RESEARCH COMMUNICATION

Evaluation of Anti-Cancer and Anti-Oxidative Potential of *Syzygium Cumini* Against Benzo[a]pyrene (BaP) Induced Gastric Carcinogenesis in Mice

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Abstract

***Syzygium cummini* extract (SCE) was used in the present study to explore anti-tumor promoting activity in a stomach carcinogenesis model in mice. For this purpose, Swiss albino mice were administered with 1 mg of benzo-a-pyrene (BaP) in 100µl sesame oil by oral gavage twice a week for 4 consecutive weeks. The animals were sacrificed 14 weeks after the last administration of BaP. Oral administration of the extract to pre-treated (i.e. SCE as 25mg/kg b. wt./ day before BaP application for 2 weeks), post-treated (i.e. SCE after BaP application for 8 weeks) and pre-post treated (i.e. SCE for 2 weeks before treatment of BaP followed by the concomitant treatment with SCE and BaP for 4 weeks during & 2 weeks after the last dose of BaP) groups provided a significant reduction in tumor incidence, tumor burden and cumulative number of gastric carcinomas along with a significant elevation of phase II detoxifying enzymes, and inhibition of lipid per oxidation in the stomach. Thus, the present data suggest that the *Syzygium cummin*i extract has anti-tumor and anti-oxidative potential against chemical induced stomach carcinogenesis.**

Keywords: Carcinogen - chemoprevention - syzygium cummini - gastric tumor - antioxidants - phase II enzymes

Asian Pacific J Cancer Prev, **11**, 753-758

Introduction

Environment pollutants are one of the main risk factors for the induction of cancer that is considered as a major public health concern and leading cause of death in both developing and developed countries. These pollutants include benzo(a)pyrene (BaP), which is a polycyclic aromatic hydrocarbon (PAH) formed by the pyrolytic process during smoking of cigarettes and other tobacco products as well as in combusted organic matter in automobile exhaust. BaP is frequently used as a representative indicator of total PAH levels. It is a complete carcinogen, as it produces initiation and promotion of carcinogenesis (Halliwell and Gutteridge, 1989).

Cancer chemoprevention is a mean of cancer control by pharmacological intervention of the occurrence of the disease using chemical compounds. Recent events suggest that new emphasis in the development of medical treatment of human disease will be intimately connected to natural products. The use of medicinal plants in modern medicine for the prevention or treatment of cancer is an important aspect. For this reason, it is significant to identify anti-tumor-promoting agents present in medicinal plants commonly used by the human population, which can inhibit the progression of tumor.

The *Syzygium cumini* (Jambul or Jamun or Jamblang) is, an evergreen tropical in the flowering plant of the family Myrtaceae, native to India, Pakistan and Indonesia. It is also grown in other areas of southern and south eastern Asia including the Philippines, Myanmar, and Afghanistan. The Jamun fruit is a small oval shaped purple colored fruit. that ripens during June-July months. These huge Jamun trees continue to give fruits for 60-70 years. The berry is oblong, ovoid, green when just appearing, pink when attaining near maturity and shining purple when fully ripe. The fruits and seeds are sweet, acidic, sour, tonic and it has lots of medicinal properties like anti-diabetes, anti-inflammatory, anti-diarrhea, anti-ringworm, anti-pharyngitis, anti-splenopathy (Deila Rosély et al., 2004, Kumar et al., 2008). It also has anti-radiation activities and reduces the radiation-induced DNA damage in the cultured human peripheral blood lymphocytes (Jagetia, 2002).

The present study has been undertaken to explore the possible anti-cancer and anti-oxidative potential of Syzygium cumini seed extract against chemical induced gastric carcinogenesis in mice.

Materials and Methods

Animal Care and Handling

Random-bred Swiss albino mice (6-7 weeks old) were used for the present experiments. These animals were maintained in the animal house at room temperature of 24°±3° and 12 hrs light: 12 hrs dark periods. Such animals

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were housed in polypropylene cages and fed standard mice feed from Aashirvaad India Ltd., Chandigarh (India). Tap water was provided for drinking, and tetracycline once in a fortnight was given to prevent them against microbial infections. Animal care and handling were performed according to the guidelines set by the World Health Organization (WHO), Geneva, Switzerland, and the INMAS Indian National Science Academy, New Delhi, India. The departmental animal ethical committee has approved this study.

Chemical

Benzo(a)pyrene was procured from Sigma Chemical Company, St. Louis, MO, USA. The BaP-induced stomach tumorigenesis in mice was performed according to the method of Wattenberg et al (1981) with minor modifications as suggested by Nagabhushan and Bhide (1987).

