

# Antigenotoxic Activity of Thai Sangyod Red Rice Extracts against a Chemotherapeutic Agent, Doxorubicin, in Human Lymphocytes by Sister Chromatid Exchange (SCE) Assay *In Vitro*

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**Background:** Nowadays, anticarcinogenic potential of pigmented brown rice and rice bran varieties have been increasingly stated. However, their mechanisms of action are still inconclusive. One of which might be their antigenotoxic activity that no study in human cells was reported before.

**Objective:** To evaluate the antigenotoxic activities of Thai Sangyod red rice extracts against a chemotherapeutic agent, doxorubicin, by sister chromatid exchange (SCE) assay in human lymphocytes *in vitro*.

**Material and Method:** Two fractions of water-soluble of Sangyod rice extracts were used: (i) the washed water extract of brown rice (WWBR) and (ii) the water extract of rice bran (WERB). Human lymphocytes were pretreated with each extracts at concentrations of 6.2, 12.5, 25, 50 and 100 µg/ml for 2 h followed by a genotoxic agent, doxorubicin (DXR) (0.1 µg/ml) for 2 h. SCE level, mitotic index (MI) and proliferation index (PI) were evaluated. Statistical analysis by Dunnett's *t*-test was performed.

**Results:** The results indicated that the pretreatment of WERB fraction only at concentration of 6.2 µg/ml could significantly decrease SCE level as compared to that of the DXR treated alone ( $p < 0.05$ ). On the other hand, WERB fraction at other concentrations and all WWBR pretreatments could not. In addition, there was no significant difference in MI and PI levels between all pretreated extracts as compared to the DXR treated alone.

**Conclusion:** Our data revealed that WERB pretreatment only at specific low concentration of 6.2 µg/ml possessed the antigenotoxic potential against genotoxic damage but not anticytotoxic induced by DXR. Further work is still needed to clarify more the antigenotoxic and anticytotoxic potentials from other fractions of Sangyod rice extracts.

**Keywords:** *Oryza sativa*, Sangyod red rice, Doxorubicin, Sister chromatid exchange (SCE), Antigenotoxicity

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Rice (*Oryza sativa* L.) is an important staple food around the world. There are at least 100,000 rice cultivars that were classified by their grain shapes and texture. The two most common sub-species (ssp.) of

rice are varieties of the *indica* group and the *japonica* group. The non sticky, long grain belongs to ssp. *indica* that is widely grown in the hot climates of the tropical, South and Southeast Asia such as India and Thailand. The ssp. *japonica* is generally grown in the dry fields of restricted temperate climates of the East Asia such as Japan and southern China. Rice is known in a variety of colors including white, black, purple, and red. Colored rice has been used as food and medicine for sixty years<sup>(1)</sup>. There are high in minerals and bioactive

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substances such as anthocyanin, flavonoid, phenolic compounds (e.g. protocatechuic acid, p-coumaric acid, caffeic acid, ferulic acid, sinapic acid, vanillic acid, methoxycinnamic acid, and inositol hexaphosphate)<sup>(2)</sup>. These bioactive compounds have been reported to have high potency as anticarcinogens or chemopreventive agents. For example, Tricin is a flavone found in rice bran demonstrated growth inhibition of breast tumor cells<sup>(3,4)</sup>. Anthocyanins e.g. peonidin 3-glucoside and cyanidin 3-glucoside exert an inhibitory effect of cell invasion on various cancer cell lines<sup>(5)</sup>. Anthocyanin-rich extract from black rice (AEBR) could suppress tumor growth and angiogenesis in nude mice<sup>(6)</sup>. A novel pentapeptide isolated from rice bran could inhibit growth of colon, breast, lung and liver cancer cells<sup>(7)</sup>.

In Thailand, the Sangyod red rice (*Oryza sativa*, L., var. *indica*) is one of special red rice, originally planted in Pattalung, a province in the South for hundred years. It has been proposed as the protected rice variety under the law and registered as goods associated with geographical indications called Sangyod rice of Muang Pattalung since 2006<sup>(8)</sup>. Its cooked rice is soft and scented. High content of minerals, vitamin B complex and bioactive compounds have been reported<sup>(9)</sup>. In addition, the antioxidant activity of the water extract of Sangyod red rice were analyzed and found three times higher than that of Dawk Mali 105, the jasmine white rice<sup>(10)</sup>. The high activity was consistent with high content of phenolic compounds and flavonoids. The natural antioxidant activity is well known for protection against oxidative stress related chronic diseases such as diabetes mellitus, cardiovascular disease, and cancer<sup>(11)</sup>.

