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ORIGINAL ARTICLE

Anticarcinogenic Effect of Brucine on DMBA-Induced Skin Cancer via Regulation of PI3K/AKT Signaling Pathway

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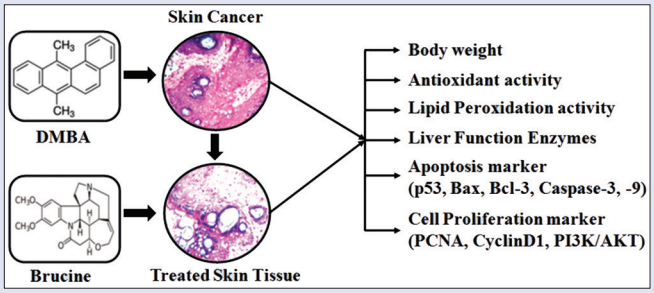
ABSTRACT

Background: Brucine is an alkaloid derived from the natural plant seeds of *Strychnos nux-vomica,* also known as a medicinal herb, and broadly employed in Chinese medicine for liver cancer. **Objectives:** The main intention of these hypothesis anticancer effects of brucine on 7,12-dimethylbenz(a)anthracene (DMBA)-induced skin cancer in mouse model through phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) regulating signaling pathway via the suppressive effect on cell proliferation and apoptotic pathways in mouse model. **Materials and Methods:** Brucine action on DMBA-induced mouse skin body weight, tumor volume, histology, biochemical, molecular marker analysis using spectrophotometric, Western blotting, and real-time polymerase chain reaction analysis. **Results:** Brucine stifled the lipid peroxidation (TBARS), suggestively augmented the levels of antioxidants (superoxide dismutase, catalase, glutathione peroxidase, and glutathione), and brought back the status of xenobiotic enzymes (Cyt-p450, Cyt-b5, and glutathione S-transferases). In brucine administered since skin tissues presented the cell proliferative protein marker expression of PI3K, and AKT were downregulated compared to the DMBA-applied skin tumor tissues protein. Further, brucine has downregulated the proliferating cell nuclear antigen, cyclin-D1, and p53 expressions. In the apoptotic expression, markers such as Bcl-2, Bax, caspase-3, and caspase-9 were upregulated compared to the DMBA-induced skin cancer. From these data, we diseased and brucine potential to suppress the proliferative cell markers induces apoptotic expressions. **Conclusion:** The current search settled that administering brucine chemopreventive and chemotherapeutic effect means for the cancer management in clinical tactic.

Key words: Apoptosis, brucine, cell proliferation, 7,12-dimethylbenz(a)anthracene, skin cancer

SUMMARY

- Brucine stifled the 7,12-dimethylbenz(a)anthracene (DMBA)-induced skin cancer.
- Brucine inhibited the phosphatidylinositol 3-kinase/protein kinase B signaling pathways via the downregulation of proliferating cell nuclear antigen, cyclin-D1, p53, and augmented expression of Bax, caspase-3, and caspase-9 markers of DMBA-induced skin carcinogenesis.



Abbreviations used: DMBA: 7,12-dimethylbenz(a)anthracene; PI3K/AKT: Phosphatidylinositol 3-kinase/protein kinase B; WHO: World Health Organization; TBARS: Thiobarbituric acid reactive substances; SOD: Superoxide dismutase; GSH: Glutathione; GPx: Glutathione peroxidase; CAT: Catalase; GR: Glutathione reductase; Cyt-p450: Cytochrome p450; Cyt-b5: Cytochrome b5; GST: Glutathione S-transferases; QRT-PCR: Quantitative real-time polymerase chain reaction; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; AKT: Protein kinase B; PI3K: Phosphoinositide 3-kinase; PCNA: Proliferating cell nuclear antigen; MDA: Malondialdehyde.

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INTRODUCTION

Skin cancer is the most communal cancer worldwide, and fair-skinned peoples are most exaggerated by this type of cancer.[1,2] It accounts for 40% of new cancer cases in India every year and 0.6% of the increase in its occurrence among the Caucasian populations in 50 years. Nowadays, we are uncovered to numerous carcinogens that lead to carcinogenesis. The prevalence of skin cancer rises with the exposure to UV rays exponentially, which surges the concern. These cancers develop quickly from benign phase to metastatic phase and grow most largely among other cancers. Skin cancer mostly comes into two categories, i.e., melanoma arises from melanocytes (melanin pigmented cells) and nonmelanoma skin cancer contains squamous and basal cell carcinomas.[3,4] However, most malignant melanoma is treatable when recognized in the early stages; it is tough to cure relapsed, accounting for 80% of all other cancer deaths. Alongside, more than 250% of augmented new melanoma cases have been identified in young adults, teenagers, and infants in the past four eras. Nonmelanoma type of skin cancer has been increased by 77% in the last 20 years. To control these types of skin cancers, existing therapies, counting expensive immunotherapy, radiotherapy, chemotherapy, and targeted therapy, are in effectual and toxic, chiefly in metastasis, because

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of their resistance. Henceforth, substitute therapy that is effective, is nontoxic, and dodges all these adverse effects is wanted for skin cancer treatment.[4]

The polyaromatic hydrocarbon of 7,12-dimethylbenz(a)anthracene (DMBA) recruits cancer progression via its metabolic form of dihydrodiol epoxide, which is covalent to DNA, and stimulates the malformed and fragmentation of DNA during their DMBA metabolism.[5] Minimal reports were printed about the brucine mechanism. Still, Saminathan *et al.*[6] clarified that brucine on DMBA-induced breast cancer can potently increase caspase-3 suppression of caspase-6, which also amplified that the ratio of Bax/Bcl-2 leads to the apoptotic induction ability.

