Chemoprotective Effect of Boeravinone B against DMBA/Croton Oil Induced Skin Cancer via Reduction of Inflammation

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Abstract: Inflammatory reactions and oxidative stress play a major role in cancer expansion. Boeravinone B (BB) had already proofed their anti-inflammatory and antioxidant effects against various animal models of disease. In this experimental research, the chemoprotective effect of BB against skin cancer caused by 7,12-dimethylbenz(a)anthracene (DMBA)/croton oil was investigated and the possible mechanism was explored. Swiss albino mice were used in the current protocol. 100 µg/100 mL acetone, DMBA was used for induction the skin cancer and, after the 2-week repeated dose of croton oil (1% in acetone) give to the mice till end of the protocol. The mice were received the oral dose of BB (1.25, 2.5 and 5 mg/kg, body weight). The body weight and tumor incidence were estimated at regular time interval. At the end of the protocol, the antioxidant, phase I, phase II, pro-inflammatory cytokines and inflammatory mediators were scrutinized. The mRNA expression of pro-inflammatory cytokines and inflammatory mediators were estimated. BB treatment significantly (p < 0.001) reduced tumor incidence, tumor yield, average latency period and tumor burden in a dose-dependent manner. BB treatment considerably (p < 0.001) reduced the levels of lipid peroxidation (LPO) and increased the level of superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) in DMBA/croton-induced skin cancer. BB treatment significantly (p < 0.001) reduced the level of phase I and phase II enzymes. BB treatment considerably reduced the cytokines include tumor necrosis factor-α (TNF-α), interleukin-18 (IL-18), interleukin-1β (IL-1β), interleukin-6 (IL-6) and inflammatory parameters such as transforming growth factor beta 1 (TGF-β1), prostaglandin E2 (PGE₂), nuclear kappa B factor (NF-κB) and cyclooxygenase-2 (COX-2) in DMBA/croton-induced skin cancer mice. BB considerably (p < 0.001) reduced the mRNA expression of pro-inflammatory cytokines and inflammatory mediators. The results of the current investigation suggest that oral administration of boeravinone B significantly reduced skin cancer in mice via reduction of inflammatory reaction.

Key words: skin cancer, 7,12-dimethylbenz(a)anthracene, boeravinone B, inflammation, oxidative stress

1 Introduction

Among the non-communicable diseases with a high global burden, cancer is the dangerous disease. Cancer incidences increase day by day due to change in the lifestyle and environment factors1,2. The body’s largest organ is the skin and is typically targeted in everyday life by carcinogens and chemical mutagens. In whole over the world, many people diagnosed with skin cancer due to the boosted exposure of ultraviolet (UV) rays and environmental pollutants3,4. The studies suggest that the skin cancer affected people double until 2050. Clinical studies indicate that cancer incidences evolve due to genetic injury arising from each internal cause, including hormones, food metab-

Abbreviations: BB; Boeravinone B, DMBA; 7,12-dimethylbenz(a)anthracene, LPO; Lipid peroxidation, SOD; Superoxide dismutase, GSH; Glutathione, GPx; Glutathione peroxidase; CAT; Catalase, TNF-α; Tumor necrosis factor-α, IL-18; Interleukin-18, IL-1β; Interleukin-1β, IL-6; Interleukin-6, TGF-β1; Transforming growth factor beta 1, PGE₂; Prostaglandin E₂, NF-κB; Nuclear kappa B factor, COX-2; Cyclooxygenase-2, UV; Ultraviolet, BCC; Basal cell carcinoma, SCC; Squamous cell carcinoma, DNA; Deoxyribose nucleic acid, GST; Glutathione S-transferase, ROS; Reactive oxygen species, LDH; Lactate dehydrogenase, H₂O₂; Hydrogen peroxide, OH; Hydroxyl radical, O₂; Superoxide anion, CYP1A1; Cytochrome P450 family 1 subfamily A member 1, CYP1B1; Cytochrome P450 family 1 subfamily B member 1, CYP450; Cytochrome P450

