

## Research Article

# Antiskin Cancer and Antioxidant Activities of Formulated Agar from Brown Seaweed *Laminaria digitata* (Hudson) in Dimethyl Benzanthracene-Induced Swiss Albino Mice

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This study explores the antiskin cancer effect of formulated agar (FA) from *Laminaria digitata* on dimethyl benzanthracene-(DMBA-) induced skin cancer mice. The agar was extracted and formulated (emulgel), and FA was biochemically characterized. The *in vitro* cytotoxicity of FA was tested using NTT 3T3 mice fibroblast cells. The mice were divided into 5 groups: group 1 served as control mice, group 2 mice were considered as DMBA-induced cancer control, group 3 mice were FA pretreated (low dose) + DMBA-induced mice, group 4 mice were FA pretreated (high dose) + DMBA-induced mice, and group 5 were positive control + DMBA-induced mice. The behaviour and biochemical markers of cancer were significantly decreased in group 2 (DMBA-induced) mice, which were brought to near normalcy by FA pretreated mice (groups 3 and 4). The levels of p53 and keratin were significantly elevated in group 2 mice and these levels were decreased in 3 and 4 mice as well. The histopathological examination of DMBA-induced mice was shown degenerated cervical patches in the skin, cirrhosis in liver, oedema in the renal tissue, and swollen and damage in cardiac tissue, which were reduced for the mice applied with FA. This confirms that FA pretreatment offered potential antiskin cancer property.

## 1. Introduction

Worldwide, 18.1 million new cases and 9.6 million deaths are due to skin cancer [1]. In India, 63% of death is due to non-communicable diseases, and cancer is one of the dominating causes, accounting for 9%. These figures will nearly double by 2040; skin cancer is almost egregious malignancies among other common cancers though not ranked among the top ten common cancers [2]. There has been a continuous increase in the prevalence of skin cancers, especially that of

cutaneous melanomas off the last few years. Despite lack of complete prevalence data, different cancer athenaeum in India announced an increasing incidence of skin cancer changing from 0.5 to 2 per 100 000 communities [3, 4]. Surgery and radiation therapy are the standard available treatments for cancer. However, the main drawback of these therapies includes damage to normal cells with a high proliferate index. Most chemotherapeutics are dispensed methodically and are cytotoxic to normal cells; hence, cancer case must undergo considerable morbidity. The traditional-

based topical administration of anticancer natural drug is a fascinating flipside for increasing drug directing and remedial benefits [5].

The dimethyl Benz (a) anthracene (DMBA) is a polycyclic aromatic hydrocarbon (PAHs), branded as an effective carcinogen and/or endocrine disruptors. PAHs are widely distributed in the environment as a result of incomplete combustion of fossil fuels and other organic molecules and are common contaminants of terrestrial and aquatic ecosystems [6]. Seaweeds are attractive sources of biomolecules such as proteins, polysaccharides, minerals, vitamins, steroids, and dietary fibers which are profitable resources for nutraceutical and pharmaceutical applications [7]. The brown seaweeds are the largest marine sources for polysaccharide extraction (44-53%) globally. Some of the valuable bioactivities revealed by the polysaccharides and their derivatives, both *in vivo* and *in vitro*, include immunomodulatory ability, anticancer, antiviral, anticoagulant, and/or antithrombotic properties. They are also proved to have potent antidiabetic, hypoglycaemic antioxidants, antibiotics, and anti-inflammatory effects. Previously, we have reported the antioxidant, anticoagulant, and mosquitocidal properties of phloroglucinol and some water-soluble polysaccharides from Indian brown seaweeds [8, 9]. But till now, there is no report on the anticancer activity of FA from seaweed (IP No. E-101/17997/dated 01.07.2019). Hence, the present study is aimed at finding out the antiskin cancer property of formulated agar from brown seaweed (*L. digitata*) against DMBA-induced mice model.

## 2. Materials and Methods

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies [9].

**2.1. Seaweed Collection.** Brown seaweed (*L. digitata*) was collected from Kanyakumari, (Lat. 8.0866 N and Long. 77.5544 E), Tamil Nadu, India, during December 2018–January 2019. The collected brown seaweed species were identified using the Central Marine Fisheries Research Institute (CMFRI) taxonomic manuals and bulletin. The collected samples were cleaned assiduously with seawater and distilled water and finally washed with distilled water. The processed seaweeds were shadow dried, powdered, and stored at  $-20^{\circ}\text{C}$  for further study.