The problems stated earlier could be simply overcome by using natural components derived from plants exposed to be safe and efficient for cancer patients. According to the WHO (World Health Organization), over 80% of peoples depend on traditional medicines as their major health-care essentials in developing countries. Several plant-based derivatives as drugs such as paclitaxel from *Taxus brevifolia*, irinotecan from *Camptotheca acuminata,* and vincristine from *Catharanthus roseus* have been formally approved by the U.S Food and Drug Administration for quite a lot type of cancer treatments. Considering this, the researchers have been rifled for plentiful plant derivatives for the treatment of skin cancer in latest years.[7] Brucine is an alkaloid derived from the natural plant seeds of *Strychnos nux-vomica,* which is also known as a medicinal herb and widely used in Chinese medicine for liver cancer.[8] It has been appealed to get rid of rheumatic pain, increase blood circulation, and use many treatments for gonorrhea, diabetes, bronchitis, and anemia.[9] Latest studies naked the antiproliferative effects of brucine in various cancer cells such as HepG2,[10] K562 and HeLa,[11] and different myeloma cells.[12] It also holds anti-inflammatory, analgesic, anti-snake venom, and antioxidant activities. Although, the reports connected to antitumor effects of brucine are still limited and ruins indefinitely.

The improved knowledge of the physiological pathways of skin cancer could clear the way for the auspicious target findings which leads to skin cancer treatment. Phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway is a noteworthy intracellular signaling pathway resulting in several growth factor receptors. It is a precise pathway that is often activated proliferation and persistence pathways in numerous cancers. It can also control the main cellular functions such as cell growth, proliferation, malignant transformation, and apoptosis.[13] DMBA is an environmental contaminant, procarcinogen, frequently found in cigarette smoke, which often affects xenobiotics through the causes of DNA breakage.[14] Furthermore, it disturbs the nucleic acid and proteins linkage and generates an exaggeration and genomic change mechanisms.

Henceforth, the anticancer effects of brucine have been explored on DMBA-induced mice to treat skin cancer by subjecting the mice toward the histopathological scrutiny, quantitative real-time polymerase chain reaction (RT-PCR), immunoblotting analysis, and biochemical analysis.

MATERIALS AND METHODS

Chemicals

DMBA and brucine were acquired from Sigma-Aldrich, USA. In the immunoblotting process, the used antibodies were obtained from Cell Signaling, USA, and total RNA was prepared by the protocol (Qiagen RNeasy mini kit).

Animals

Male mice (4–6 weeks of age and weighing 30–35 g) were reserved in a room with controlled humidity (55%–65%) and temperature (26°C ± 2°C) with fresh tap water and a standard pellet diet. The mice were imperiled to 12 h light and dark cycles and fingered as per the norms of IAEC (NSMC2019AC-102).

Tumor induction and assessment

Hair depilatory creams were pragmatic on the posterior side of each mouse and left untreated for 2 days. That selected area has not grown the hair on the skin, further using DMBA induced the tumor formation by smearing DMBA topically on skin and then checked out 8 weeks.

Animal experimental design

DMBA-induced mouse, skin carcinogenesis model was used in accordance to Xu *et al.*[13] DMBA 25 μg in 0.1 mL acetone/mouse was applied twice weekly for 8 weeks, while Group II mice were received no other treatment.

The animals were treated twice a week topically and haphazardly separated into four groups consisting of six mice each: Group I – control (100 μl acetone per mice), Group II – DMBA alone (5 μg/mice in 100 μl acetone), Group III – DMBA + brucine (5 μg + 50 mg/kg), and Group IV – brucine alone (50 mg/kg). End of the experiment, the animals' mean body and tumor weight was logged as well as tumor incidence was also calculated.

Histopathology analysis

The vital organs (skin and liver) of mice were taken and endangered to histopathological estimation. The removed tissues were secured in 10% formalin and fixed in paraffin wax. After that, the blocks were sectioned of 4–6 mm thickness and then stained with H and E.[15] Finally, the stained sections were considered under a light microscope.

Biochemical evaluation

End of the experiment, the animals were forfeited by cervical dislocation, and the skin tissues were subjected to biochemical assessment. Skin tissue homogenate was prepared by using 10% lysis buffer with pH 7.4. Total protein was appraised by Lowry *et al.*[16]

The production of thiobarbituric acid reactive substances (TBARS) was established per Ohkawa *et al.*[17] The levels of condensed antioxidant enzymes superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR), cytochrome p450 (Cyt-p450) and cytochrome b5 (Cyt-b5), and glutathione S-transferases (GST) were assessed according to Kakkar *et al.*;[18] Beutler and Kelly, 1963;[19] Rotruck *et al.,* 1975;[20] Carlberg and Mannervik;[21] Sinha;[22] Omura and Sato, 1964;[23] and Habig *et al.,* 1974,[24] respectively.