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olism and spontaneous body mutations, or external factors, including chemical exposure, alcohol intake, radiation and tobacco consumption\(^1\)\(^{-3}\). In developing countries, cancer is the second leading disease, responsible for about 1 in every 4 deaths. It is well documented that skin carcinogenesis is most widespread in all types of cancers and its prevalence is expected to increase considerably. Skin cancer is two types such as melanoma skin cancer and non-melanoma skin cancer\(^4\)\(^{-5}\). The first type of skin cancer, such as melanoma, is considered the most dangerous type of skin cancer and the second type of cancer is non-melanoma skin cancer. Non-melanoma skin cancer is further divided into 2 types of cancer such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)\(^6\)\(^{-7}\). Reported evidence suggests that UV-B radiation has a major cause of both types of non-melanoma skin cancer. 20% of SCC and 80% of BCC skin cancer cases diagnosed in all nonmelanoma skin cancer (NMSC) cases globally\(^8\)\(^{-10}\).

Therefore, the available treatment for skin cancer is a synthetic drug, but most of the treatment having limitation due to induces the serious side effects, including infertility, hair loss, nephrotoxicity, thromboembolic complications, neurotoxicity, myocardial infarction and nausea. Due to the limitation of synthetic drugs, urgent need the more effective treatment with a less side effect on eradication and prevention of skin cancer\(^1\)\(^{-3}\).

DMBA is a persuasive skin carcinogenesis and its expand the incidence of skin cancer, along with the use of skin cancer promoter such as croton oil. The 3 phases are involved in the expansion of skin cancer are initiation, promotion and progression\(^3\)\(^{-4}\). DMBA induced skin cancer involved in various processes such as DMBA metabolite via cytochrome P450 family 1 subfamily A member 1 (CYP1A1) and cytochrome P450 family 1 subfamily B member 1 (CYP1B1) and cytochrome P450 (CYP450) enzyme to formed dihydrodiol-epoxide (carcinogen) or 1,2-epoxide-3,4-diol DMBA, which further cause the deoxyribose nucleic acid (DNA) adducts. The ultimate carcinogen that mediates carcinogenesis leads to mutations required for tumor growth by inducing reactive oxygen species (ROS) overproduction, oxidative DNA damage, and chronic inflammation\(^4\)\(^{-6}\).

Chemoprevention is the mechanism of halting, reversing and avoiding the occurrence of various cancer forms by natural drugs or synthetic drugs\(^8\)\(^{-9}\). Various develop and developing countries use plant-based medicine as the first line medicine to treat the cancer. Plant based drug having low toxicity, cost effectiveness, antioxidant, anti-inflammatory properties and high safety against different types of cancer\(^4\)\(^{-4}\). Till date, various drugs obtained such as fruits, dietary supplement, vegetables, fruits, minerals and phytochemicals obtained from natural sources have been scrutinized for treating cancers. Comprehensive research has documented several natural dietary and botanical compounds with anti-cancer properties in recent decades.

Boeravonone B is major rotenoid of B. diffusa, has been reported to show the anticancer potential against various cancers. BB exhibited the therapeutic effects for treating the spondylosis, rheumatoid arthritis, acute myoskeletal disorders, osteoarthritis, atherosclerosis, psoriasis, systemic lupus erythematosus in mammals\(^10\)\(^{-11}\). BB was also used in the treatment of pain in the various organ of mammals. The immunomodulation properties of BB are being studied extensively in order to avoid the negative effects on autoimmune diseases. BB already confirm their antioxidant and anti-inflammatory effect against various diseases, but its not scrutinized against the skin cancer\(^10\)\(^{-11}\). In this experimental study, we try to explore the chemoprotective effect of BB against the DMBA/croton oil induced skin cancer and explore the possible mechanism.

2 Material and Methods

2.1 Chemical

DMBA, BB and croton oil were purchased from Sigma Aldrich Co. (St Louis, USA).

2.2 Experimental animal

Swiss albino mice (24 ± 5 g; 7–8 old week) were selected for the current experimental study. For the current experimental study, the mice were kept under standard laboratory conditions (25 ± 2°C; 65 ± 5% relative humidity; 12/12 hr light cycle). The mice were kept in the polypropylene cages and received the standard pellet diet. The whole study was conducted in accordance with the university guidelines.

2.3 Toxicant preparation

The mice resting phase of growth selected for the current experimental study. First, removed the dorsal hair from the caudal and cervical portion. After that, the mice were received the 100 μg/100 μL concentration of DMBA was used for inducing skin cancer. Croton oil was used as a promotor of cancer. Croton oil used as the promotor for skin cancer. Croton oil was dissolved in acetone for the preparation of 1% solution\(^1\)\(^{-4}\).

