RESEARCH COMMUNICATION

Effects of *Rhinacanthus nasutus* Kurz on Colon Carcinogenesis in Mice

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Abstract

***Rhinacanthus nasutus* Kurz., a Thai medicinal plant which possess antiproliferative and pro-apoptotic effects on human cancer cells, was examined for chemopreventive potential against colonic neoplasms induced by azoxymethane (AOM) combined with dextran sodium sulfate (DSS) in mice. Male ICR mice were given a single intraperitoneal administration of AOM (10 mg/kg body weight) followed by 2% DSS in their drinking water for a week. Water extract of the roots of *R. nasutus* (RNR) was given to the animals intragastrically daily in the initiation and promotion phases. The one hundred mice were divided into 8 groups, one group treated with AOM plus DSS serving as a control. Four other groups received AOM/DSS and RNR at 100 or 500 mg/kg body weight for 5 weeks (initiation phase study) and for 14 weeks (promotion phase study). Another two groups were given RNR alone at 100 and 500 mg/kg body weight and the last group was maintained untreated. At the end of the study, we found that the incidence and multiplicity of colonic tumors in mice fed with RNR both at 100 and 500 mg/kg body weight in initiation phase were higher than those in the control group. Moreover, RNR feeding during the promotion phase also gave similar results. Our results suggest that water extract of the roots of *R. nasutus* Kurz. has no preventive potential against colon carcinogenesis induced by AOM/DSS in mice, rather increasing the incidence of colonic tumors when given during initiation and promotion phases. Further study on RNR should provide more information on mechanisms of its tumor promotion activity.**

Key Words: *Rhinacanthus nasutus* - colon cancer - AOM/DSS mouse model - promotion

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Introduction

Since 2000, cancer has been the leading cause of death in Thailand and the incidence of colorectal cancer has been increasing in tandem with the Westernized dietary habits of Thai people. According to the cancer registry in Thailand, colorectal cancer is the third ranking of cancer incidence in male and is the fifth ranking in female (Khuhaprema et al., 2007). Although there is no magic bullet that can completely and selectively destroy cancer, a paradigm shift is obviously needed for the management of cancer. One of the most pragmatic and realistic ways of reducing the risk of cancer is chemoprevention (Szarka et al., 1994), a promising strategy for cancer prevention by the use of either synthetic or naturally occurring chemicals. At present, there are a large number of chemicals that have been found to exhibit antimutagenic and anticarcinogenic effects in numerous animal and cell culture studies. A vast variety of dietary and medicinal phytochemicals have possessed substantial chemopreventive properties (Dragsted et al., 1993; Surh, 2003).

Rhinacanthus nasutus Kurz. (family Acanthaceae) has been used in Thai Folk remedies for the treatment of various diseases including cancer (Wu et al., 1988; Kodama et al., 1993; Sendl et al., 1996; Kerman et al., 1997; Wu et al., 1998a,b). *Rhinacanthus* plant is well known as the sources of flavonoids, steroids, triterpenoids, anthraquinones, lignans and especially naphthoquinone analoges. Naphthoquinone compounds have been reported to possess in vitro antiproliferative activity towards various cancer cells as well as inhibited HeLaS3 cells growth by apoptotic induction (Wu et al., 1988; 1998b; Gotoh et al., 2004; Siripong et al., 2006a,b). Rhinacanthone has also shown antitumor activity against Dalton's lymphoma ascitic cells bearing mice (Thirumrugan et al., 2000). In addition, rhinacanthin-C, a main naphthoquinone ester, and the active chloroform and aqueous extracts of the roots and stems have significantly suppressed the growth of Meth-A-sarcoma-bearing mice (Siripong et al., 2006c). This study was aimed at a determination of the chemopreventive effect of *R. nasutus* Kurz. roots extract against colon carcinogenesis using a mouse model induced by a