**2.2. Extraction of Agar.** A 10 g of powdered seaweed sample was transferred to the beaker and mix up with 100 ml of high-pure Milli-Q water then autoclaved at  $121^{\circ}\text{C}$  for 1 h for extraction of water-soluble polysaccharides. Further, the water-soluble polysaccharides were filtered through Whatmann No. 1 filter paper to eliminate seaweed debris present in the extract, and then, it was centrifuged at 5000 rpm for 15 min at RT. The filtered extract was kept at RT, and it gets gelled. The gelled samples were dried out at  $60^{\circ}\text{C}$  overnight and stored in  $-20^{\circ}\text{C}$  for further experiments [10].

**2.3. Formulation of Agar (FA).** The carbopol 934 was liquefied slowly while stirring in 60 ml of Milli-Q water for 1 h to evade agglomeration. Carbopol was then neutralized with

triethanolamine (5.6 ml) with stirring. This was followed by the addition of methylparaben (0.6 g) and propylparaben (0.07 g) with continuous exhaustive stirring until medium components were homogeneous, then mannitol (17.5 g), lysine (0.88 g), and glycerol (8.75 ml) were added; finally, the gelled agar (100 g) core component of the formulation was added. The mixture was mixed well, and it was stirred for 30 min (Magnetic stirrer, PCE Pvt. Ltd. India) until a clear consistent gel base was obtained. The formulated agar was stored in an air-tight container under freeze conditions [11].

**2.4. Characterization of FA.** Organoleptic characteristics of the FA, such as color, consistency texture, sterility, and homogeneity, were assessed by visual examination. Besides, several physicochemical characteristics were also analyzed [12–16]. The data represented here was for triplicate determinations.

**2.5. FT-IR Spectroscopy of FA.** The FT-IR spectroscopic analysis of FA from brown seaweed was performed using a Bruker Alpha instrument (USA) measured in the range of  $4000$  to  $400\text{ cm}^{-1}$  at 30 scans at RT. The samples were thoroughly mixed up with KBr, and the spectra were taken [8].

**2.6. Microstructure Studies of FA by Scanning Electronic Microscope (SEM).** The morphological features of the FA were studied with a JSM-5600 LV scanning electron microscope (JEOL, Tokyo, Japan). The dried sample was mounted on a metal stub, and the images were taken at an accelerating temperature ( $200^{\circ}\text{C}$ ,  $400^{\circ}\text{C}$ ,  $600^{\circ}\text{C}$ , and  $1200^{\circ}\text{C}$ ) with magnifications 5.00 KX, 2.50X, 2.52 KX, and 1.00 KX [17].

**2.7. MTT Assay of FA.** The cell viability of FA was determined by MTT assay. Freshly cultured NIT 3T3 mouse fibroblast cell line cells were trypsinized, collected, counted, and seeded in a 96-well plate. After 24 h incubation, cells were treated with various concentrations of FA. Then, the cells were incubated in 5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$  for 24 h. Later,  $100\ \mu\text{l}$  of MTT was added, and incubation was extended for more 4 h. Finally, the medium was removed and  $100\ \mu\text{l}$  of DMSO were used to solubilize the formazan crystals. The samples were read at 570 nm (Tecan Multimode Multiwall Plate Reader, Austria). Percentage cytotoxicity was calculated as described earlier [10].

**2.8. In Vivo Anticancer Assay.** The skin cancer was experimentally induced in female Swiss albino mice following the ethical clearance obtained (IAEC4/Proposal: 31/A.Lr:13/Dated: 20.12.18). The animals were divided into 5 groups as mentioned in Table 1. Control mice received normal food and water *ad libitum*. The 5-FU (100 mg/100 ml acetone)-treated mice served as a positive control. The FA was applied topically weekly twice 30 min before exposure for 63 days. The physical parameters like measurement of tumor size, body weight, and food and water intake of the experimental mice groups were measured during the entire experimental period. At the end of the experimental period, the mice were sacrificed and the skin, liver, kidney, and heart were dissected, then the organs were stored under  $-20^{\circ}\text{C}$  until further use.