Syzygium cummini extract preparation

The fruits of *Syzygium cummini* were collected from local market after proper identification (Voucher No. RUBL-20425) by a competent botanist in herbarium of the Department of Botany, University of Rajasthan, Jaipur (India). The pulp was removed from the fruit and the seed were washed properly and shade dried. After this, fruits were powdered in a mixture and the extract was prepared by refluxing with the double distilled water (DDW) for 36 (12x3) hrs at 40ºC. The extract was cooled and concentrated by evaporating its liquid contents. The required dose for treatment was prepared by dissolving the extract in DDW at a dose level of 25mg/kg body weight.

Experimental Design

Swiss albino mice of 6-8 weeks-old were divided into the following groups:

Group I: Sterile tap water (STW) treated (Negative Control). The animals of this group received sterile tap water (STW) as drinking source throughout the study period.

Group II: Syzygium cumini treated (Drug treated Control). Animals of this group were administered Syzygium cumini extract with the dose of 25 mg/kg/b. wt/day orally for 2 weeks.

Group III: Sesame oil (SMO) treated (Vehicle treated Control). The animals of this group were injected with 100 µl/animal of sesame oil (SMO) by oral gavage twice a week for 4 consecutive weeks.

Group IV: Carcinogen (BaP) treated (Positive Control). The animals of this group were administered with 1 mg of BaP in 100 µl sesame oil by oral gavage twice a week for 4 consecutive weeks (total eight administrations). These were sacrificed 14 week after the last administration of BaP.

Group V: SCE treated (Pre-treatment group; Experimental-I). The animals of this group received 4 mg/ml of ASE as sole source of drinking water for 5 days a week, for 2 weeks. 48 hrs. before the first administration of BaP or sesame oil, the SCE source was withdrawn and the mice were provided with sterile tap water as drinking for rest of the study period.

Group VI: SCE treated (Post-treatment group; Experimental-II). The animals of this group were provided sterile tap water during the carcinogen treatment for 4 weeks. 48 hrs. after the last administration of BaP in sesame oil, the animals were administered with SCE at a dose (25mg/kg/b.wt./day) of for 8 weeks.

Group VII: SCE treated (Pre-post treatment group; Experimental-III). The animals of this group received the dose 25mg/kg/b.wt./day of SCE for 2 weeks before treatment with BaP followed by the concomitant treatment of SCE and BaP for 4 weeks during and 2 weeks after the last dose of BaP in sesame oil.

The body weights of the animals from each group were recorded at the beginning and at the termination of the experiments. The mice from all the groups were sacrificed at 14 weeks after the last dose of BaP for the following study:

1. Morphological. a) Tumor incidence. This is the number of mice carrying at least 1 tumor expressed as percentage incidence. b) Tumor yield. This refers to the total number of tumors per group and the mean number of tumors per mouse. c) Cumulative number of papillomas. The total number of tumors that had appeared by the end of the experiment was considered the cumulative number of papillomas. d) Tumor burden. The average number of tumors per tumor bearing mouse was calculated as tumor burden.

2. Biochemical. The whole stomach was taken out for each mouse for the study of following biochemical parameters. Biochemical analysis was performed in the above groups at the time of termination of the experiment. The parameters that were assayed are described below.

Lipid peroxidation (LPO)

The level of LPO in stomach was measured in terms of thiobarbituric acid reactive substances by the method of Okhawa et al (1979). Briefly, thiobarbituric acid (0.8%), sodium dodecyl sulfate (0.1%), and acetic acid (20%) were added to 100 ml. of the tissue homogenate for 60 min.it was cooled and extracted with N-butanolpyridine and the optical density of LPO was recorded at 532 nm. The content of LPO was expressed in nmol/mg.

Glutathione (GSH)

The level of reduced GSH was estimated by the method of Moron et al (1979). The GSH content in the stomach was measured Spectrophotometrically using Ellman's reagent with 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) as a coloring agent, according to the method of Beutlar et al., (1963). The absorbance was recorded at 412 nm with levels expressed as nmol/mg of protein.

Catalase (CAT)

Catalase activity was assayed in the stomach by the method of Aebi et al (1984). The content was estimated at 240 nm by monitoring the disappearance of H_2O_2

Total Proteins

Total protein contents in stomach were measured by the method of Lowry et al (1951). The absorbance was recorded at 680 nm.

