Alleviative Effect of Herbal Extract Containing Phytoconstituents of *Bambusa vulgaris* and *Opuntia ficus-indica* against AOM/DSS-Induced Colorectal-Carcinoma-Bearing Mice

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Abstract

The study includes preliminary phytochemical screening and assessing alleviative effects of ethanolic extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits (EEBO) on azoxymethane/dextran sodium sulfate (AOM/DSS)-induced colorectal cancer in rodents. Acute oral toxicity study was performed to find the therapeutic dose of EEBO. In animals, cancer was induced by single injection of AOM 10 mg/kg intraperitoneally followed by supply of three repeated cycles of 2.5% of DSS solution. Then animals were treated with EEBO from third day of experiment and treatment was continued throughout the experimental period. The anticancer effect was assessed by studying body weight (b. wt) changes, macroanatomy, antioxidant level, and microanatomy of colon. The phytochemical evaluation revealed the presence of alkaloid, glycoside, carbohydrate, and flavonoid in bamboo extract, whereas glycoside and flavonoid in cactus extract. The results found that EEBO increases the b. wt and level of antioxidants like sodium dismutase, glutathione, and catalase and meanwhile decreases malondialdehyde. Macroanatomy study indicated a decrease in the numbers of adenoma and inflammatory signs in the treated group when compared with diseased group. Histopathology revealed that the presence of EEBO improved the colon histoarchitecture similar to normal and masked the crypt appearance. Decreased chromosomal aberrations and micronucleus numbers were observed in EEBO treated group. Overall, results suggest that the EEBO 400 mg/kg exhibited highest activity compared with 200 and 100 mg/kg and the effect may be attributed to its antigenotoxic or antioxidant effect.

Keywords

► *Bambusa vulgaris*
► colorectal cancer
► *Opuntia ficus-indica*


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Introduction

Colorectal cancer (CRC) is the third most leading cancer globally. It is a disease of cell characterized by uncontrolled proliferation and abnormal growth of cells in colorectal region. According to American Cancer Society, one million people are diagnosed with CRC every year globally, and it is estimated that 101,420 colon cancer cases, 40,180 new cases of rectal cancer, and 51,020 cases of death occurred in 2019. Incidence of CRC is increasing in developing countries due to their forwarding lifestyles related to westernization like smoking, alcohol consumption, obesity, poor physical activities, and consumption of red and processed meat. Hence, it is one of the major reasons of mortality and morbidity in the world.1 CRC initially develops as polyps, abnormal noncancerous tissue on colon inner lining. After some decades, few polyps, especially adenomatous polyps (adenoma), grow slowly and become cancerous. Less than 10% of adenoma is capable of invading other organ. Adenoma starts proliferating from glandular cells and lubricates the colon by producing mucus. The cancer arising from inner lining of colon is called as adenocarcinoma. The formed cancer grows to colon wall that can be carried in blood vessels and lymph nodes and is able to metastasize to other parts of the organ.2 Treatment of this epithelial cancer is a new approach in chemoprevention. Improvement in imaging, chemotherapy, and surgery helps to advance the treatment over past decades. The current treatments are limited, costly, and generally show side effects like bone marrow depression, alopecia, chemo brain, cold intolerance, numbness, tingling or blistering of hand and feet (common with oxaliplatin), bleeding, fatigue, frequent bowel movements, diarrhea, constipation, bloating, urogenital dysfunctions and colostomy from surgery, skin irritation, bladder irritation, rectal irritation, secondary tumor formation, and infertility in case of pelvic area exposed to radiation.3,4 Hence, our study was planned to evaluate a combined herbal extract of fruits of Opuntia ficus-indica (OFI) and shoots of Bambusa vulgaris (BV), which are consumed normally in diet and used as treatment choice in many diseases. Several studies have demonstrated the effectiveness of plant parts of BV and OFI in different types of cancer. According to literature review, various parts of OFI and its isolates (betanin, indicaxanthin, etc.) show antiproliferative activity by inducing apoptosis, cell cycle arrest, antigenotoxicity, and by inactivation of tumor suppressor gene.5–9 The presence of adenine and octadecatricenoic acid in bamboo shoot has been reported for dose-dependent anticancer activity.10 Hence, with this background, work was focused on combined herbal extract of BV and OFI against azoxymethane/dextran sodium sulfate (AOM/DSS)-induced CRC in mice, which mimics similar mechanism of repeated colonic inflammation-induced cancer in humans.