2.4 Experimental design

2.4.1 Experimental protocol

The mice were divided into following groups and shown in Table 1. Each group contains the 10 mice.

The mice were received treatment orally for the 16 weeks. The body weight and food intake of all mice were estimated at regular time interval. The mice were observed weekly for the identify the incidence of tumors on the dorsal surface and mice having tumor incidence more than 2 mm was recorded.
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2.4.2 Tumor assessment
At the end of the experimental protocol, the mice were macroscopically scrutinized for estimating skin cancer. At the end of the study, the skin tumor was successfully removed and scrutinized for estimating tumor incidence as given formula

\[
\text{Tumor incidence} (\%) = \frac{\text{Number of mice showing carcinogenic response}}{\text{Total number of animal}} \times 100
\]

Mean tumor volume = \( \frac{4}{3} \pi r^3 \)

Mean tumor burden = \( \frac{\text{Mean tumor volume} \times \text{Mean number of tumors}}{\text{Total number of lesions}} \)

2.4.3 Sample preparation
For estimating biochemical parameters, the different biochemical parameters were estimated during the pre, post and promotion stages of skin carcinogenesis, the mice were sacrificed at regular time duration (2, 10 and 22 weeks). For the preparation of homogenate, skin tumor homogenate was prepared using the Teflon fitted Potter Elveinhem homogenizer in 0.2 M tris base (pH-7.4). A small part of tissue homogenate was kept in the 4°C.

2.4.4 Biochemical parameters
Biochemical parameters such as GPx, GSH, SOD, LPO and CAT were estimated following a previously reported method with a minor correction\(^5\). Biochemical

2.4.5 Phase I and Phase II enzymes
Phase I enzymes such as cytochrome b5 and cytochrome P450; phase II enzymes such as glutathione S-transferase (GST) were estimated using the previously reported method with minor modification\(^6,7,12\).

2.4.6 Pro-inflammatory cytokines and inflammatory mediators
Pro-inflammatory cytokines viz., IL-1β, IL-18, IL-6 and TNF-α were estimated using the ELISA kits following the manufacture protocol (Abcam) following the manufacture protocol. For isolating total RNA, TRIndol\(^\circ\) Reagent was used. A PrimeScript RT reagent kit was used for conducting the reverse transcription PCR following the manufacture instruction (Takara Biotechnology Co, Ltd. Dalian, China) and further used the subsequent reaction for cDNA. For conducting qPCR, the SYBR Premix Ex Taq II. Table 2 showed the primer sequences used in the current experimental protocol. The primers were synthesized as follows. GAPDH used as the internal standard.

2.4.7 Real time reverse transcription (qPCR)

2.5 Statistical analysis
The result of the current experimental study presented as mean ± standard error of the mean (SEM). GraphPad Prism was used for statistical analysis (Version 7.0, USA).

### Table 1: The drug treatment.

| S. No | Group | Name | Dose       |
|-------|-------|------|------------|
| 1     | I     | Vehicle Control | Saline     |
| 2     | II    | DMBA control (100 μg/100 μL animal) + Croton oil (1%) | Saline |
| 3     | III   | DMBA control (100 μg/100 μL animal) + Croton oil (1%) + BB | 2.5 mg/kg |
| 4     | IV    | DMBA control (100 μg/100 μL animal) + Croton oil (1%) + BB | 5 mg/kg |
| 5     | V     | DMBA control (100 μg/100 μL animal) + Croton oil (1%) + BB | 10 mg/kg |