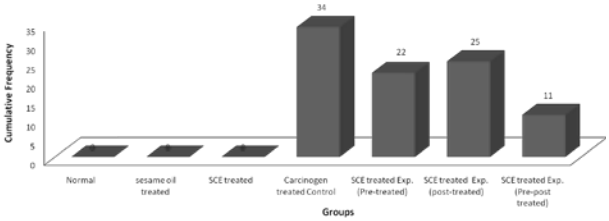

Figure 1. Variations in Cumulative Frequency during BaP- Induced Gastric Carcinogenesis with / without SCE Administration.

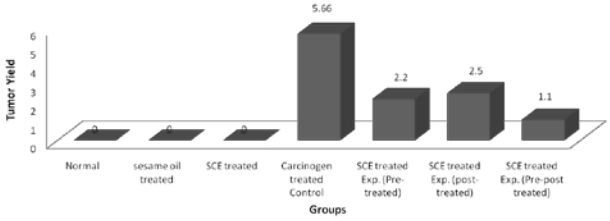

Figure 2. Variations in Tumor Yield during BaP-Induced Gastric Carcinogenesis with / without SCE Administration

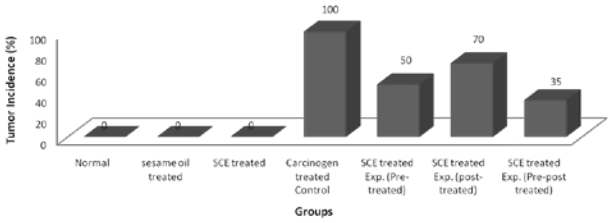

Figure 3. Variations in Tumor Incidence in during BaP- Induced Gastric Carcinogenesis with / without SCE Administration

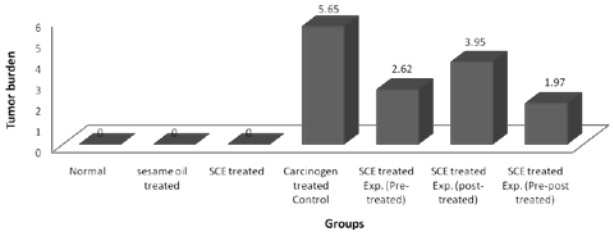

Figure 4. Variations in Tumor Burden during BaP-Induced Gastric Carcinogenesis with / without SCE Administration

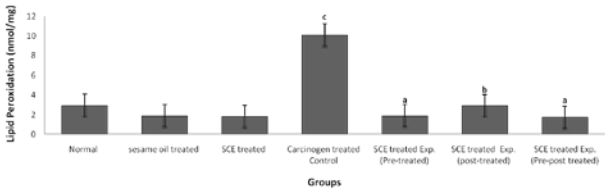

Figure 5. Variations in Lipid Peroxidation Level during BaP- Induced Gastric Carcinogenesis with / without SCE Administration. Significant level - Normal v/s Carcinogen treated control; Carcinogen treated control v/s SCE treated experimental (Pre-, Post-, Pre-post group) - a = p<0.05, b=p<0.01, c=p<0.001

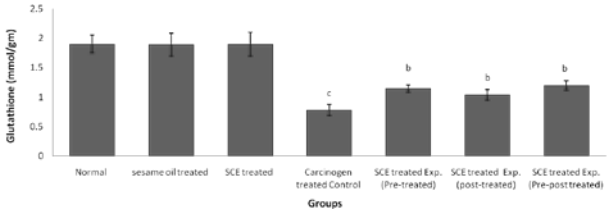

Figure 6. Variations in Glutathione Level during BaP- Induced Gastric Carcinogenesis with / without SCE Administration. Significant level - Normal v/s Carcinogen treated control; Carcinogen treated control v/s SCE treated experimental (Pre-, Post-, Pre-post group) - b=p<0.01, c=p<0.001

Results

Morphological Analysis

Tumor incidence (number of mice carrying at least 1 tumor) is depicted in Figure 1 In Group IV (Carcinogen-treated control), tumor incidence was found to be 100%. In the animals of Group V (Experimental-I), tumor incidence was observed as 50%. In group VI (Experimental-II), in which animals received SCE orally (25 mg/kg by b wt. /day) after BaP application for 8 weeks, the tumor incidence was recorded as 70%. In Group VII (Experimental-III), tumor incidence was scored as 35%. Like this, all experimental groups, administered SCE, had considerably lower tumor incidences then the carcinogen treated control.

Tumor yield (i.e. average number of papillomas per mouse) Figure 2). In Group IV (Carcinogen-treated control), tumor yield was found to be 5.66. In the animals of Group V (Experimental-I), tumor yield was observed as 2.2. In Group VI (Experimental-II), in which animals received SCE orally (25 mg/kg by bwt. /day) after BaP application for 8 weeks, the tumor yield was recorded as 2.5 while in Group VII (Experimental-III), it was scored as 1.1.

