proliferative effect are through regulation of apoptosis and angiogenesis. Argarwal et al. (2003), demonstrated that IP_6 inhibit NF-kappa B, which is active in advanced and androgen-independent human prostate cancer cells (DU145), showed strong inhibition in cell proliferation and apoptosis. In addition, IP_6 has been shown to significantly increase caspase-3 activity in an experimental mouse prostate model (Sharma et al., 2003).

In summary, phytic acid (IP_6) is a common dietary polyphosphorylated carbohydrate, significantly decreased growth of colorectal cancer *in vitro*. The mechanism by which IP_6 as a strong anti-proliferative modulates cell growth is by disruption of cell cycle progression and altering early and late apoptotic activity. Our findings further supports that IP_6 extracted from rice bran has the potential to become useful for prevention and therapy of cancers. Further *in vivo* and human studies are needed to evaluate safety and clinical utility of this agent in patients with colorectal cancer.

ACKNOWLEDGEMENTS

This research was funded by Ministry of Agriculture of Malaysia. Special thanks to BERNAS, Sekinchan, Malaysia for supplying the rice bran samples.

REFERENCES

Agarwal C, Dhanalakshmi S, Singh RP, Agarwal R (2003). Inositol hexaphosphate inhibits constitutive activation of NF- kappa B in androgen-independent human prostate carcinoma DU145 cells. Anticancer. Res., 23: 38-55.

American Cancer Society (2008). Cancer facts and figures 2007–2008. American Cancer Society.

Andreeff M, Goodrich DW, Pardee AB (2000). Cell Proliferation, Differentiation, and Apoptosis. Cancer Medicine BC Decker Inc. 5th Edition.

Camire AL, Clydesdale FM (1982). Analysis of phytic acid in foods by HPLC. J. Food. Sci., 47: 575-578.

Deschner EE, Lewis CM, Lipkin M (1963). *In vitro* study of human rectal epithelial cells. I. Atypical zone of H3 thymidine incorporation in mucosa of multiple polyposis. J. Clin. Invest., 42: 1922–1928.

Deschner EE, Lipkin M (1975). Proliferative patterns in colonic mucosa in familial polyposis. Cancer. 35: 413–418.

El-Sherbiny YM, Cox MC, Ismail ZA, Shamsuddin AM, Vucenik I (2001). G_0/G_1 arrest and S phase inhibition of human cancer cell lines by inositol hexaphosphate (IP_6). Anticancer. Res., 21: 2393–2403.

Fox CH, Eberl M (2002). Phytic acid (IP_6), novel broad-spectrum anti – neoplastic agent: a systematic review. Complementary and Therapies in Med. 10: 229–234.

Fruhbeck G, Alonso R, Marzo F, Santidrian SA (1995). Modified method for the indirect quantitative analysis of phytate in foodstuff. Analytical. Biochem., 225: 206–212.

Howe GR, Benito E, Castello R (1982). Dietary intake of fiber and decreased risks of cancers of the clon and rectum: Evidence from the combined analysis of 13 case–control studies. J. Natl. Cancer. Instit., 84: 1887–1896.

Hu W, Wells JH, Shin TS, Godber JS (1996). Comparison of isopropanol and hexane for extraction of vitamin E and oryzanols from stabilized rice bran. J. Am. Oil. Chem.' Soc., 12: 1653–1673.

Huang C, Ma WI, Hecht SS, Dong Z (1997). Inositol hexaphosphate inhibits cell transformation and activate protein 1 activation by targeting phosphatidylinositol-3 kinase. Cancer. Res., 57: 2873–2878.

Jenab M, Thompson LU (2000). Phytic acid in wheat bran affects colon morphology, cell differentiation and apoptosis. Carcinogenesis, 21: 1547–1552.