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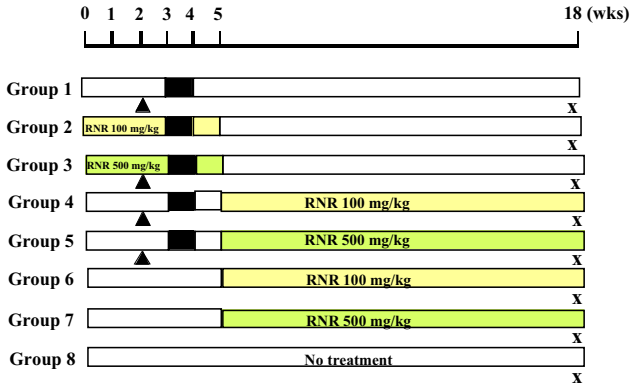

Figure 1. Experimental Protocol for the Mouse Colon Carcinogenesis Model Induced by AOM plus DSS. Triangles, AOM, 10 mg/kg, i.p.; black boxes, 2% DSS in drinking water; Xs, sacrifice

combined treatment of azoxymethane (AOM) plus dextran sodium sulfate (DSS) both in the initiation and promotion phases of carcinogenesis.

Materials and Methods

Plant extracts

Dried roots of *R. nasutus* Kurz. (RNR) were ground and extracted with distilled water by refluxing. The filtrates were evaporated and lyophilized and then kept as the dried materials at -20˚C until use.

Chemicals

AOM was purchased from Sigma Aldrich (U.S.A.) and DSS (molecular weight 36,000) was purchased from MP Biomedicals (U.S.A.).

Animals and diets

One hundred male ICR mice, aged 4 weeks old, were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. Animals were maintained at the Laboratory Animal Facility of the National Cancer Institute according to the Institutional Care Guidelines. All animals were housed in the filter-top plastic cages in a clean conventional room at 23±2 ˚C and humidity 50±20% with a 12-h light/dark cycle. They were provided with a pellet diet (Perfect Companion Group Co. Ltd., Thailand) and water *ad libitum*.

Experimental Design

After acclimatization, the mice were randomly divided by weight into 8 groups and 5 groups were given AOM/DSS and/or the water extract of the roots of *R. nasutus* (RNR) daily at a doses of 100 or 500 mg /kg body weight (in 0.3% carboxymethylcellulose in normal saline) as shown in Figure 1. Group 1 was assigned as control group which received a single intraperitoneal injection of AOM at a dose of 10 mg/kg body weight at week 2nd and one week later, animals were administered with 2% DSS in drinking water for one week, then no further treatment til the end of the experiment. Groups 2 and 3 were assigned as the experimental groups in the initiation phase. They were given RNR at a doses of 100 and 500 mg/kg body weight, respectively, intragastrically daily for 2 weeks prior to giving AOM, during one-week DSS administration and also 1 week after completion of DSS. Groups 4 and 5 were assigned as the experimental groups in the promotion phase receiving RNR at a doses of 100 and 500 mg/kg body weight, respectively, starting 1 week after completion of DSS administration until the end of experiment at week 18th. Groups 6 and 7 which were assigned as medicinal plant control groups were given only RNR daily at a doses of 100 and 500 mg/kg body weight, respectively, for 14 weeks. Group 8 was assigned as negative control group. All animals were sacrificed at week 18th. At necropsy, the colons were excised, cut open longitudinally along the main axis, washed with saline and then examined for the presence of tumors. The tumors were then counted, measured for their sizes, and thereafter fixed with 10% buffered formalin prior to performing histopathological examination by conventional methods. The classification of neoplastic lesions was verified according to the criteria described previously (Turusov and Mohr, 1994).

Statistical Analysis

The significance of differences in the incidences of tumors between the experimental and control groups was assessed by the statistical techniques described by Peto et al., 1980, whereas that of the number of tumors per mice, body and liver weights and the length of colon were analyzed using One-Way ANOVA and Krusskal-Wallis H Test.