Results

Phytochemical Studies

BV extract was dark brown colored and semi-solid in nature. Percentage yield was found to be 13.08%. OFI was reddish brown colored and with semi-solid consistency, and percentage yield was found to be 15.12%. The qualitative phytochemical screening revealed the presence of alkaloid, carbohydrate, and glycosides in BV and glycosides, polyphenols, amino acids, and flavonoid in OFI.

Acute Oral Toxicity

No adverse sign and lethality were seen in acute toxicity study for 14 days of experimental period and also no delayed toxicity was observed with ethanolic extract of Bambusa vulgaris shoots and Opuntia ficus-indica fruits (EEBO).

Anticolectal Cancer Activity

Observation on Body Weight

Body weight (b. wt) is an important marker of CRC. Change in b. wt during experiment is represented in ►Fig. 1. Decreased b. wt was seen in AOM/DSS-treated animal compared with normal. During 10 weeks of study, gradual increase in b. wt was observed in normal group that was supplied with normal diet and water and without any treatment. Gradual decrease in b. wt was seen in diseased group; b. wt of mice in EEBO 100 and 200 mg/kg groups was lower compared with normal but higher than diseased group. The mice treated with EEBO 400 mg/kg prevented weight loss as well as increased b. wt similar to normal group when compared with the diseased. Standard group showed increase in b. wt during experiment but not as significant as EEBO 400 mg/kg and normal.

Macroscopic Analysis

In ►Fig. 2, we found that there were no features of inflammation or neoplasia in normal colon. Treatment of animals with AOM/DSS induced severe inflammation and marginal adenoma in distal part of the colon. The incidence of these features was significantly reduced in animals receiving EEBO. The standard was exerted in its action by complete elimination of neoplastic characteristics. However, EEBO 400 mg/kg inhibited the signs of neoplasia and inflammation strongly, followed by 200 and 100 mg/kg compared with diseased group.

Antioxidant potential of EEBO is represented in ►Table 1. Diseased control group showed reduction in the level of free radical scavengers like glutathione (GSH), sodium dismutase (SOD), and catalase activity when compared with normal control group indicating that oxidative stress was induced by AOM/DSS. The elevated level of oxidative stress markers like malondialdehyde (MDA) was found to be high in the diseased...
control group when compared with normal control group. Treatment with EEBO 400 and 200 mg/kg significantly restored the effects of AOM/DSS-induced changes on the levels of GSH, SOD, and MDA. However, only 400 mg/kg was able to reverse the changes in catalase level.

Microanatomy of Colon

Fig. 3 indicates the microanatomy of colorectal region. There was no evidence of inflammation, injury, and neoplasms in colon of normal mice. The inflammation was characterized by inflammatory infiltrate and crypt damage in diseased control group. The presence of EEBO in genotoxicant-treated mice improved the histoarchitecture similar to normal and masked the crypt appearance. EEBO 400 mg/kg exhibited highest activity compared with 200 and 100 mg/kg.

Table 1 Effect of ethanolic extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits on colon antioxidant level

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (μmol/g of tissue)</th>
<th>Catalase molecule of H₂O₂ consumed/min/gram</th>
<th>MDA (nmol/mg of tissue)</th>
<th>SOD (μmol/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5.487 ± 0.19</td>
<td>0.657 ± 0.03</td>
<td>16.40 ± 1.044</td>
<td>6.86 ± 0.589</td>
</tr>
<tr>
<td>Cancer control</td>
<td>2.332 ± 0.10ᵃ</td>
<td>0.187 ± 0.02ᵃ</td>
<td>49.22 ± 1.72ᵃ</td>
<td>2.85 ± 0.515ᵃ</td>
</tr>
<tr>
<td>5-FU 10 mg/kg</td>
<td>4.884 ± 0.22ᵇ</td>
<td>0.547 ± 0.041ᵇ</td>
<td>15.60 ± 0.864ᵇ</td>
<td>6.115 ± 0.754ᵇ</td>
</tr>
<tr>
<td>EEBO 100 mg/kg</td>
<td>4.0277 ± 0.31ᵇ</td>
<td>0.240 ± 0.03ᵐˢ</td>
<td>44.01 ± 1.59ᶜ</td>
<td>3.73 ± 0.50ᶜ</td>
</tr>
<tr>
<td>EEBO 200 mg/kg</td>
<td>4.653 ± 0.23ᵇ</td>
<td>0.256 ± 0.03ᵐˢ</td>
<td>32.46 ± 1.25ᵖ</td>
<td>5.54 ± 0.345ᵃᵇ</td>
</tr>
<tr>
<td>EEBO 400 mg/kg</td>
<td>5.366 ± 0.21ᵇ</td>
<td>0.489 ± 0.02ᵇ</td>
<td>13.96 ± 0.71ˢᵇ</td>
<td>5.92 ± 0.531ᵇ</td>
</tr>
</tbody>
</table>

Abbreviations: EEBO, extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits; 5-FU, 5 Fluorouracil; GSH, glutathione; MDA, malondialdehyde; SEM, standard error of the mean; SOD, sodium dismutase.