However, the mechanisms of action of anticancer by colored rice are still inconclusive. One of which might be their antigenotoxic activities. To further evaluate the antigenotoxic activities of Sangyod red rice, we performed a sister chromatid exchange (SCE) assay in human lymphocytes *in vitro*. Two types of Thai Sangyod rice extracts were analyzed, the washed water of brown rice (WWBR) and the water extract of rice bran (WERB).

## Material and Method

### Chemicals

5'-Bromo-2'-Deoxyuridine, Bizbenzimidazole 33258 were purchased from Sigma-Aldrich (St Louis, MO, USA). RPMI 1640 medium with HEPES and L-glutamine and fetal bovine serum were from Hyclone, Utah, USA. Giemsa stain was from Biotech reagents, Thailand, Phytohemagglutinin (PHA) L, Penicillin-

Streptomycin and Colcemid were from Seromed, Germany. Doxorubicin (DXR) was from Pfizer (Pharmacia & Upjohn Company, NY, USA).

### Sangyod rice extracts

Sangyod brown rice and rice bran were obtained from a local polishing mill in Pattalung, Thailand. Extraction of the WWBR and WERB were performed at the Division of Applied Thai Traditional medicine, Faculty of Medicine, Thammasat University, Thailand. Briefly, the WWBR was prepared by soaking brown rice with distilled water (rice:water = 1:2, wt/wt) and repeated 3 times. The washed water extract were then pooled, filtered, lyophilized and kept in a freezer (-18°C). The percentage yield of this extract was 0.07%. The WERB was prepared by boiling rice bran with distilled water (rice:water = 1:2) at 70°C for 30 min and filtered. The water extract was lyophilized and kept in a freezer (-18°C). The percentage yield of this extract was 38.2%.

### Determination of total phenolic compounds, vitamin B1 and $\gamma$ -oryzanol

Analysis of total phenolic compounds was determined by Folin-Ciocalteu methods<sup>(12)</sup>. Briefly, twenty five milligrams of crude extract was dissolved in 25 ml methanol. 200  $\mu$ l of these samples were mixed with 200  $\mu$ l of methanol, 2 ml of Folin-Ciocalteu reagent and 1.6 ml of sodium carbonate solution (7.5 g/100 ml), and the mixture were measured at 765 nm after 30 min. Gallic acid was used as a standard and the total phenolic compounds were expressed as GAE (gallic acid equivalents) in milligrams per gram dry weight of extracts. Vitamin B1 and  $\gamma$ -Oryzanol was determined by quantitative HPLC<sup>(13,14)</sup>.

### Sister chromatid exchange assay

#### Cell culture

Fresh blood samples were obtained by venipuncture from 4 healthy volunteers, age 25-35 years with no recent exposure to radiation or drugs. These studies were approved by our institutional ethical committee (MTU-E-1-49/51). Lymphocyte-enriched buffy coat was cultured in 5 ml culture medium using standard blood culture conditions as previously described<sup>(15)</sup>. At 24 h after initiation of the culture, the lymphocytes were treated with the extracts at various concentrations in plain RPMI 1640 culture medium for 2 h at 37°C, then the supernatant medium were discarded. The treated lymphocytes were continued to treat with doxorubicin (0.1  $\mu$ g/ml) for 2 h at 37°C. After

treatment, all cultures were centrifuged and the treated lymphocytes were continued to culture at 37°C in the dark with the previously saved medium and bromodeoxyuridine solution was added (final concentration at 5 µM).

#### Cell culture harvest, staining and scoring

The cell culture was harvested at 77 hr after initiation. Fluorescent plus Giemsa technique was used for staining procedure<sup>(15)</sup>. Three independent experiments were performed. From each experiment, twenty-five cells per dose per experiment showing the second metaphase-staining pattern (differential stained chromosomes) were scored from coded slides for the frequencies of SCEs to evaluate the antigenotoxic activity. Mitotic indice (MI) determined as the total number of mitotic cells/1,000 cells) and proliferation indice (PI) determined as (MI + 2MII + 3MIII)/100 cells were evaluated.