Quantitative real-time polymerase chain reaction

As per the manufacturer's protocol, total RNA was extracted via Qiagen RNeasy mini kit. In cDNA conversion, a reverse transcription system was employed, and then, the RT-PCR (Applied Biosystems) amplification was made according to the instructions of the manufacturer. The primers were found from Sigma, USA, and given in Table 1. β-actin was used as the housekeeping gene to compute the ΔCt values, and then, the fold change of protein was considered.

Immunoblotting analysis

The proteins were divided using SDS-PAGE (10%) and were moved to a nitrocellulose membrane. The blots were searched overnight with anti-PI3K, AKT, proliferating cell nuclear antigen (PCNA), and cyclin-D1. Then, it was followed by secondary antibody incubation conjugated with horseradish peroxidase. Immunoreactive bands were observed using an enhanced chemiluminescence detection system.

Software J was employed for the densitometric analysis. The blot was normalized against β-actin.

RESULTS

Effect of brucine on tumor and mean body weight

The effect of brucine on the DMBA mouse model the difference of mean body and tumor weight was chronicled [Figure 1 and Table 2]. In Group II, the animal body weights were significantly diminished compared with Group I, whereas tumor weight was augmented, and a 100% tumor occurrence rate was also distinguished. The animal tumor weights knowingly decreased, and body weight was amplified in the brucine-treated group compared to Group II. There is no noteworthy difference in the body weight and no signs of tumor frequency in the brucine-alone treated group compared with the control group.

Level of thiobarbituric acid reactive substances and stress markers

The levels of TBARS, GSH, GPx, CAT, and SOD in experimental mice's skin cancer tissues [Figure 2]. TBARS level was suggestively augmented, and SOD, CAT, GPx, and GSH levels knowingly lessened on Group II (DMBA-exposed mice) compared with the control group. The brucine-treated mice have recovered the antioxidant's prominence to the average level. There are no adverse effects seen in the brucine-alone treated group.

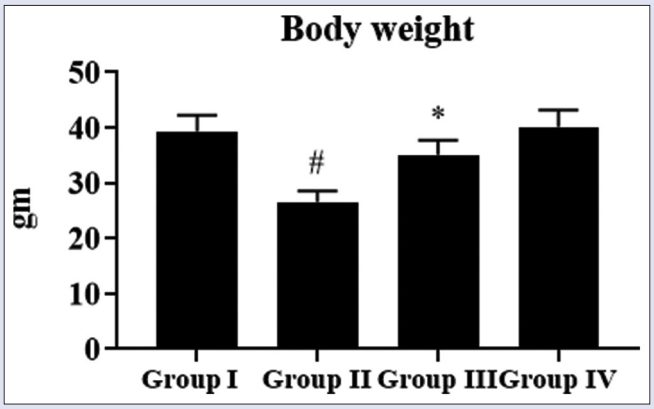

Figure 1: The body weight changes of control and experimental rats. Values are expressed as mean ± standard deviation for six animals in each group. Values not sharing a common superscript letter differ significantly at $^{\#,*}P < 0.05$

Evaluation of detoxification enzymes

The levels of Cyt-b5 and Cyt-p450 enzymes were pointedly augmented compared with the control group. However, GR and GST have been abridged in Group II by comparing them with the control. In addition to this, the brucine-treated mice presented normal detoxification enzymes, and no unique variations were seen in Group IV [Figure 3].

Histopathological evaluation

The total appearance and histopathological fluctuations of the skin and liver in skin cancer-induced experimental mice were analyzed [Figure 4]. The normal epithelial layers of skin tissues were seen in Group I and IV mice (control and brucine alone). Well-transformed skin cancer was obtainable in Group II mice (DMBA alone) with the keratin pearl development distinct dissemination of cancer cells underneath the skin layer. There was a noteworthy normal cellular morphology by hyperplastic and hyperkeratosis papillomatous lesion mild and temperately in Group III mice (DMBA and brucine).

The liver tissue section displayed perceptible inflammation by infiltrating neutrophils and lymphocytes at the inflamed site [Figure 5]. In Group II, the cell damage was formed in the liver tissues section compared to the other three groups. These cell damages were measured by necrotic appearances such as membrane breakdown, cytoplasmic loss, and vagaries in pyknosis nucleus appearance. Furthermore, Group II was exhibited the Bowman's capsule dilated and sinusoidal, glomerular cellularity augmented and microvascular steatosis also found. In contrast, Bowman's capsule and dilation in Group III and no more any deviations were detected brucine treated with DMBA-applied tissues.[25]

Table 1: List of specific gene primers used for real-time polymerase chain reaction