### Table 2: List of the primers.

| S. No | Primers          | Sequences                        |
|-------|------------------|----------------------------------|
|       |                  | Forward                          | Reverse                          |
| 1     | TNF-α            | 5'-CGAAAGGAGGAGTGAAGTGC-3'       | 5'-AAACATACAGGCGCTAGC-3'         |
| 2     | IL-1β            | 5'-ACATAG AGAGAGGACAGAC-3'       | 5'-CAGCGTAGATTACTAGTTCG-3'       |
| 3     | IL-6             | 5'-GAGAGACGAGTGGGCAC-3'          | 5'-CTCAAGTGAGAAGGCAACGCTAG-3'    |
| 4     | IL-18            | 5'-CTGATGAGGCTGCAAGAAG-3'        | 5'-TTCCTCAACGCGTAAAGGAC-3'       |
| 5     | TGF-β1           | 5'-TCGTTGAGACTCTGAAGAACC-3'      | 5'-TGGCTGACTTACAAACACGCTA-3'     |
| 6     | NF-κB            | 5'-TGGACAGCAAATCCGCCCTG-3'       | 5'-TGGTTGAAATGAAGTCGCATCCT-3     |
| 7     | COX-2            | 5'-CCGGGTACAATCGACACTAT-3'       | 5'-GGCGCTACAGCATAAGCA-3'         |
| 8     | GAPDH            | 5'-AACGGTGTCACAGACAGGCTCA-3'     | 5'-TCCACCTGACAGACACAAACA-3'      |

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3 Results

3.1 Effect of BB on tumors and morphological alteration

The Vehicle control group did not demonstrate any tumor on the skin of the mice. DMBA/croton oil-induced mice exhibited the number of tumors on the skin (55.43 ± 2.34) and tumor weight 1.35 ± 0.45. BB treatment (1.25 and 2.5 mg/kg) demonstrated the reduced number of tumors 43.56 ± 2.12, 30.04 ± 2.35 with a weight of 1.12 ± 0.43 and 0.74 ± 0.12. DMBA/croton-oil-induced skin cancer mice treated with BB (5 mg/kg) exhibited the 10.34 ± 1.03 (number of tumors) with 0.23 ± 0.05 weight of the tumor (Table 3). BB treatment exhibited a dose-dependent reduction in skin cancer.

Table 3 The effect of boeravinone B on tumor variation of DMBA induced skin cancer mice.

| S. No | Group                  | Number of tumor | Tumor Weight (mg) |
|-------|------------------------|-----------------|-------------------|
| 1     | Vehicle Control        | –               | –                 |
| 2     | DMBA Control           | 55.43 ± 2.34    | 1.35 ± 0.45       |
| 3     | DMBA + BB (1.25 mg/kg)| 43.56 ± 2.12*   | 1.12 ± 0.43*      |
| 4     | DMBA + BB (2.5 mg/kg) | 30.04 ± 2.35**  | 0.74 ± 0.12**     |
| 5     | DMBA + BB (5 mg/kg)   | 10.34 ± 1.03*** | 0.23 ± 0.05***    |

The result was compared with the DMBA/croton-induced skin cancer mice; where *p < 0.05 consider as significant, **p < 0.01 consider as more significant and ***p < 0.001 consider as extreme significant.

3.2 Effect of BB on body weight

Figure 1 exhibited the effect of BB on the body weight of DMBA/croton-oil-induced skin cancer mice. Vehicle control mice showed increased body weight compared with the initial body weight. In the country, DMBA/croton induced mice demonstrated reduced body weight compared to the other group and initial body weight. DMBA/croton-induced skin cancer mice treated with BB (1.25 and 2.5 mg/kg) showed increased body weight compared to initial body weight and DMBA/croton oil-induced control group mice. BB (5 mg/kg) treated mice exhibited increased body weight as the vehicle group mice.

3.3 Effect of BB on biochemical parameters

Figure 2 demonstrated the effect of BB on biochemical parameters of normal and treated mice. DMBA/croton oil-induced skin cancer mice exhibited increased levels of LPO (Fig. 2a) and reduced level of GSH (Fig. 2b), GPx (Fig. 2c) and SOD (Fig. 2d) compared with vehicle and other treated group mice. BB treatment considerably (p < 0.001) boosted the levels of LPO and suppressed the levels of GSH, GPx and SOD.