TABLE 1: Experimental design.

Group 1	Normal control mice (normal diet and water)
Group 2	Cancer-induced mice (100 nM DMBA dissolved in 200 $\mu$ l acetone was applied topically weekly twice for four weeks)
Group 3	FA (low dose)—60 mg/ml in 200 $\mu$ l acetone was applied topically weekly twice for four weeks, 30 min before DMBA exposure (100 nM DMBA dissolved in 200 $\mu$ l acetone was applied topically weekly twice)
Group 4	FA (high dose)—120 mg/ml in 200 $\mu$ l acetone was applied topically weekly twice for four weeks, 30 min before DMBA exposure (100 nM DMBA dissolved in 200 $\mu$ l acetone was applied topically weekly twice)
Group 5	Positive control: 5-FU-100 mg/ml in 100 ml acetone was applied topically weekly twice for four weeks, 30 min before DMBA exposure (100 nM DMBA dissolved in 200 $\mu$ l acetone was applied topically weekly twice)

TABLE 2: Stability of FA.

S. no.	Parameters/tests	Stability of FA storage conditions		
		25°C $\pm$ 2°C Months (0–3)	32°C $\pm$ 2°C Months (0–3)	40°C $\pm$ 2°C Months (0–3)
1	Color	No change in color	No change in color	No change in color
2	Odor	No change	No change	No change
3	Homogeneity	Good	Good	Good
4	Consistency	Smooth	Smooth	Smooth
5	pH	6	6	6
6	Viscosity ( $m^2/s$ )	0.385	0.382	0.381
7	Microbial load (bacteria)	No microbial growth is observed at 24, 48, and 72 h	No microbial growth is observed at 24, 48, and 72 h	No microbial growth is observed at 24, 48, and 72 h
8	Sterility	No microbial contamination	No microbial contamination	No microbial contamination
9	Vibrational test	No phase of separation	No phase of separation	No phase of separation
10	Centrifugation test	No phase of separation	No phase of separation	No phase of separation

Time period (three months).

**2.9. Measurement of Tumor in Experimental Mice.** Measurement of tumor development and the effect of FA on tumor development was evaluated by using Vernier caliper. The length, width, and height of the tumor were measured for during the experimental days.

**2.10. p53 Protein Level Measurement.** Determination of p53 protein level in mice skin tissues and isolated cells were estimated by using mouse p53 ELISA kit.

**2.11. Isolation and Determination of Primary Keratinocytes.** The epidermis of experimental mice were isolated by incubation in dispase for up to 72 h that was cut subsequently into small pieces with sterilized scissors then incubated for 30 minutes in 0.025% DNase in DMEM at RT with gentle agitation. Afterwards, the mixture was filtered through a 100  $\mu$ m cell strainer, and the cells were pelleted by centrifugation. Cells were resuspended in 3:1 defined keratinocyte serum-free medium containing appropriate growth supplements, seeded on plates previously coated with collagen, then characterized by spectroscopy [18].

**2.12. Collagen and Elastin Contents Determination.** The amount of collagen and elastin in the dermis was measured using a Sircol assay kit based on the fact that the sirius red dye binds to the side chains of the amino acids in collagen.

TABLE 3: Texture analysis of FA.

Moisture (%)	48.28 $\pm$ 0.21
Hardness (g)	243.43 $\pm$ 0.60
Adhesiveness (N/mm)	61.50 $\pm$ 0.40
Cohesiveness	0.952 $\pm$ 0.0.12
Springiness (mm)	3.520 $\pm$ 0.012
Gumminess (N)	3.699 $\pm$ 0.10

Both soluble and covalently crosslinked insoluble collagen fractions were obtained by successive treatment of the skin with 10 volumes of 5 types of a buffer. The collagen extracted with buffers A and B was categorized as soluble collagen and that extracted with buffers C, D, and E were categorized as insoluble collagen [19, 20].

**2.13. Enzymatic and Nonenzymatic Assessments.** The levels of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured in mice skin tissues determined by using commercial kits from Sigma, USA.