Gene name	Primer sequences
Bax	F 5′-GGGACGAACTGGACAGTAACA-3′ R 5′-CCGCCACAAAGATGGTCAC-3′
Bcl-2	F 5’-GGAGAGTGCTGAAGATTG-3′ R 5′-ACTTCCTCTGTGATGTTGTA-3′
Caspase-3	F 5′-CAACATTTTTCAGAGGGGATCG-3′ R 5′-GCATACTGTTTCAGCATGGCAC-3′
Caspase-9	F 5’-CGAACTAACAGGCAAGCAGC-3’ R 5’-ACCTCACCAAATCCTCCAGAAC-3’
p53	F 5’-GCAGCGCCTCACAACCTCCGTCAT -3’ R 5’-GCAGCGCCTCACAACCTCCGTCAT -3’
β-actin	F 5’-GGTCACCAGGGCTGCTTTTA-3’ R 5’-GGATCTCGCTCCTGGAAGATG-3’

Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2

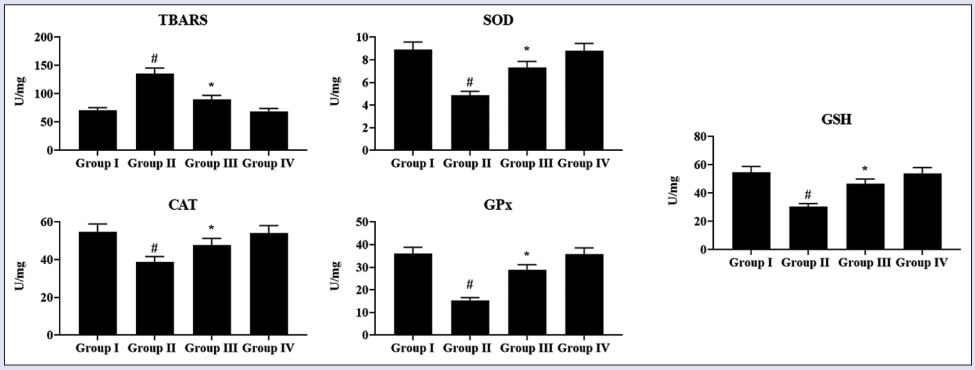

Figure 2: Effect of brucine on levels of thiobarbituric acid reactive substances and enzymatic antioxidants in the skin tissues of the experimental rats. Values are expressed as mean ± standard deviation for six animals in each group. Values not sharing a common superscript letter differ significantly at $^{\#,*}P < 0.05$

Evaluation of p53 and apoptosis-related genes

Figure 6 displays the effect of p53 and apoptosis-related genes markers were examined. The p53 levels have been upregulated by 1.5-fold after the exposure of DMBA. During the brucine and DMBA administration, the mRNA level of p53 was downregulated by 1-fold. Furthermore, apoptosis-related genes such as Bcl-2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2), and caspase-3 and caspase-9 have also been analyzed to gauge Group II (DMBA-exposed mice). Here, except Bcl-2, lingering all other apoptosis-related genes was downregulated. However, the fold change of caspase-9, caspase-3, and Bax was upregulated, respectively, whereas Bcl-2 was downregulated on the occurrence of brucine and DMBA.

Expression of protein kinase B, phosphatidylinositol 3-kinase, cyclin-D1, and proliferating cell nuclear antigen

The expression signaling molecules AKT and phosphoinositide 3-kinase (PI3K) and cell markers PCNA and cyclin-D1 [Figure 7]. It is renowned that the expression of the protein was augmented in Group II (DMBA-treated mice). After the brucine administration with DMBA, the protein expression was reduced when compared with Group II. There were no distinct fluctuations seen in the brucine-alone treated group.

DISCUSSION

Skin cancer is a mutual type of cancer that fallouts in the maximum mortality and morbidity.[26] Cancer occurrence is a stimulating phenomenon for oncology researchers and clinicians. The tardy prognosis, costly drugs, and lower therapeutic effects in radiotherapy and chemotherapy force the peoples with in adequate medical facilities to have an alternative to the products of the pharmacopoeia. However, there is no precise technical indication capable of confirming biological properties for these products. In this study, the anticancer activity of brucine was assessed through the DMBA-induced skin carcinogenesis via PI3K/Akt-regulated apoptotic pathway mechanism. Traditionally, the herbal plants employed to derive brucine have been applied for various cancer treatments. DMBA is a carcinogenic substance that stimulates carcinogenesis by accretion of reactive oxygen species (ROS) and overstressing the normal cell to cancer cells. The contemporary study is first happening by inducing skin cancer in 4–6 weeks of mice using the carcinogenic substance (DMBA) at numerous doses. The mice were established with skin cancer after exposure to a DMBA comparable with the numerous other research reports.[26,27] The DMBA-induced mice display an outstanding loss in body weight due to oxidative stress prompted by epithelial mucosa inflammation and feature the pathological variations for the progression of skin cancer. Consequently, oxidative stress leads to the over ROS stimulation with abridged free radical mechanism has stirred the cancer pathogenesis by inducing lipid peroxidation (LPO), damaging DNA through varying gene expression and numerous biochemical mechanisms.[28-30] The contemporary study proved that treating the skin cancer-induced mice with brucine expressions a momentous amelioration effect by inhibiting ROS generation, inflammatory gene markers and summary inflammatory mediators. The administration of brucine delayed the tumor occurrence further in the promotion phase stated in earlier research articles. Brucine shows antiangiogenic, antioxidant, antihyperlipidemic, and antidiabetic activities. Additionally, brucine also unprotected anticancer activity in numerous chemically induced cancers by avoiding oxidative stress, preneoplastic lesion, and biotransforming