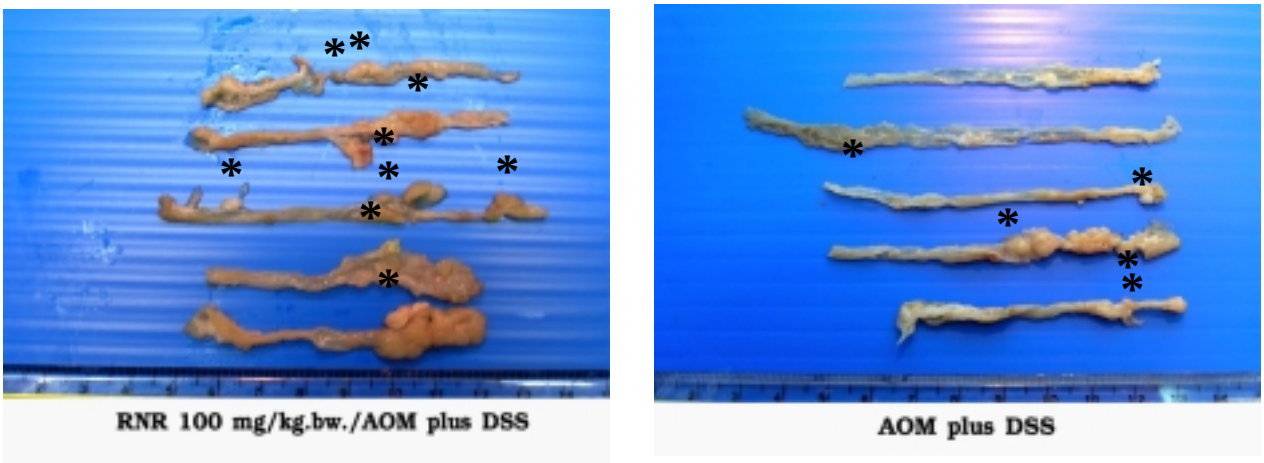

Figure 2. Macroscopic View of Mouse Colons with Gross Tumors Indicated (*, tumor)

Table 1. Effect of RNR on the Body, Liver weights, Length of Colon and Lesion Development in Mice Treated with AOM plus DSS

Group No	Treatment	No. of Mice	B.W. (g)	Liver weight (g) Absolute	Relative	Length Colon (cm)	Colon Tumour Incidence (%)	Multiplicity
1	AOM/DSS	10	41.4±4.7[a]	2.3±0.4[a]	5.4±0.8[a]	9.7±1.1[a]	6/10 (60.0)	1.7±0.8[b]
2	RNR 100 mg/kg/ AOM/DSS	13	42.7±3.2	2.2±0.2	5.0±0.6	9.1±1.0	11/13 (84.6)	4.2±2.1
3	RNR 500 mg/kg/AOM/DSS	13	41.7±4.3	2.3±0.3	5.0±0.5	9.6±1.7	9/13 (69.2)	3.3±1.6
4	AOM/DSS/ RNR 100 mg/kg	16	38.7±2.8	2.3±0.3	5.6±0.8	8.8±1.0	14/16 (87.5)	5.6±1.9*
5	AOM/DSS/ RNR 500 mg/kg	15	40.2±4.1	2.3±0.4	5.7±1.1	9.1±1.2	10/15 (66.7)	3.3±1.5
6	RNR 100 mg/kg	5	41.5±3.6	2.3±0.4	5.4±0.8	9.7±1.1	0/5 (0.0)	0.0
7	RNR 500 mg/kg	5	40.5±4.0	2.3±0.2	5.6±0.3	10.8±1.7	0/5 (0.0)	0.0
8	No treatment	10	40.3±6.6	2.3±0.6	4.8±2.0	10.5±1.8	0/10 (0.0)	0.0

[a]Data are Mean+SD values; [b]Data are Mean±SE values; *p<0.05

Results

General observations

Bloody stools were noted in a few mice in groups which receiving 2% DSS and their body weight were slightly decreased during the period of treatment. After week 7, anal prolapse was found in a few mice treated with AOM/DSS in groups 1, 4 and 5. The mean body weight and length of the colon of mice fed AOM/DSS plus RNR were not significantly different from those of the control group which received AOM/DSS alone (Table 1). In addition, the mice in group 4 which were fed with RNR 100 mg/kg body weight after AOM/DSS showed the lowest body weight and length of colon, while untreated mice showed the lowest relative liver weight.