**2.14. Histopathology.** The skin, liver, kidney, and heart of each group of mice were fixed in 10% formalin for

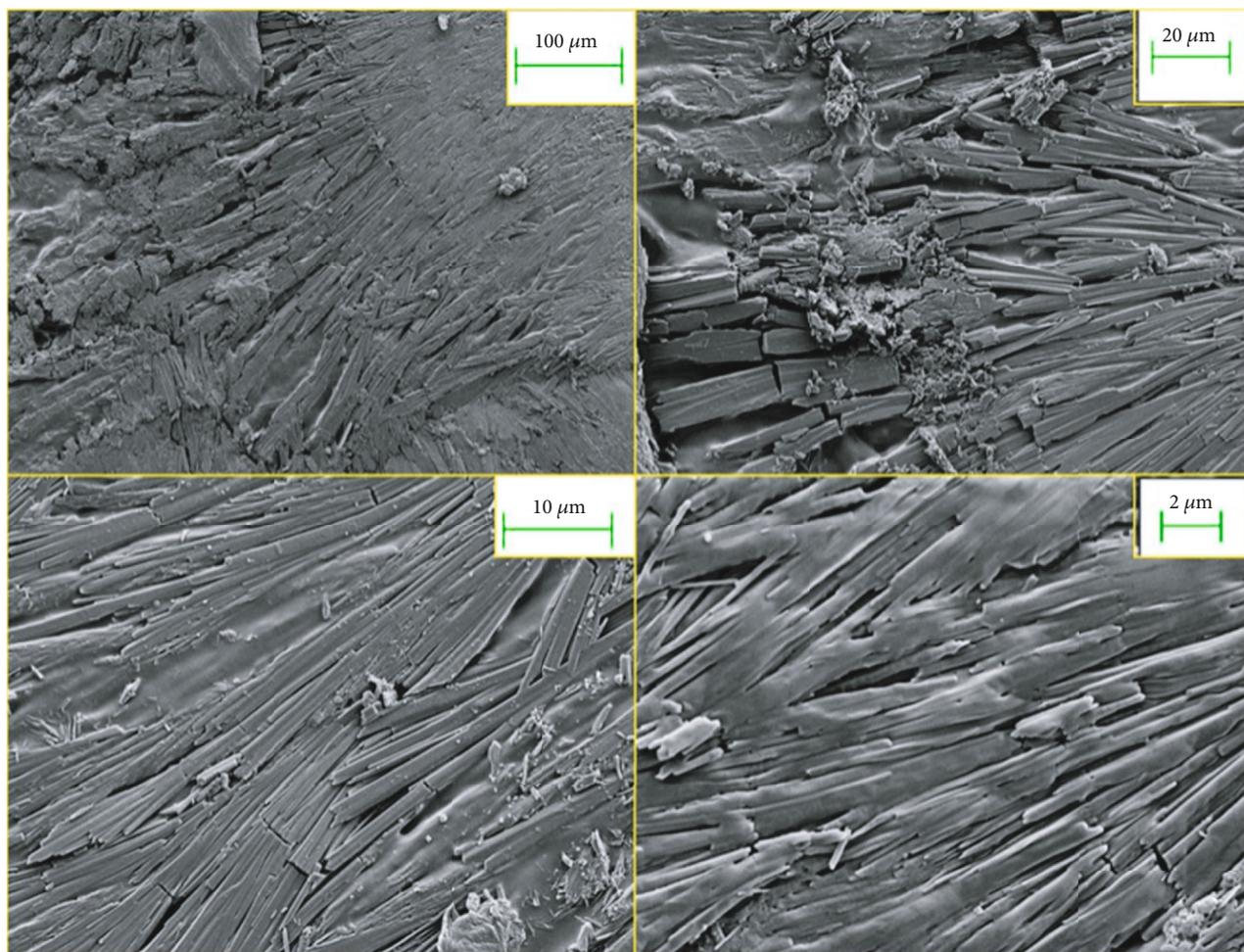


FIGURE 1: SEM of FA from brown seaweed (The FA (2-10  $\mu\text{m}$ ) exhibits regular, layered, patched and smooth, and flat continuous sheet appearance).

haematoxylin and eosin staining (H&E). Serial sections of 20  $\mu\text{m}$  thickness were cut and mounted on slides and stained. The fixed samples were trimmed and dissected in the crosssections and were processed. The samples were fixed in stretches of formalin and alcoholic formalin, 95% alcohol, absolute alcohol, xylene, and liquid paraffin wax. Crosssections were selected from the three plates per sample. The morphological changes were detected under the microscope [21].