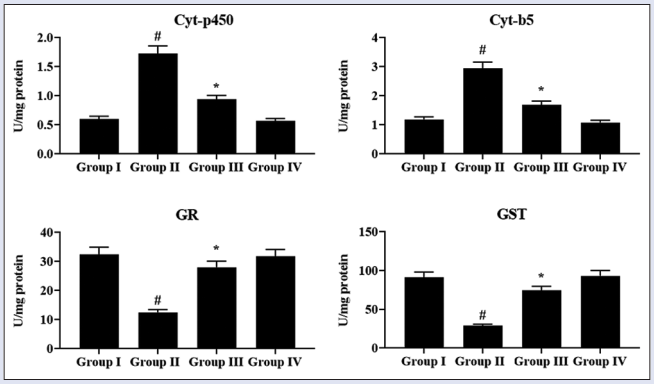

Figure 3: Effect of brucine on levels of non-enzymatic antioxidants in the skin tissues of the experimental rats. Values are expressed as mean ± standard deviation for six animals in each group. Values not sharing a common superscript letter differ significantly at $^{\#,*}P < 0.05$

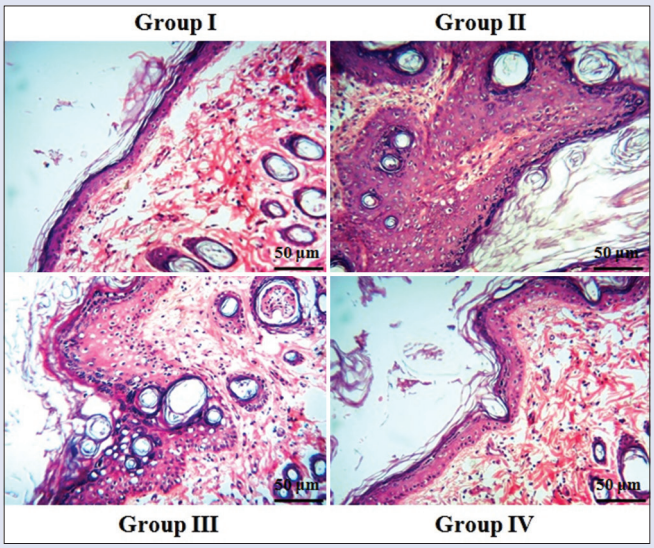

Figure 4: Antitumor effects of brucine on 7,12-dimethylbenz(a) anthracene-induced skin cancer rats. Histopathological profiles of representative skin tissue control and experimental rats (H and E). Scale bar = 50 µm

Table 2: Effect of brucine on tumor incidence, tumor volume, and tumor burden of experimental mice

Groups	Treatments				
	Number of mice	Tumor incidence (%)	Tumor number	Tumor volume	Tumor burden
Group I	6	-	-	-	-
Group II	6	100	16±1.19	520.13±39.48	8322.08±637.32
Group III	6	-	-	-	-
Group IV	6	-	-	-	-

Values are expressed as mean±SD of six mice. Values not sharing a common superscript letter differ significantly at $P<0.05$. SD: Standard deviation

enzymes' abnormal activation. The existing study recognizes that brucine parades a chemopreventive activity in skin cancer-induced mice. The two extreme main stages (Initiation and promotion of cancer oncogenesis) have been induced by LPO through a free radical chain reaction. The malondialdehyde (MDA) has been prepared from LPO when it is level surges. Additionally, the LPO substances are carcinogenic and mutagenic with the control group.[30] The mice bare to the DMBA on the skin tend to induce ROS and LPO, leading to cancer.

Altogether, the oxidative stress of Group I mice illustrations improved TBARS (LPO), and eminences of SOD, GPx, GSH, and SOD amount were concentrated on mice throughout the experiment. Treatment of brucine is for exciting the free radical enzymes in tumor cells. The established enzyme activity in the skin tissues, while downregulating the ROS and LPO formation were the incidence of papilloma, has been condensed in the brucine-treated skin cancer region. The histopathology analysis leaks that DMBA has induced severe injury on the skin surface. The brucine re-established the injured dermal and epidermal skin layers of the mice in Group III. Conversely, the hypodermis region displays main variations and is generated as a chemopreventive agent to inhibit ROS production by *in vivo* model. At last, the administration of brucine slows down the rise and upholds the LPO levels in DMBA-induced skin cancer-bearing mice, and it also planned that it interruption the skin cancer frequency.