Table 4 The effect of boeravinone B on morphological alterations of DMBA induced skin cancer mice.

| S. No | Group                  | Tumor Yield | Tumor Burden | Average Latent Period | Tumor Incidence (%) |
|-------|------------------------|-------------|--------------|-----------------------|---------------------|
| 1     | Vehicle Control        | –           | –            | –                     | –                   |
| 2     | DMBA Control           | 6.12 ± 0.23 | 6.59 ± 0.34  | 10.12 ± 1.03          | 100                 |
| 3     | DMBA + BB (1.25 mg/kg)| 4.03 ± 0.20*| 5.46 ± 0.32*| 11.83 ± 1.04*         | 74.12               |
| 4     | DMBA + BB (2.5 mg/kg) | 2.52 ± 0.12***| 4.45 ± 0.27***| 12.84 ± 1.14***      | 57.64               |
| 5     | DMBA + BB (5 mg/kg)   | 1.02 ± 0.04***| 3.12 ± 0.12***| 13.45 ± 1.83***      | 32.98               |

The result was compared with the DMBA/croton-induced skin cancer mice; where *p < 0.05 consider as significant, **p < 0.01 consider as more significant and ***p < 0.001 consider as extreme significant.

Dunnet’s test was used for the statistical analyses. Where *p < 0.05 use as the significant.
Figure 3 showed the reduced levels of CAT (Fig. 3a), vit C (Fig. 3b) and GST (Fig. 3c) in the DMBA/croton-induced skin cancer mice and BB treatment substantially \((p < 0.001)\) dose-dependently increased the level of CAT, GST and vit C.

### 3.4 Effect of BB on phase I and phase II enzymes

Figure 4 showed the level of cytochrome P450, cytochrome B5 and total protein in the experimental mice. DMBA/croton oil-induced skin cancer mice exhibited down-regulation of the level of cytochrome P450 (Fig. 4a), total protein (Fig. 4b) and cytochrome B5 (Fig. 4c) compared with the vehicle and BB treated group mice. Dose dependently treatment of BB considerably \((p < 0.001)\) up-regulated the level of cytochrome P450, cytochrome B5 and total protein.

### 3.5 Effect of cytokines

During the cancer, the inflammatory reaction boosted due to the expansion of disease. A similar result was observed in the current experimental investigation. DMBA/croton oil-induced skin cancer mice exhibited increased level of cytokine level includes TNF-\(\alpha\) (Fig. 5a), IL-1\(\beta\) (Fig. 5b), IL-6 (Fig. 5c) and IL-18 (Fig. 5d) compared to other group mice. BB treatment significantly reduced the level of cytokines and suggesting the anti-inflammatory effects.

Fig. 1 showed the effect of boeravinone B on the body weight against the DMBA/croton oil-induced skin cancer mice. The result was compared with the DMBA/croton-induced skin cancer mice; where *\(p < 0.05\) consider as significant, **\(p < 0.01\) consider as more significant and ***\(p < 0.001\) consider as extreme significant.

Fig. 2 showed the effect of boeravinone B on the antioxidant level against the DMBA/croton oil-induced skin cancer mice. a: LPO, b: GSH, c: GPx and d: SOD. The result was compared with the DMBA/croton-induced skin cancer mice; where *\(p < 0.05\) consider as significant, **\(p < 0.01\) consider as more significant and ***\(p < 0.001\) consider as extreme significant.
On the estimation of mRNA expression of cytokines, mRNA cytokines such as TNF-α (Fig. 6a), IL-1β (Fig. 6b), IL-6 (Fig. 6c) and IL-18 (Fig. 6d) boosted in the DMBA/croton oil-induced skin cancer mice and BB treatment considerably ($p < 0.001$) suppressed the cytokines mRNA levels.

### 3.6 Effect of inflammatory mediators

Inflammatory mediators such as COX-2, PGE$_2$, TGF-β1 and NF-κB, boosted during cancer. In our experimental research, a similar momentum was observed. DMBA/croton-induced skin cancer mice showed the boosted level of COX-2 (Fig. 7a), PGE$_2$, TGF-β1 (Fig. 7b) and NF-κB (Fig. 7c) and BB treatment significantly ($p < 0.001$) reduced the...
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Fig. 5 showed the effect of boeravinone B on the pro-inflammatory cytokines against the DMBA/croton oil-induced skin cancer mice. a: TNF-α, b: IL-1β, c: IL-6 and d: IL-18. The result was compared with the DMBA/croton-induced skin cancer mice; where *p<0.05 consider as significant, *p<0.01 consider as more significant and *p<0.001 consider as extreme significant.