Note: All values are expressed as mean ± SEM; n = 6.

ᵃp < 0.01 when compared with normal control.

ᵇp < 0.01 when compared with disease control.

ᶜp < 0.05 when compared with disease control.

ᵐˢWhen compared with disease control.

Antigenotoxic Effect of EEBO

Chromosomal Aberration Assay

Fig. 2 and Fig. 4 show effects of EEBO on chromosomal aberrations. Single injection of cyclophosphamide (CP) significantly increased the number of chromosomal aberrations in animals. Oral administration of EEBO 400 mg/kg significantly decreased the number of aberrations induced by CP. The % inhibition of chromosomal aberration was found to be 79.50%, 63.68%, and 71.44% by EEBO 400, 200, and 100 mg/kg, respectively.

Micronucleus Assay

The effect of EEBO on micronucleus is represented in Table 3 and Figs. 5 to 8. Animals treated with CP showed a significant increase in mean number of micronucleus
polychromatic erythrocytes (MNPCE), % of MNPCE, and % of polychromatic erythrocytes (PCE) ($p < 0.01$) when compared with control group. Animals when treated with EEBO 100, 200, and 400 mg/kg showed significant decrease in the mean of MNPCE, % of MNPCE, and % PCE when compared with control group.

### Discussion

Approximately 4.6% of men (1 in 22) and 4.2% of women (1 in 24) will be diagnosed with CRC in their lifetime with the incidence of 1.23 million new cases every year worldwide.$^{11}$ Natural products such as turmeric, tea, onion oil, soybean, fenugreek, and orange juice have demonstrated anticancer effects on CRC. Similarly, we established the activity of EEBO for their activity against neoplasm, as these herbs are edible with no side effects. To support this study, we followed The Organization for Economic Co-operation Development (OECD) Guideline 423 to perform acute oral toxicity test and results showed that EEBO was nontoxic and safe up to 2,000 mg/kg. The b. wt changes were observed throughout the experimental period. Antioxidant levels and macroscopic and microscopic analyses were performed for the excised colorectal region. B. wt loss represents the prognosis and progression of tumor in colon. EEBO 100 and 200 mg/kg recovered lost weight, whereas 400 mg/kg recovered lost weight as well as increased the b. wt like normal group. AOM/DSS can lead to colorectal damage by elevating

### Table 2

<table>
<thead>
<tr>
<th>S. no</th>
<th>Grouping</th>
<th>Mean ± SEM</th>
<th>Different aberrations in %</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle control</td>
<td>7.5 ± 0.34</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>CP 50 mg/kg</td>
<td>68.3 ± 1.43$^a$</td>
<td>42</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>CP + EEBO 100 mg/kg</td>
<td>19.5 ± 0.84$^b$</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>CP + EEBO 200 mg/kg</td>
<td>24.8 ± 1.16$^b$</td>
<td>16</td>
<td>6</td>
<td>3</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>CP + EEBO 400 mg/kg</td>
<td>14.0 ± 1.12$^b$</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: Asso., centromeric association; Brea., chromatid breaks; CP, cyclophosphamide; EEBO, extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits; Fra., fragmentation; Gap., chromatid gap; Inh., inhibition; SEM, standard error of the mean.

Note: All values are expressed as mean ± SEM; $n = 6$.

$p < 0.01$ when compared with normal control.

$p < 0.01$ denotes it is statistically significant as compared with cyclophosphamide group.