**2.15. Statistical Analysis.** All the statistical analyses were presented by using software using the SPSS software (SPSS, IBM, version 20.0). Each test is performed from all animals, and all the results were expressed as the means plus-minus their corresponding standard deviation (SD). A value of  $p < 0.05$  was measured to specify a significant difference between groups.

### 3. Results and Discussion

**3.1. Extraction Yield and FA.** The agar yield from *L. digitata* was found to be 40%. This is considered the maximum yield when compared to a previous report [22], which indicated that the agar yield from brown seaweeds could vary. The

yield difference is largely due to the species, season period, and geographical location. A cost-effective hot water extraction method with comparable yield was successfully developed for agar extraction and its formulation to develop antiskin cancer property of FA from brown seaweed (*L. digitata*) [23]. Screening for new drugs from natural sources mainly from marine environment to discover anticancer activities is valuable as they are likely to be more safe, robust, and effective. Since agents of synthetic origin may have various adverse effects. Natural products have been used as remedies for skin cancer prevention and other traditional treatments as well. Sulfated polysaccharides (SP) from marine sources were investigated to have good antioxidant, antitumor, and immunomodulating activities. It was suggested that, next to the pharmacological activities of SP, their physicochemical properties, like good water solubility, thickening, and solidity over a wide pH range, offer extra benediction for their use as a preservative in topical application products [10].

**3.2. Characterization of FA.** Stability studies of FA were essential to ensure product quality, efficacy, and safety. Thus, these studies tend to give the development and improvement

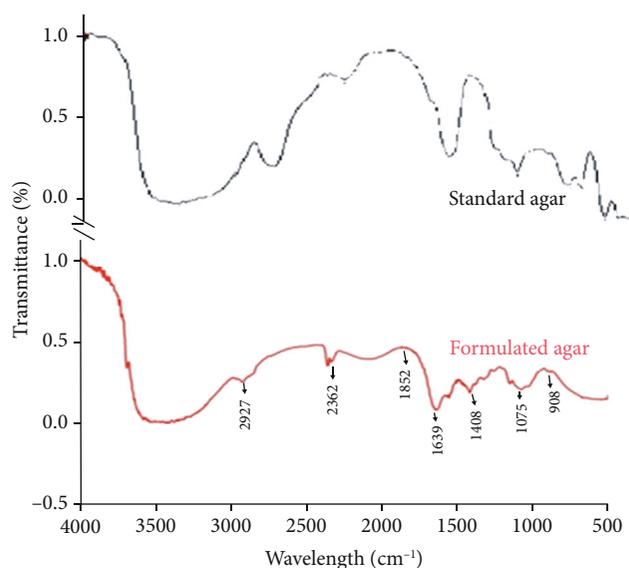
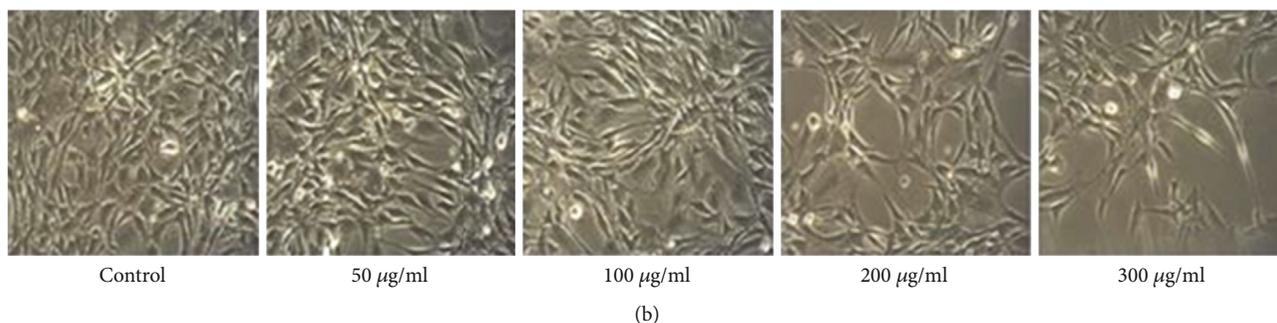
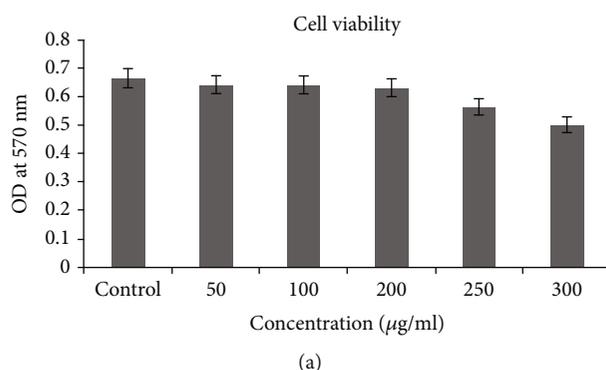