Fig. 6 showed the effect of boeravinone B on the mRNA expression of pro-inflammatory cytokines against the DMBA/croton oil-induced skin cancer mice. a: TNF-α, b: IL-1β, c: IL-6 and d: IL-18. The result was compared with the DMBA/croton-induced skin cancer mice; where *p<0.05 consider as significant, *p<0.01 consider as more significant and *p<0.001 consider as extreme significant.

level of inflammatory mediatory dose dependently manner.

DMBA/croton-induced skin cancer mice showed increased expression of inflammatory parameters and BB treatment significantly (p<0.001) down regulated the expression of inflammatory parameters expression (Fig. 8).

4 Discussion

Scientists and clinicians faced the major challenge for treating cancer disease. Recently, radiotherapy and chemotherapy are the first choice for treating cancer, but all therapy having the limitations and various side effects[1, 2]. However, the researcher focuses on their research to scrutinize the new protective therapy to suppress the global
cancer burden is gaining momentum. Plant based drugs have showed the chemotherapeutic effects due to presence of anti-inflammatory and antioxidant nature\textsuperscript{13, 14}.

The chemical induced the skin carcinogenesis method is mostly used for estimating the biochemical and genetic alteration. In the current experimental procedure, DMBA was used for the initiate carcinogenesis due to systemic absorption from the skin\textsuperscript{1, 4}. A previous report suggests that polycyclic aminohydrocarbons (PAH) systematic fastest absorb from the skin. In the process of skin cancer, DMBA metabolic activates in the hepatic tissue via phase I detoxification enzymes (cytochrome P450), which further convert into 3, 4-diol-1, 2-epoxide that covalently binds to the DNA and form DNA adducts and finally lead to the mutation\textsuperscript{12, 13}. Croton oil (12-O-tetradecanoylphorbol-13-acetate) is commonly used for promoting skin cancer via

Fig. 7 showed the effect of boeravinone B on the inflammatory mediators against the DMBA/croton oil-induced skin cancer mice. a: COX-2, b: PGE\(_2\), c: TGF-\(\beta\)1 and d: NF-\(\kappa\)B. The result was compared with the DMBA/croton-induced skin cancer mice; where *\(p<0.05\) consider as significant, *\(p<0.01\) consider as more significant and *\(p<0.001\) consider as extreme significant.

Fig. 8 showed the effect of boeravinone B on the mRNA expression of inflammatory mediators against the DMBA/croton oil-induced skin cancer mice. a: COX-2, b: TGF-\(\beta\)1 and c: NF-\(\kappa\)B. The result was compared with the DMBA/croton-induced skin cancer mice; where *\(p<0.05\) consider as significant, *\(p<0.01\) consider as more significant and *\(p<0.001\) consider as extreme significant.

\[\text{Fig. 7}\] showed the effect of boeravinone B on the inflammatory mediators against the DMBA/croton oil-induced skin cancer mice. a: COX-2, b: PGE\(_2\), c: TGF-\(\beta\)1 and d: NF-\(\kappa\)B. The result was compared with the DMBA/croton-induced skin cancer mice; where *\(p<0.05\) consider as significant, *\(p<0.01\) consider as more significant and *\(p<0.001\) consider as extreme significant.

\[\text{Fig. 8}\] showed the effect of boeravinone B on the mRNA expression of inflammatory mediators against the DMBA/croton oil-induced skin cancer mice. a: COX-2, b: TGF-\(\beta\)1 and c: NF-\(\kappa\)B. The result was compared with the DMBA/croton-induced skin cancer mice; where *\(p<0.05\) consider as significant, *\(p<0.01\) consider as more significant and *\(p<0.001\) consider as extreme significant.
increase the generation of hydroperoxides and reactive oxygen species (ROS) in keratinocytes.

Previous investigation showed that serum lactate dehydrogenase (LDH) activity is directly proportional to the skin cancer expansion and its aggressiveness. Furthermore, increased level of LDH occurred due to boosted glucose utilization by cancer cells. In this study, we have observed the boosted level of LDH in DMBA control group mice compared to normal mice. LDH is considered the prognostic and diagnostic marker, due to the prediction of patient survival and advanced melanoma. The level of LDH (cytosolic enzymes) boosted in the serum during skin cancer and similar results were observed in the DMBA induced skin cancer in mice. The level of LDH was boosted by damaging the cellular and induction of skin ulceration.