Pathological findings

Macroscopic view of the colons of the mice in group 1 through group 5 showed nodular or polyploidy-like tumors, of varying sizes, at the middle and distal part of colons (Figure 2). Their histopathology showed mainly simple tubular adenocarcinoma. The mice in groups 6-8 did not have any tumors in the colon. The incidence and multiplicity data for colonic neoplasms are also summarized in Table 1. An incidence of tumors in group 1 (AOM/DSS) was 60% with a multiplicity of 1.7±0.8. In groups 2 & 3 which were fed RNR at 100 and 500 mg/kg body weight and AOM/DSS in the initiation phase study, the incidences of tumors was higher 84.6% and 69.2%, respectively, but not significantly, than those in the control group 1. In addition, mice given RNR during the promotion phase study (Groups 4 & 5) also showed the same results (87.5% and 66.7%, respectively). However, only the multiplicity of tumors in the low dose group (group 4, 5.6±1.9) was significantly higher than that in the control group 1 (P=0.026).

Discussion

In the present study, water extract of the roots of *R. nasutus* Kurz. (RNR) when given to mice both in the initiation and promotion phases at the doses of 100 and 500 mg/kg body weight which were equivalent to 3 and 15 times of human use had no effect on the growth and liver weight of mice. Unfortunately, we found that RNR could not inhibit carcinogenesis induced by AOM plus DSS either given in the initiation or promotion phase. Despite the recent studies that various *R. nasutus* extracts such as petroleum ether, hexane and chloroform have shown antimutagenicity towards AFB_1 (Rojanapo et al., 1990) and the chloroform extract and eight naphthoquinone compounds (rhinacanthins and rhinacanthone) isolated from *R. nasutus* exhibited antiproliferative activity towards various cancer cell lines (Siripong et al., 2006a). Interestingly, three main naphthoquinone esters: rhinacanthin-C, -N and -Q isolated from RNR have been found to induce the apoptosis against human cervical carcinoma cells, HeLaS3 (Siripong et al., 2006b). Moreover, rhinacanthin-C and rhinacanthone showed antitumor activity against Sarcoma ascitic cells and Dalton's lymphoma in mice, respectively (Gotoh, et al., 2004; Thirumurugan, et al., 2000) as well as liposomal rhinacanthin-C, -N and -Q at a dose of 5 mg/kg body weight also significantly suppressed the tumor growth in Met-A sarcoma-bearing mice and prolong survival time (Siripong et al., 2006c). A water extracts of the roots and stems, chloroform extract and rhinacanthin-C isolated from the roots of *R. nasutus* Kurz. also inhibited mammary gland carcinogenesis induced by 7,12 dimethylbenz(a)anthracene in rats (Siripong et al., 2008). These information indicated that RNR may have chemopreventive effect in many systems except colon carcinogenesis.

The mechanism by which RNR had no preventive effect in colon carcinogenesis in mice is not clear. However, these results are fairly similar to that of neem flowers which could inhibit liver and mammary gland carcinogenesis induced by aflatoxin B_1 and 7,12 dimethylbenz(a)anthracene, respectively, whereas significantly increased the incidence of colonic neoplasm induced by AOM in rats (Tepsuwan et al., 2002; Kupradinun et al., in preparation). Results of neem flowers indicated organ different in chemopreventive potential. However, based on the overall findings, it was possible that RNR may act as a tumor promoter or anticarcinogen, depending upon the test species, initiating agent and exposure protocol. Further study of the chemopreventive effect of water and organic extracts of roots as well as active main compounds of *R. nasutus* should be performed.

In conclusion, these results suggest that water extract of the roots of *R. nasutus* Kurz. had no preventive potential against colon carcinogenesis induced by AOM plus DSS in mice when given either in the initiation or promotion

phase. These findings also suggest that *R. nasutus* Kurz. may have a different chemopreventive effect on different organs since in the mammary gland model it could significantly decreased the incidence and multiplicity of tumors.

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