Cumulative number of papillomas (Figure 3).No tumor was observed in the animals of Groups I, II and III. Cumulative number of papillomas in carcinogen treated control was recorded as 34. The counts of cumulative number of such papillomas were highly reduced to 22, 25, and 11 in the experimental group V, VI & VII respectively as compared with carcinogen treated control (i.e. Group IV).

Tumor Burden (Figure 4). In Group IV (Carcinogen-treated control), tumor burden was found to be 5.65. On the other hand, the same was recorded as 2.62 (Group V), 3.95 (Group VI) and 1.97 (Group VII) in the animals which were treated with SCE alone with carcinogen.

Biochemical Analysis

Lipid peroxidation (Figure 5). A significant increase (P<.001) in the level of LPO was recorded in Group IV (Carcinogen treated control) as compared with Groups I and II Administration of SCE significantly (P<0.001) reduced the level of lipid peroxidation in all the SCE treated experimental mice (Groups V-VII) in comparison to the carcinogen treated control (Group IV).

Reduced glutathione (Figure 6).Treatment with Syzygium cummini extract resulted in an enhanced level of the non enzymatic antioxidant protein GSH in stomach of animals in groups V to VII as compared with the carcinogen treated control (Group IV). On contrary, a significant decrease (P<0.001) in the level of GSH was recorded in Group IV (carcinogen treated control) animals as compared with Groups I, II and III.

Catalase (Figure 7). The activity of catalase was observed as significantly (P<0.001) declined in

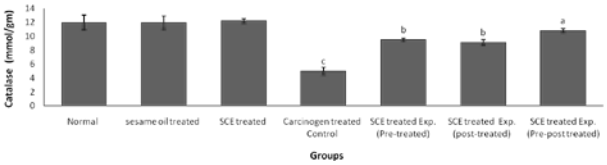

Figure 7. Variations in Catalase Level during BaP-Induced Gastric Carcinogenesis with / without SCE Administration. Significant level - Normal v/s Carcinogen treated control; Carcinogen treated control v/s SCE treated experimental (Pre-, Post-, Pre-post group) - a = p<0.05, b=p<0.01, c=p<0.001

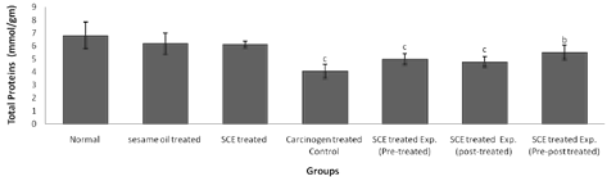

Figure 8. Variations in Total Proteins Level during BaP- Induced Gastric Carcinogenesis with / without SCE Administration. Significant level - Normal v/s Carcinogen treated control; Carcinogen treated control v/s SCE treated experimental (Pre-, Post-, Pre-post group) - b=p<0.01, c=p<0.001

carcinogen treated control (Group IV) as compared to normal (Groups I), drug treated control (Group II) and sesame oil treated (Group III). A significantly increased (P<0.001) CAT activity was recorded in the stomach of SCE-treated experimental animals (Groups V-VII) then the carcinogen treated controls (Group IV).

Total Proteins (Figure 8). A significantly increase (P<0.001) in total proteins activity was recorded in the stomach of SCE-treated experimental animals (Groups V-VII) as compared with the carcinogen treated controls (Group IV).

Discussion

Stomach cancer is the second most prevalent malignancy in the world and chemoprevention has evolved as an effective strategy to combat this dreadful disease (Velmurugan et al., 2005). Fruits, vegetables, vitamins, common beverages and several medicinal plants/herbs with diversified pharmacological properties have been shown to be a rich source of cancer chemopreventive agents (Wattenberg et al., 1992). In the present piece of research work, an attempt was made to evaluate the modulatory effects of an Indian medicinal plant *Syzygium cummini* against B(a)P induced forestomach tumorigenesis.

B(a)P, an extremely potent pro-carcinogen, is metabolized by biotransformation enzymes to a variety of metabolites that are responsible for initiating carcinogenesis (Choi et al., 1994). Biotransformation enzymes have broadly been divided into two categories namely phase- I and phase-II. The former constitutes cytochrome P-450 based mono-oxygenase system which is responsible for initiating conversion of pro-carcinogens to several of their metabolites including ultimate carcinogens. Glutathione-S-transferase (GST) is a major phase II detoxifying enzyme that primarily functions in catalyzing the active carcinogenic metabolites to endogenous ligand-reduced glutathione (GSH) favoring their elimination from the body of the organisms (Hartman et al., 1990). The balance between the phase-I carcinogen-activating enzymes and the phase-II detoxifying enzymes is critical to determining an individual's risk for cancer (Wilkinson et al., 1997). There is substantial evidence that chemopreventive agents including medicinal plants exert their anti-carcinogenic effects by modulation of phase-I and phase-II xenobiotic biotransformation enzymes (Subapriya et al., 2005).