In this experimental study, BB exhibited reduced level of LDH due to its dual natural such as a pro-oxidant and antioxidant effects. According to Kumar et al., potent antioxidant pyto-constituents are capable of exploiting the difference between the healthy and cancer cells and finally they kill the cancer cells and helping the healthy cells. These types of the substance have the capability to act like pro-oxidant or antioxidants.

It is well documented that ROS played a significant role in the expansion of various chronic diseases such as cancer. It creates an imbalance between cellular proliferation and apoptosis. Literature suggests that when apoptosis mechanisms are blocked in cells, it turns into the uninhibited cellular proliferation and finally induces tumor expansion. The cellular redox potential changes toward oxidative stress when the antioxidant regulation mechanisms are depleted or overwhelmed, raising the potential damage to proteins, lipids and nucleic acids. During the cancer condition, boosted the ROS level due to the increase the production of hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (OH) and superoxide anion (O$_2^-$). During the DMBA induced skin cancer, ROS play an imperative role in the expansion of different signaling cascades and lipid peroxidation. LPO levels reflect the oxidative stress degree in carcinogenesis and boosted the different stages of cancer expansion. DMBA induced skin cancer mice exhibited the boosted the level of MDA and reduced the level of non-enzymatic antioxidants, including GSH and enzymatic antioxidant such as GPx, SOD and CAT compared to normal mice.

Reducing the concentration of endogenous reduced glutathione exhibits enhanced consumption of antioxidants to neutralize the huge amount of ROS. SOD and CAT scavenge free radicals and suppress ROS formation. GST (phase II enzyme) plays a significant role in the initiation the detoxification process by neutralizing the electrophiles active site through binding with the thiol (-SH group) of glutathione. In the current experimental study, we have found that the reduced level of GST and GSH in the DMBA induced skin cancer mice and BB treatment significantly boosted the level of GST and GSH and suggested the anticancer effect by increasing the drug detoxification system.

The boosted level of H$_2$O$_2$ commonly observed after DMBA treatment, which exhibits a close relationship between ROS generation includes superoxide anion and tumor expansion. The continuous generation of H$_2$O$_2$ enhanced the level of endogenous antioxidants showed the BB having the ability to inhibit oxidative stress, which is closely related to the expansion of different stages of cancer and inflammation. During the skin cancer, DMBA induces inflammation, which is triggered by the activation of various pro-inflammatory cytokines and leukocytes infiltration. The estimation of macrophages and neutrophils infiltration commonly used as an inflammatory biomarker.

During the skin cancer, boosted the activity of xanthine oxidase (XO) due to the synthesis of xanthine dehydrogenase and conversion xanthine dehydrogenase to xanthine oxidase. Its also correlated with the hyperplasia degree. In the current experimental study, we have observed the boosted level of xanthine oxidase in the DMBA induced skin cancer and BB treatment considerably reduced the level of xanthine oxidase. An inflammatory reaction plays an important role in the expansion and promotion of cancer. Previous research suggests that the boosted level of inflammatory mediators and pro-inflammatory cytokines plays a significant role in skin cancer promotion. During the skin cancer, DMBA boosted the level of ROS and NF-kB activation, which further leading to up-regulation in uncontrolled transcriptional and boosted the level of cytokines, COX-2 and PGE$_2$. DMBA induced skin cancer mice exhibited increased levels of pro-inflammatory and inflammatory mediators and BB treatment significantly reduced the level of pro-inflammatory and inflammatory mediators. BB treatment considerably altered inflammatory responses (infiltration of neutrophil, cutaneous, hyperplasia and edema) and COX-2, PGE$_2$ and NF-κB. IL-6 (pro-inflammatory cytokines) and NO play a role like messenger in cell-to-cell signaling and play a significant role in the expansion of skin cancer.

5 Conclusion

The result obtained from the current experimental protocol suggest that the boeravinone B reduced the skin cancer incidence in the DMBA/croton-induced skin cancer group mice. BB considerably reduced the tumor weight; tumor yield along with the tumor incidence. BB showed the chemoprotective effect against the DMBA/croton-induced skin cancer mice might be due to

- Improved the level of the endogenous antioxidant enzymes
Reduced the level of phase I and phase II enzymes
Reduced the level of proinflammatory cytokines and inflammatory mediators
Reduced the mRNA expression of proinflammatory cytokines and inflammatory mediators

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