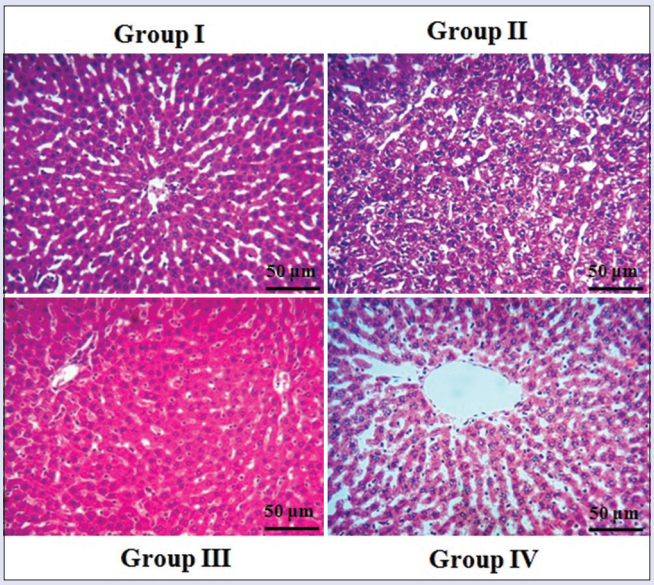

Figure 5: Histopathological changes in the liver tissue control and experimental rats (H and E). Scale bar = 50 µm

Generally, cell metabolism GSH enzyme markers play a key role in preventing and developing free radical production.[31] We have been recommended that the GSH was reduced on Group I mice, but it was proceeded in the brucine-treated mice which indicates the free radical scavenging activity. It was described to be a chemopreventive activity in other research articles.[32] Antioxidants are employed to protect the stress induced by ROS and help the conversion of oxidative reactions in cancer formation. GPx, CAT, and SOD are antioxidant catalysts that are against ROS.[33] The effects of GPx, CAT, SOD, and GSH have been explored on the brucine-treated mice compared with the control.

The phase I metabolism enzymes are accountable for converting chemically induced carcinogens to their lively intermediary, which were employed throughout the experiments.[34] Enhanced level of Cyt-b5 and Cyt-p450 enzymes biomarker for the analyzed the cancers by the carcinogen-treated mice. Brucine has been deposited the modulator effects of Cyt-b5 and p450 status compared to normal skin cancer tissues. The staining revelations that the GST, GSH, and GR proceeded the status in biological tissues. In addition, GST also formed a way to possible the carcinogen xenobiotics by ligands for eradicating the biotransformation enzymes.

Furthermore, initiator caspase-9 and effector caspase-3 activities have been examined in the experimental mice for the study of the apoptotic effects of the brucine. Apoptotic effects of brucine are largely reliant on the effector, i.e., caspase-3 activation condensed COX-2 and increased intracellular Ca^{2++}.[7] Mitochondrial membrane damage and cytochrome c leakage have also been clarified in the results of brucine treatment. Dependably, the strong dosage of brucine and its time-dependent manner of caspase-9 and caspase-3 proteolysis cleavage displays prominent advantages in skin cancer-induced experimental mice. It is also found that brucine has been inhibited by PCNA/cyclin-D1 expression by comparing with the DMBA-treated group. The converse connection between apoptosis and angiogenesis might elucidate the stimulation of antiangiogenic and pro-apoptotic factors. Cyclin-D1 overexpression and age-related cyclin-D1 in dysplastic regions of skin cancer found that cyclin-D1 might have a part as an age-dependent primary event in tumorigenesis of skin cancer.[35]

Many phytocompounds have antioxidant, anti-inflammatory, and immunomodulatory characters to control skin cancers. Harris *et al.*,

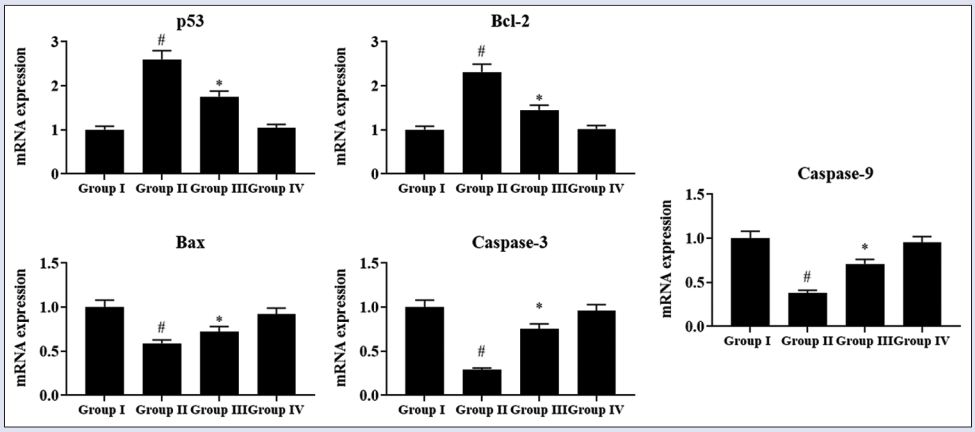

Figure 6: The mRNA protein levels in the experimental rats' skin tissues were analyzed by real-time polymerase chain reaction technique. Values are expressed as mean ± standard deviation for six rats in each group. Values not sharing a common superscript letter differ significantly at $^{\#,*}P < 0.05$