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**Fig. 3** Normal colon (A) displayed colon structure that is free from inflammation, injury, and neoplasm. Diseased colon (B) indicated damaged crypt structure, irregular glandular structure, blood vessel formation, and adenoma with severe atypia. 5-FU treated group (C) showed colon histarchitecture similar to normal. Ethanolic extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits (EEBO) 100 mg/kg (D) displayed crypt ablation and inflammatory infiltrate. EEBO 200 mg/kg (E) moderately inhibited crypt ablation, inflammation, and other damages. Treatment with EEBO 400 mg/kg (F) proved typical crypt and glandular structure, and other unremarkable injury. 5-FU, 5 Fluorouracil.
oxidative free radicals (MDA) and by destroying defense antioxidants (GSH, catalase, and SOD). EEBO protected antioxidants and destroyed free radical formation to protect colon tissue from AOM/DSS-induced damage. Macroanatomy study showed normal colons were devoid from tumor feature as they are not exposed to any treatment. In cancer control group we found the immarginal adenoma in distal part of colorectal region along with remarkable inflammatory features. Treatment with extracts overcomes the features of neoplasia due to its alleviative activity against CRC. Microscopic view of colon of different groups also suggested the anticancer activity of extracts. Antigenotoxic studies are

![Figure 4](image)

**Fig. 4** Chromosomal aberration. CP, cyclophosphamide; EEBO, Ethanolic extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits.

**Table 3** Effect of ethanolic extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits on micronucleus

<table>
<thead>
<tr>
<th>Groups</th>
<th>MNPCE/2,000</th>
<th>% MNPCE</th>
<th>% PCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>1.5 ± 0.42</td>
<td>0.07 ± 0.021</td>
<td>18.5 ± 1.147</td>
</tr>
<tr>
<td>Cyclophosphamide (CP)</td>
<td>14.66 ± 0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.0 ± 1.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EEBO 100 mg/kg + CP</td>
<td>4.83 ± 0.87</td>
<td>0.24 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.8 ± 1.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EEBO 200 mg/kg + CP</td>
<td>4.66 ± 0.98</td>
<td>0.23 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.0 ± 1.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EEBO 400 mg/kg + CP</td>
<td>3.33 ± 0.42</td>
<td>0.16 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.6 ± 0.76&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: EEBO, extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits; MNPCE, micronucleus polychromatic erythrocytes; PCE, polychromatic erythrocytes; SEM, standard error of the mean.

Note: All values are expressed as mean ± SEM; *n* = 6.

<sup>a</sup>*p* < 0.01 when compared with normal control.

<sup>b</sup>*p* < 0.01.

<sup>c</sup>*p* < 0.05 denotes it is statistically significant as compared with cyclophosphamide group.

![Figure 5](image)

**Fig. 5** Chromosomal aberration assay: (A) vehicle control, (B) cyclophosphamide (CP) control 50 mg/kg, (C) CP + ethanolic extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits (EEBO) 100 mg/kg, (D) CP + EEBO 200 mg/kg, and (E) CP + EEBO 400 mg/kg.
Fig. 6 Micronucleus assay: (A) vehicle control, (B) cyclophosphamide (CP) control 50 mg/kg, (C) CP + ethanolic extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits (EEBO) 100 mg/kg, (D) CP + EEBO 200 mg/kg, and (E) CP + EEBO 400 mg/kg.

Fig. 7 Mononuclear polychromatic erythrocytes number. CP, cyclophosphamide; EEBO, Ethanol extract of *Bambusa vulgaris* shoots and *Opuntia ficus-indica* fruits; MNPCE, micronucleus polychromatic erythrocytes; SEM, standard error of the mean.

Fig. 8 Impact on polychromatic erythrocytes in response to treatment groups at aforementioned doses.
useful to study the effect of test article on genetic damage caused by toxicants. According to OECD guidelines 474 and 475, rodent bone marrow micronucleus test and chromosomal aberration test, we evaluated antigenotoxicity of EEBO. An increase in the frequency of MNPCEs and chromosomal aberrations in CP-treated animals is an indication of induced genetic damage. Doses of EEBO 100, 200, and 400 mg/kg per os (p.o.) neither increased mean number of mono-nuclear cells peripheral mononuclear cells (MNPCEs) nor increased % of PCE while reduced chromosomal aberration in mice indicating nonclastogenicity. All doses of EEBO exhibited significant reduction in aberrations.

Conclusion
In conclusion, EEBO exhibited anti-CRC activity and was found to be dose dependent, that is, maximum dose of 400 mg/kg showed highest activity followed by 200 and 100 mg/kg. The chemopreventive action of this herbal combination might be attributed to its antigenotoxicity or anti-oxidant property.