Kelloff GJ, Boone CW, Steele VE, Fay JR, Lubet RA, Crowell JA,

Sigrnan CC (1994). Mechanistic considerations in chemopreventive drug development. J. Cell. Biochem., 20: 1–24.
Mans DRA, Da Rocha BA, Schwartsmann G (2000). Anti-cancer drug discovery and development in Brazil: target plant as a rational strategy to acquire candidate anti-cancer compounds. The Oncologist. 5: 185-198.
Matejuk A, Shamsuddin A (2010). IP_6 in Cancer Therapy: Past, Present and Future. Current Cancer Therapy Rev., 6: 1
National Institute of Environmental Health Sciences (NIEHS) (2001). Report of the International Workshop on *in vitro* Methods for assessing Acute Systemic Toxicity. NIH publication No. 01-4499. NIEHS, Research Triangle Park, NC.
Norazalina S, Norhaizan ME, Hairuszah I, Norashareena MS (2010). Anticarcinogenic efficacy of phytic acid extracted from rice bran on azoxymenthane-induced colon carcinogenesis in rats. Experimental. Toxicol. Pathol., 62: 259-268.
O'Dell BL, deBoland AR, Koirtyohann SR (1972). Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. J. Agric. Food. Chem., 20: 718-721.
Potter JD (1993). Colon cancer do the nutritional epidemiology, the gut physiology and the molecular biology tell the same story? J. Nutr., 123: 418–423.
Ramezanzadeh FM, Rao RM, Windhauser M, Prinyawiwat-kul RT, Marshall WE (1999). Prevention of hydrolytic rancidity in bran during storage. J. Agric. Food. Chem., 47: 3050–3052.
Scalmati A, Lipkin M (1993). Proliferation and differentiation biomarkers in colorectal mucosa and their application to chemoprevention studies. Environ. Health. Perspect., 99: 169–173.
Shamsuddin AM, Elsayed AM, Ullah A (1988). Suppression of large intestinal cancer in F344 rats by inositol hexaphosphate. Carcinogenesis., 9: 577– 580.
Shamsuddin AM, Wah A (1989). Inositol hexaphosphate inhibits large intestinal cancer in F344 rats 5 months after induction by azoxymethane. Carcinogenesis., 10: 625-626.
Shamsuddin AM, Baten A, Lalwani ND (1992). Effects of inositol hexaphosphate on growth and differentiation in K-562 erythroleukemia cell line. Cancer Letter., 64: 195-202.
Shamsuddin AM, Yang GY, Vucenik I (1996). Novel anti-cancer functions of IP_6: growth inhibition and differentiation of human mammary cancer cell lines in vitro. Anticancer. Res., 16: 3287-3292. carcinoma cell line. Anticancer. Res., 18: 1479-1484. Shamsuddin AM (1999). Metabolism and cellular function of IP_6: a review. Anticancer Res., 19: 3733-3736.
Sharma G, Singh RP, Agarwal R (2003). Growth inhibitory and apoptotic effects of inositol hexaphosphate in transgenic adenocarcinoma of mouse prostate (TRAMP-C1) cells. Int. J. Oncol., 23: 1413.
Shamsuddin AM, Vucenik I, Cole KE (1997). IP_6: a novel anti-cancer agent. Life. Sci., 61: 343-354.
Shamsuddin AM, Said IT (1998). Up-regulation of the tumor suppressor gene p53 and WAF1 gene expression by IP_6 in HT-29 human colon.
Singh RP, Sharma G, Mallikarjuna GU, Dhanalakshmi S, Agarwal C, Agarwal R (2004). *In vivo* suppression of hormone-refractory prostate cancer growth by inositol hexaphosphate: induction of insulin-like growth factor binding protein-3 and inhibition of vascular endothelial growth factor. Clin. Cancer Res., 10: 244-250.
Singh RP, Agarwal R (2005). Prostate cancer and inositol hexaphosphate: efficacy and mechanisms. Anticancer Res., 25: 2891-2903.
Suffnes M, Pezzuto JM (1990). Assays Related to Cancer Drug Discovery. In: Methods in Plant Biochemistry: Assays for Bioactivity, Hostettmann, K. (Ed.). Vol. 6. Academic Press, London, pp. 71-133.
Tanaka K, Yoshida T, Kasai Z (1972). Subcellular particles isolated from phytic acid in rice and wheat grains. Plant Cell Physiol., 15: 147-151.
Tantivejkul K, Vucenik I, Eiseman J, Shamsuddin AM (2003). Inositol hexaphosphate (IP_6) enhances the anti-proliferative effects of adriamycin and tamoxifen in breast cancer. Breast Cancer Res. Treat., 79: 301-312.
Thompson CB (1995). Apoptosis in the pathogenesis and treatment of disease Sci., 267: 1456–1462.
Tian Y, Song Y (2006). Effects of inositol hexaphosphate on proliferation of HT-29 human colon carcinoma cell line. World J. Gastroenterol., 12: 4137-4142.
Vucenik I, Yang GY, Shamsuddin AM (1995). Inositol hexaphosphate and inositol inhibit DMBA-induced rat mammary cancer. Carcinogenesis, 16: 1055-1058.
Vucenik I, Kalebic T, Tantivejkul K, Shamsuddin AM (1998). Novel anticancer function of inositol hexaphosphate: inhibition of human rhabdomyosarcoma *in vitro* and *in vivo*. Anticancer Res., 18: 1377-1384.
Vucenik I, Shamsuddin AM (2003). Cancer inhibition by inositol hexaphosphate (IP_6) and inositol: from laboratory to Clin. J. Nutr., 133: 3778-3784.
Vucenik I, Ramakrishna G, Tantivejkul K, Anderson LM, Ramljak D (2005). Inositol hexaphosphate (IP_6) blocks proliferation of human breast cancer cells through a PKC delta-dependent increase in p27Kip1 and decrease in retinoblastoma protein (pRb) phosphorylation. Breast Cancer Res. Treat., 91: 35-45.
Yang GY, Shamsuddin AM (1995). IP_6-induced growth inhibition and differentiation of HT-29 human coloncancer cells: involvement of intracellular inositol phosphates. Anticancer Res., 15: 2479–2487.