#### Statistical analysis

Raw data obtained from the SCE assays were transformed to stabilize the variance by the procedures of Whorton et al 1984<sup>(16)</sup>. Dunnett's t-test was performed to analyze the difference between the mean of the treated groups and of the control groups using the transformed data.

$$\text{Transformed SCE (SCE}_t\text{)} = \text{square root SCE}$$

### Results

#### Analysis of the WWBR and WERB extracts

The percentage yield, total phenolic, vitamin B1 and γ-oryzanol content of each extracts were shown in Table 1. WWBR showed higher antioxidant compound such as total phenolic content and γ-oryzanol than WERB. Thus, the method of water extraction with high temperature could reduce total phenolic content, γ-oryzanol and water soluble vitamin (vitamin B1). However, percentage of yield of WWBR showed less than that of the WERB.

#### Antigenotoxic and anticytotoxic studies of the WWBR

All pretreatment of WWBR at concentrations of 6.2, 12.5, 25, 50 and 100 µg/ml for 2 h followed by DXR (0.1 µg/ml) could not significantly reduced the SCE level induced by DXR treatment alone. The SCE values of DXR treatment and all WWBR pretreatments were significantly different from that of the negative control (plain RPMI) ( $p < 0.05$ ) as shown in Fig. 2. All MI and PI values from all WWBR pretreatments against DXR did not show significant different from DXR treated alone but significant different from the negative control (plain RPMI) ( $p < 0.05$ ) as shown in Table 2.

#### Antigenotoxic and anticytotoxic studies of the WERB

Only WERB pretreatment at concentration of 6.2 µg/ml could significantly reduced the SCE level induced by DXR treatment alone ( $p < 0.05$ ) as shown in Fig. 3. Pretreatment of WERB at other concentrations of 12.5, 25, 50 and 100 µg/ml could not. All MI and PI values from all WERB pretreatments against DXR did not show significant different from DXR treated alone

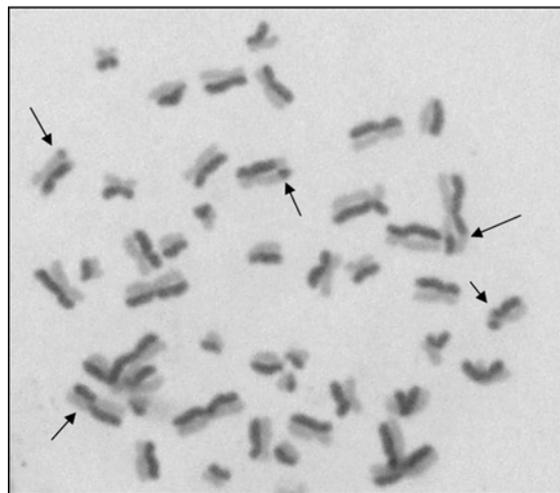
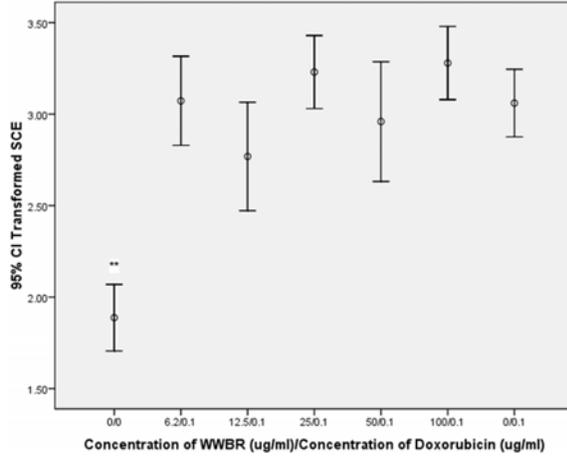


Fig. 1 An example of a metaphase cell with 5 SCEs

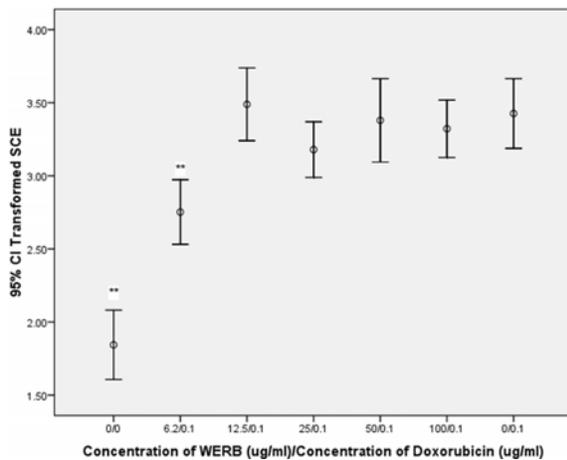
Table 1. The percent yield, total phenolic, vitamin B1 and γ-oryzanol contents of Sangyod rice extracts