In the present study, the exposure of mice to the carcinogen BaP caused high incidence of forestomach tumors, while the sesame oil treatment did not induce any tumor in the recipient animals. In BaP alone treated group tumor multiplicity, tumor incidence, tumor burden, tumor yield as well as cumulative number of papillomas was found to be quite high in comparison to SCE+BaP treated group (Experimental). The results of the present investigation are also supported by the others (Wattenberg et al, 1980; Azuine and Bhide, 1992; Deshpande et al., 1997; Agha et al., 2001) who have used the different plant extracts to reduce chemical induced carcinogenesis in there finding (Dasgupta et al., 2004; Gangar et al., 2006).

Oxidative stress is potentially harmful to cells, and thus free radicals and other reactive oxygen species (ROS) are implicated in the etiology and progression of many diseases, including cancer. However antioxidant mechanism that scavenges ROS, by means of low-molecular-weight antioxidants or antioxidant enzyme systems, protects organism from the damaging effects of oxidative stress. Excessive oxidative stress and depleted antioxidant damage cellular components interfere with critical cellular activities and result in lipid peroxidation (Conklin, 2000). Similarly, in the present study, the higher level of LPO in BaP treated group was observed in stomach whereas the same was significantly decreased in SCE treated groups. Earlier workers (Parashar et al., 1994; Singh et al., 2004; Sancheti and Goyal, 2006) have also reported an elevation in the level of LPO in skin and liver after DMBA/ croton oil application on skin of mice. The oxidative stress induced by the elevated LPO might be responsible for the high incidences of gastric tumors in the present study.

Glutathione acts as a most important antioxidant in living systems because it is a remover of H_2O_2 lipid peroxides and their products like 4-hydroxinental (Bagchi et al., 2000). In the present study, a decrease in the level of GSH in stomach in the BaP treated mice has been observed, and this may be because of the enhanced oxidative damage, enhanced use of GSH by the enzyme GPx, and a reduction in the activities of the GSH-synthesizing enzymes such as glucose-6-phosphatedehydrogenase and GPx, which neutralize the hydroxyl radicals and singlet oxygen. As it is present in high concentration in the cells, it protects cells from free radical damage (Gopalakrishnan et al., 1996). GSH level was observed significantly higher in SCE treated mice than the carcinogen alone treated ones.

Antioxidants are potent scavengers of free radicals and serve as inhibitors of neoplastic processes (Mayer et al., 2000). Similarly, plants such as *Alstonia scholaris*

(Singh et al., 2006), *Trigonella foenum greacum* (Ranu et al., 2008), *Emblica officinalis* (Sancheti et al., 2007) and *Rosemarinus officinals* (Jahan et al., 2009) have also been reported to enhance GSH as well as to reduce the LPO level during chemical induced skin carcinogenesis in mice. It confirms the anti oxidative activity of various active constituents found in the fruit extract of *Syzygium cumini* used in the present study.

The protein level with the BaP was lower and with the SCE (Bap+SCE) it was found significantly higher. The similar result was found by (Dasgupta et al., 2003) defining the protein level in DMBA induced skin cancer.

It has been observed that catalase is a hemeprotein, which catalyzes the reduction of hydrogen peroxides and protect the tissue from the highly reactive hydroxyl radicals (Li et al., 2000). The catalase level depleted with the BaP but the same significantly increased in SCE treated Groups. Saha and Das also reported the depletion of catalase activity in DMBA/ croton oil treated mice whereas an increase in the level of this enzyme was observed in experimental group treated by various plant extracts.

Fatty oils (lauric, myristic, palmitic, stearic , oleic , linoleic , malvalic , stericulic & vernolic acid), phytosterols (β-sitosterol, tanins predominantly corilagin, ellagitannins, ellagic acid, galloyl-glatoside and gallic acid), phenolic compounds (quercetin, ferulic acid) have been identified as constituents of *Syzygium cumini* (Kumar et al., 2009). All these components may be collectively responsible for the anti cancer properties of such plant extract as evident in the present study.

In conclusion, the results of the present study support to the traditional use of *S. cumini* seed extract for the treatment of various diseases. It may be concluded that the extract of this plant may be further explored for its preventive and therapeutic uses against chemical induced gastric carcinogenesis.

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