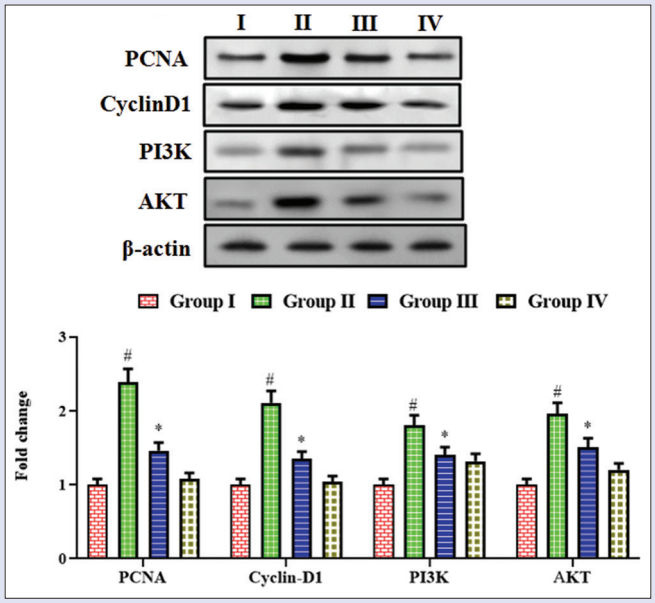

Figure 7: Effect of brucine on levels of signaling molecules and cell markers in the skin tissues of the experimental rats by Western blot. Values are expressed as mean ± standard deviation for six rats in each group. Values not sharing a common superscript letter differ significantly at $^{\#,*}P < 0.05$

2016,[36] have conveyed that quercetin acts anti-melanoma via causes cell viability at abridged doses and stimulates cell death at augmented doses. Olson and Whiteman[37] have stated that it induces the c-Fos gene release by UVB, and suppresses the PI3K, which suppresses cancers. Kaempferol has the superlative compound to arrest the mechanism cell cycle in the G2/M cycle phase.[38] Similarly, in our current study, brucine was control progression of different mechanisms which control the promotion of caspase activity, suppression of effects of tumor development of proteins such as PI3K, AKT Bcl-2, and Bax. In the existing study, brucine administered with DMBA skin tissues was inhibited the activation/regulation of PI3K/AKT expressions, and induced the expression of p53 and Bcl-2 when Bax, cas-3, and cas-9 were leaked the levels.

Caspase activation is completed while initiating the ROS permeabilization of the outer membrane of mitochondria into the cytosol from the inner membrane.[39] Finally, in Group III, it means brucine persuaded apoptotic protein marker production such as Bax, cas-9, cas-3, and lessened the leakage of PCNA, cyclin-D1, PI3K, and AKT. So *et al.* enlightened that even a short-term PI3K/Akt pathway inhibition could withstand the occurrence of skin cancer even after the treatment accomplished a long time.[40] This maintained that short-range exposure of skin cancer to PI3K marker can importance in chemotherapy or chemoprevention. Accordingly, it evades toxicity and its corresponding side effects that often arise from chronic treatment.

CONCLUSION

The contemporary examination suggests that the supplementation of brucine at different steps of cancer has the abilities for chemoprevention through xenobiotic enzymes modulations, antioxidants, anti-LPO, and overwhelm the inflammation. The anti-cancerous, anti-oxidative, and anti-inflammatory effects of brucine ensue a potential therapeutic and chemopreventive targeting for skin cancer. More studies are wanted to find out the precise functionalities of brucine in numerous molecular pathways relating to the potential of anticancer activities.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Linares MA, Zakaria A, Nizran P. Skin cancer. Prim Care 2015;42:645-59.
2. Brunssen A, Waldmann A, Eisemann N, Katalinic A. Impact of skin cancer screening and secondary prevention campaigns on skin cancer incidence and mortality: A systematic review. J Am Acad Dermatol 2017;76:129-39.e10.
3. Linos E, Swetter SM, Cockburn MG, Colditz GA, Clarke CA. Increasing burden of melanoma in the United States. J Invest Dermatol 2009;129:1666-74.
4. Xie R, Chung JY, Ylaya K, Williams RL, Guerrero N, Nakatsuka N, *et al.* Factors influencing the degradation of archival formalin-fixed paraffin-embedded tissue sections. J Histochem Cytochem 2011;59:356-65.
5. Melendez-Colon VJ, Luch A, Seidel A, Baird WM. Cancer initiation by polycyclic aromatic hydrocarbons results from formation of stable DNA adducts rather than apurinic sites. Carcinogenesis 1999;20:1885-91.
6. Saminathan U, Pugalendhi P, Subramaniyan S, Jayaganesh R. Biochemical studies evaluating the chemopreventive potential of brucine in chemically induced mammary carcinogenesis of rats. Toxicol Mech Methods 2019;29:8-17.
7. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2014;4:177.
8. Bensky D, Gamble A. Chinese Herbal Medicine. Seattle: Eastland Press; 1986. p. 646.
9. Bisset NG, Phillipson JD. The African species of *Strychnos*. II. The alkaloids. Lloydia 1971;34:1-60.
10. Deng XK, Yin W, Li WD, Yin FZ, Lu XY, Zhang XC, *et al.* The anti-tumor effects of alkaloids from the seeds of *Strychnos nux-vomica* on HepG2 cells and its possible mechanism. J Ethnopharmacol 2006;106:179-86.
11. Cai BC, Wang TS, Kurokawa M, Shiraki K, Hattori M. Cytotoxicities of alkaloids from processed and unprocessed seeds of *Strychnos nux-vomica*. Zhongguo Yao Li Xue Bao 1998;19:425-8.
12. Rao PS, Ramanadham M, Prasad MN. Anti-proliferative and cytotoxic effects of *Strychnos nux-vomica* root extract on human multiple myeloma cell line-RPMI 8226. Food Chem Toxicol 2009;47:283-8.
13. Xu C, Huang MT, Shen G, Yuan X, Lin W, Khor TO, *et al.* Inhibition of 7,12-dimethylbenz (a) anthracene-induced skin tumorigenesis in C57BL/6 mice by sulforaphane is mediated by nuclear factor E2-related factor 2. Cancer Res 2006;66:8293-6.
14. Mukherjee S, Koner BC, Ray S, Ray A. Environmental contaminants in pathogenesis of breast cancer. Indian J Exp Biol 2006;44:597-617.
15. Md Roduan MR, Abd Hamid R, Mohtarrudin N. Modulation of cancer signalling pathway (s) in two -stage mouse skin tumorigenesis by annonacin. BMC Complement Altern Med 2019;19:238.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
18. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;21:130-2.
19. Beutler E, Kelly BM. The effect of sodium nitrite on red cell GSH. Experientia 1963;19:96-7.
20. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973;179:588-90.
21. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol 1985;113:484-90.
22. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-94.
23. Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 1964;239:2370-8.
24. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130-9.