Sangyod Rice Extracts	Percent yield	Total phenolic content (mg Gallic acid equivalent/g of the extract)	Vitamin B1 (mg)/100 g of the extracts	γ-oryzanol (mg)/100 g of the extracts
WWBR	0.07	6.75 ± 0.02	91 ± 0.06	164 ± 0.03
WERB	38.2	3.07 ± 0.04	68 ± 0.04	118 ± 0.01



\*\*p < 0.05 (significant different from the positive control, doxorubicin 0.1 µg/ml alone)

**Fig. 2** Antigenotoxic activities of the washed water brown rice (WWBR) pretreatments followed by Doxorubicin (n = 3)



\*\*p < 0.05 (significant different from the positive control, doxorubicin 0.1 µg/ml alone)

**Fig. 3** Antigenotoxic activities of the water extract of rice bran (WERB) against doxorubicin (n = 3)

but significant different from the negative control (plain RPMI) (p < 0.05) as shown in Table 3.

### Discussion and Conclusion

Brown rice and rice bran of colored rice are in a popular concern of their high potency of anticancer<sup>(5,6,17)</sup>. High antioxidant compounds such as anthocyanin, phenolic compounds and phytic acid were found in hydrophilic fraction. Purple rice bran had greater hydrophilic antioxidant activity than that of its

**Table 2.** Mitotic index (MI) and proliferation index (PI) of WWBR pretreatments followed by doxorubicin in human lymphocytes *in vitro* (n = 3)

Concentration of WWBR (µg/ml)	Doxorubicin (µg/ml)	MI ± SEM	PI ± SEM
0	0	26.9 ± 3.6 <sup>#</sup>	5.9 ± 0.5 <sup>#</sup>
6.25	0.1	18.9 ± 1.4	2.6 ± 0.3
25	0.1	14.2 ± 1.5	2.1 ± 0.2
100	0.1	19.7 ± 1.7	3.4 ± 0.4
0	0.1	18.1 ± 2.3	3.2 ± 0.6

<sup>#</sup>p < 0.05

**Table 3.** Mitotic index (MI) and proliferation index (PI) of WERB pretreatments followed by doxorubicin in human lymphocytes *in vitro* (n = 3)

concentration of WERB (µg/ml)	Doxorubicin (µg/ml)	MI ± SEM	PI ± SEM
0	0	26.8 ± 3.6 <sup>#</sup>	5.9 ± 0.5 <sup>#</sup>
6.25	0.1	19.5 ± 0.5	3.7 ± 0.1
12.5	0.1	13.5 ± 0.5	2.8 ± 0.2
25	0.1	15.8 ± 2.2	2.7 ± 0.5
50	0.1	18.9 ± 0.6	3.6 ± 0.4
100	0.1	19.9 ± 2.5	3.1 ± 0.4
0	0.1	18.1 ± 2.3	3.2 ± 0.6

lipophilic antioxidants<sup>(18)</sup>. In the present study, antigenotoxicity of water extracts from Sangyod red rice against DXR were determined to clarify the mechanism of action of its possibility of anticancer. Interestingly, only pretreatment of water extract of rice bran (WERB) at a specific dose of 6.2 µg/ml demonstrated the antigenotoxic activity against DXR (0.1 µg/ml). No antigenotoxic potential against DXR of the pretreatments of the water extracts of brown rice (WWBR) was found. Choi et al (2010) also reported that anthocyanin-rich bilberry extract could just alleviate the doxorubicin induced toxicities in rats and mice but were insufficient to completely counteract the DXR's toxicities<sup>(19)</sup>. Moreover, the mechanism of genotoxicity of doxorubicin is not only by producing free radical damage but also capable of intercalation of DNA in order to damage the cells<sup>(20,21)</sup>. Therefore, it might be possible that only the antioxidant potency of the compounds were not strong enough to have antigenotoxic potential against DXR. Other main bioactive compounds might be more important to

possess these activities. The authors result also demonstrated that the WWBR fraction though contained higher amount of the antioxidant such as total phenolic, vitamin B1 and  $\gamma$ -oryzanol but still could not prevent genotoxic damage induced by DXR. In contrast, the WERB fraction with fewer antioxidant components could induce antigenotoxic potential against DXR. The other main bioactive compound (s) in the WERB fraction for its antigenotoxic activity are needed to verify further.

In conclusion, the preliminary finding that the water extract from Sangyod rice bran possesses the antigenotoxic activity in human lymphocytes could lead to understand more the mechanism of anticancer or chemoprevention against cancer by rice extracts. Further study of various rice cultivar extracts are needed to verify the best product from rice to be used as anticancer agent or chemopreventive agent.