Materials and Methods
Preliminary Phytochemical Study
For the proposed study, fresh juvenile shoots of BV and fruits of OFI were collected from Kanakapura, Ramanagar District, India. Collected plant parts were authenticated by botanist Dr. Kempegowda, Rural Degree College, Kanakapura. The culm sheaths and hard fibrous portion that was at the basal end of the sheath were removed; however, tender sheaths at the top left were attached. In fruits of cactus, the minute and barbed spines were removed using brush. Both the plant parts were washed with clean water, sliced into small pieces, and air dried under shade. The dried materials were grinded to coarse powder using electrical mixer and stored in air tight container for further analysis. The extraction was performed by Soxhlet hot extraction using ethanol as solvent at 40°C for 15 hours. Extracts were filtered, concentrated, and yield values were calculated with reference to air dried materials. The color and consistency of extracts were also noted and then subjected to various phytochemical screening as described by Kokate et al.

Experimental Animals
Inbred 6 to 8-week-old albino male mice weighing between 25 and 30 g were used in the study and maintained as per committee for the purpose of control and supervision of experimentation on animals (CPCSEA) guidelines. The institutional animal ethical committee (IAEC) approval was obtained prior to the initiation of study.

Acute Oral Toxicity Study
The acute oral toxicity study was performed as per the OECD 423 guideline to establish therapeutic dose of EEBO. The EEBO 2,000 mg/kg was administered to animals in a single dose by oral gavage and observed continuously for 30 minutes, then frequently for 4 hours during first day, and clinical examination was made at least once each day for 14 days. All observations were systematically recorded for each animal.

Anticancer Activity
Mice were divided into six groups as follows, and each group contained 10 animals.

- Group 1: Animals received vehicle
- Group 2: Animals received AOM/DSS
- Group 3: Animals received AOM/DSS with fluorouracil 29 mg/kg
- Group 4: Animals received AOM/DSS with EEBO 100 mg/kg
- Group 5: Animals received AOM/DSS with EEBO 200 mg/kg
- Group 6: Animals received AOM/DSS with EEBO 400 mg/kg

Animals were injected with AOM intraperitoneally (i.p.) 10 mg/kg b. wt on first day of experiment. EEBO treatment was started from the third day and continued up to 70 days. One week later, 2.5% of DSS was supplied in place of drinking water for 7 days and the DSS solution was replaced with regular water for next 2 weeks (DSS cycle-1). After completing DSS cycle-1, the drinking water was replaced with 2.5% of DSS for 7 days again, with a recovery period of 2 weeks with drinking water (DSS cycle-2); the DSS cycle was repeated again to complete the third round. At the end of the study, animals were euthanized, and colorectal region was removed and flushed with phosphate buffer. Colon was opened longitudinally to visualize the tumor feature and it was preserved in 10% formalin solution.

The following parameters were assessed to determine anticancer activity.

Body Weight Changes
B. wt change is an important biomarker of CRC. The weight of animals was checked and noted once in every week for 10 weeks.

Macroanatomy of Colorectal Region
Determination of tumor size and number indicates cancer stage. Features of neoplasia, inflammation, and crypt appearance in colon were observed.

Enzymatic Antioxidant Level in Colon
Antioxidants like MDA, GSH, SOD, and catalase level were measured according to Rai et al.

Microanatomy of Colorectal Region
After treatment period, histopathology of colorectal region was performed to analyze the morphological and microscopic changes. Hematoxylin (H) and Eosin (E) stain were used to assess crypt and tumor pathology.

Antigenotoxic Effect of EEBO
In vivo micronucleus assay and chromosomal aberration test are recognized as genotoxic tests. Antigenotoxic agent
inhibits mutagenicity, which caused increase in the number of PCE and mono-nuclear cells (MNC) compared with control.

**Grouping**

Group 1: Phosphate buffered solution on three consecutive days
Group 2: CP 50 mg/kg on second day i.p.
Group 3: EEBO 100 mg/kg p.o. on three consecutive days + CP on second day
Group 4: EEBO 200 mg/kg p.o. on three consecutive days + CP on second day
Group 5: EEBO 400 mg/kg p.o. on three consecutive days + CP on second day

Chromosomal aberration assay and micronucleus assay were carried out according to OECD guidelines 475 and 474, respectively.13,14

**Statistical Analysis**

The data were statistically analyzed using one-way analysis of variance: Dunnett test. The comparison was made between control and treatment groups. Statistical difference of variance: Dunnett test. The comparison was made between control and treatment groups. Statistical difference was considered significant at \( p < 0.05 \). All values were expressed in terms of mean \( \pm \) standard error of the mean.

**Conflict of Interest Statement**

Authors do not have any conflict of interest.

**Acknowledgments**

Not applicable.

**Ethics Committee Approval**

All experimental studies were approved by the IAEC of the University (IAEC certificate no: XXI/MSRFPH/M-05/12.09.2018).

**References**