25. Kou P, Marraiki N, Elgorban A, Du Y. Fucoxanthin modulates the development of 7, 12-dimethyl benz (a) anthracene-induced skin carcinogenesis in swiss albino mice *in vivo*. Pharmacogn Mag 2020;16:681-8.
26. Singh M, Suman S, Shukla Y. New enlightenment of skin cancer chemoprevention through phytochemicals: *In vitro* and *in vivo* studies and the underlying mechanisms. Biomed Res Int 2014;2014:243452.
27. Das RK, Bhattacharya S. Inhibition of DMBA-croton oil two-stage mouse skin carcinogenesis by diphenylmethyl selenocyanate through modulation of cutaneous oxidative stress and inhibition of nitric oxide production. Asian Pac J Cancer Prev 2004;5:151-8.
28. Gopalakrishnan T, Ganapathy S, Veeran V, Namasivayam N. Preventive effect of D-carvone during DMBA induced mouse skin tumorigenesis by modulating xenobiotic metabolism and induction of apoptotic events. Biomed Pharmacother 2019;111:178-87.
29. Liou GY, Storz P. Reactive oxygen species in cancer. Free Radic Res 2010;44:479-96.
30. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014;2014:360438.
31. Sati J, Mohanty BP, Garg ML, Koul A. Pro-oxidant role of silibinin in DMBA/TPA induced skin cancer: 1H NMR metabolomic and biochemical study. PLoS One 2016;11:e0158955.
32. Seshadri VD. Brucine promotes apoptosis in cervical cancer cells (ME-180) via suppression of inflammation and cell proliferation by regulating PI3K/AKT/mTOR signaling pathway. Environ Toxicol 2021;36:1841-7.
33. Ali H, Dixit S, Ali D, Alqahtani SM, Alkahtani S, Alarifi S. Isolation and evaluation of anticancer efficacy of stigmasterol in a mouse model of DMBA-induced skin carcinoma. Drug Des Devel Ther 2015;9:2793-800.
34. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010;4:118-26.
35. Bernatoniene J, Kopustinskiene DM. The role of catechins in cellular responses to oxidative stress. Molecules 2018;23:965.
36. Harris Z, Donovan MG, Branco GM, Limesand KH, Burd R. Quercetin as an emerging anti-melanoma agent: A four-focus area therapeutic development strategy. Front Nutr 2016;3:48.
37. Olsen CM, Whiteman DC. Risk stratification for melanoma. Oncotarget 2019;10:1868-9.
38. Lee KM, Lee KW, Jung SK, Lee EJ, Heo YS, Bode AM, *et al.* Kaempferol inhibits UVB-induced COX-2 expression by suppressing Src kinase activity. Biochem Pharmacol 2010;80:2042-9.
39. Wei Q, Matanoski GM, Farmer ER, Hedayati MA, Grossman L. DNA repair and aging in basal cell carcinoma: A molecular epidemiology study. Proc Natl Acad Sci U S A 1993;90:1614-8.
40. So PL, Wang GY, Wang K, Chuang M, Chiueh VC, Kenny PA, *et al.* PI3K-AKT signaling is a downstream effector of retinoid prevention of murine basal cell carcinogenesis. Cancer Prev Res (Phila) 2014;7:407-17.