FIGURE 2: FT-IR spectrum of formulated agar.

FIGURE 3: MTT assay of FA (Cells treated above 300 µg/ml concentration showed a significant ( $p < 0.05$ ) death of cell; compared with control (a, b)).

of formulations, creating the product validity and observing its physical, chemical, and microbiological characteristics. The FA extracted from the *L. digitata* is slightly soluble in distilled water and its dispersal acquiesces a light brown color, slick solution. The FA was virtually insoluble in polar and nonpolar solvents like acetone, chloroform, and ethanol. Stability may be affected by environmental factors such as pH, light, temperature, air, and transport which can induce harsh damage on the product constituents. The FA stability param-

eters such as no color and odor change, good consistency, and homogeneity were smooth, and there was no microbial contamination. All these features indicate the sterility of FA in Table 2. The pH value of FA remains substantial during storage which was considered adequate to avoid the risk of irritation upon application to the skin. Hence, there was no change in the pH; the FA gel could be suitable for topical application. Centrifugation tests and vibrational tests are the main tools for estimating and predicting the shelf life of

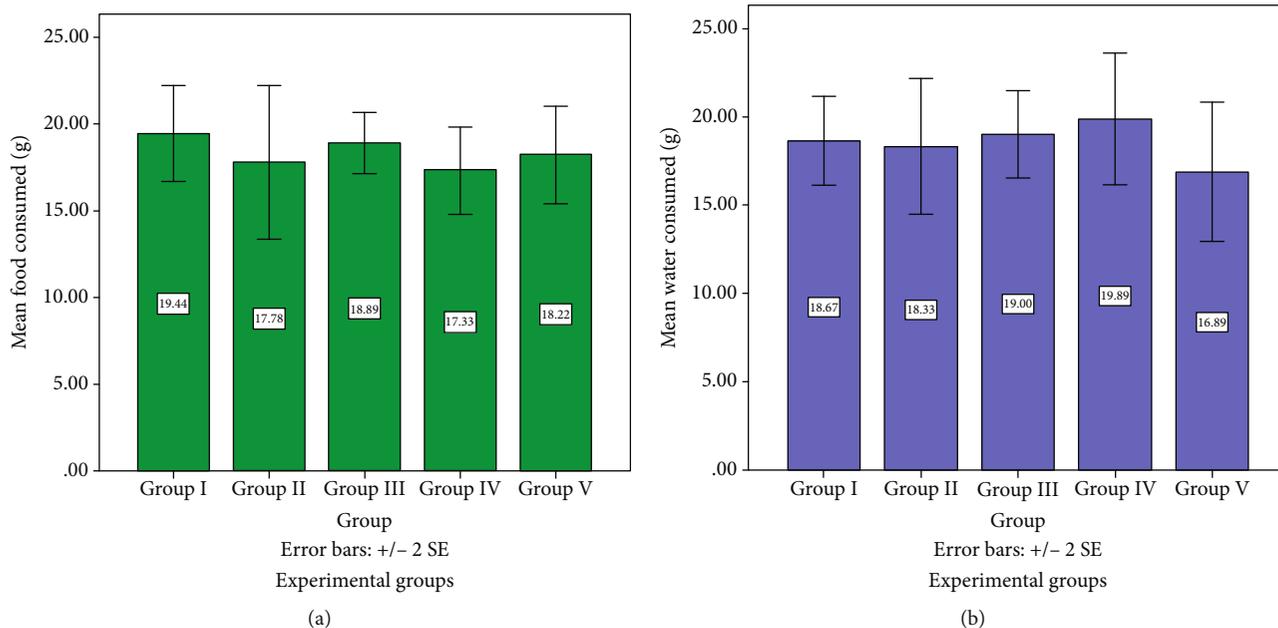


FIGURE 4: (a, b) Food and water intake of experimental mice (DMBA-induced mice significantly ( $p < 0.05$ ) reduced the food and water intake; FA-treated mice significantly ( $p < 0.05$ ) increased the food and water intake).

the formulated product. From our data, we found that the FA does not show any indication of phase separation during the study period, which are consistent with the findings of Andrade et al. [24].

**3.3. Texture Analysis of FA.** The texture analyses (moisture, hardness, adhesiveness, cohesiveness, and springiness) of FA were described in Table 3. The texture analysis results of FA are considered high when compared to the results of Reshma [23], who had extracted the agar by using hot water from brown seaweed. The difference in the texture