#### Potential conflicts of interest

None.

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## การศึกษาฤทธิ์ต้านพิษในระดับจีนของสารสกัดจากข้าวไทยสีแดงพันธุ์สังข์หยดที่มีต่อสารเคมีบำบัดดอกไซโรบิซินในเม็ดเลือดขาวของมนุษย์ในหลอดทดลองด้วยวิธีซิสเตอร์โครมาติดเอกซ์เชนจ์แอสเสย์

ตรีทิพย์ รัตนวรชัย, สุমন ธิติโอฬาร, เสริมเกียรติ ทานุชิต, เฉลิม จันทรสม, ศกุนตลา อุตมา, อรุณพร อธิรัตน์

**ภูมิหลัง:** ปัจจุบันการกล่าวอ้างถึงฤทธิ์ต้านมะเร็งของข้าวมีสีในรูปข้าวกล้องและรำข้าวมีเพิ่มขึ้น อย่างไรก็ตามกลไกการออกฤทธิ์ดังกล่าวยังไม่มีการสรุปเป็นที่แน่ชัด กลไกหนึ่งที่น่าเกี่ยวข้องคือกลไกการต้านพิษในระดับจีนในข้าว ซึ่งยังไม่มีรายงานการศึกษาในเซลล์มนุษย์มาก่อน

**วัตถุประสงค์:** เพื่อประเมินฤทธิ์ของสารสกัดจากข้าวสังข์หยดสีแดงของไทยในการต้านการเกิดพิษในระดับจีนที่เกิดจากเหนียวน้ำของ สารเคมีบำบัดดอกไซโรบิซินด้วยวิธี sister chromatid exchange (SCE) assay ในเม็ดเลือดขาวของมนุษย์ในหลอดทดลอง

**วิธีการศึกษา:** ทดสอบสารสกัดน้ำจากข้าวสังข์หยด 2 ชนิด ชนิดหนึ่งเป็นน้ำข้าวข้าวกล้อง (WWBR) อีกชนิดหนึ่งเป็นส่วนสกัดน้ำจากรำข้าว (WERB) เป็นการทดสอบต่อเซลล์เม็ดเลือดขาวของมนุษย์ โดยเฉพาะเลี้ยงเซลล์กับสารสกัดแต่ละชนิดที่มีความเข้มข้น 6.2, 12.5, 25, 50 และ 100 ไมโครกรัม/มิลลิลิตร เป็นเวลา 2 ชั่วโมงก่อนการกระตุ้นให้เกิดความผิดปกติในระดับจีนด้วยสารดอกไซโรบิซินที่เป็นพิษในระดับจีน (0.1 ไมโครกรัม/มิลลิลิตร) โดยเฉพาะเลี้ยงต่อเป็นเวลา 2 ชั่วโมง วิเคราะห์ความผิดปกติในระดับโครโมโซมในรูป SCE level, mitotic index (MI) และ proliferation index (PI) แล้ววิเคราะห์ผลทางสถิติด้วยวิธี Dunnett's t-test

**ผลการศึกษา:** ผลการศึกษาพบว่าสารสกัด WERB ที่ความเข้มข้น 6.2 ไมโครกรัม/มิลลิลิตร สามารถลดระดับ SCE ที่เกิดจากการกระตุ้นด้วยดอกไซโรบิซิน อย่างมีนัยสำคัญที่  $p < 0.05$  โดยไม่พบฤทธิ์ที่ความเข้มข้นอื่นของสารสกัด WERB หรือสารสกัด WWBR นอกจากนั้น ยังไม่พบความแตกต่างอย่างมีนัยสำคัญของค่า MI และ PI ระหว่างกลุ่มที่ให้สารสกัดก่อนการให้สารดอกไซโรบิซินและกลุ่มควบคุมผลบวกที่ให้สารดอกไซโรบิซินเพียงอย่างเดียว

**สรุป:** ผลการศึกษาสรุปว่าการให้สารสกัด WERB ก่อน ที่ความเข้มข้น 6.2 ไมโครกรัม/มิลลิลิตร แสดงคุณสมบัติปกป้องพิษระดับจีนแต่ไม่สามารถปกป้องพิษระดับเซลล์ของสารดอกไซโรบิซินได้ งานวิจัยเพื่อศึกษาให้กระจ่างในฤทธิ์ต้านพิษระดับจีนและระดับเซลล์ของสารสกัดข้าวสังข์หยดด้วยส่วนสกัดอื่นๆ จึงยังเป็นที่ต้องการต่อไป