numbers is likely due to the method of extraction and formulated nature of the sample. Texture analysis is one of the most essential unique inspection criteria for pharmaceuticals since it contributes important identification and exclamation knowledge. Characteristics with small intraclass variations and large interclass variations of differing textures are an essential product requirement. Here, we explored characteristics focused on those techniques that have been a standard in texture analyses or applied for repeated material examination.

**3.4. Phytochemical Composition of FA.** The phytochemical composition of FA includes crude protein amount (3.79%), total carbohydrates (12.33%), and element composition comprising of Ca (1.5%), P (0.18%), N (2.74%), K (2.99%), Mn (16.50 ppm), Zn (50.40 ppm), Fe (30.0 ppm), Cu (75.41 ppm), and Co (3.00 ppm), respectively. The element contents of FA were somewhat high when compared to the previous report of Fleurence et al. [25]. The observed difference in the mineral contents is owing to species difference and method selection for quantification. The mineral composition of FA grants antioxidant properties which could be partially responsible for its anticancer effect. Mineral composition typically is alerted by unusual environmental and

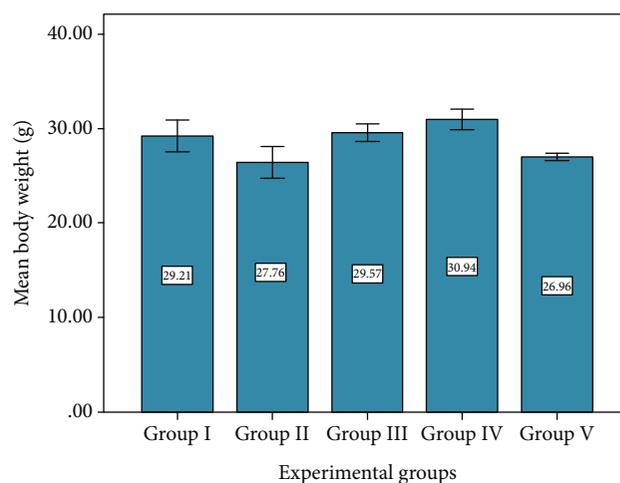


FIGURE 5: Body weight of experimental mice (DMBA-induced mice significantly ( $p < 0.05$ ) reduced the body weight; FA-treated mice significantly ( $p < 0.05$ ) increased the body weight).

physiological conditions, various processing techniques, and methods of mineralization. The macrominerals for example K, Ca, Na, and Mg are present in marine algae at very high levels than in land sources. Further, the level of Fe and Cu were reported to be higher in seaweeds than spinach and meat. The polysaccharides are major components of brown seaweed, accounting for 40% of the dry weight. The hot water extract of agar biomass is loaded in low molecular weight, water-soluble polysaccharides, (WSC) and insoluble in aqueous medium [10].

### 3.5. Structural Characterization

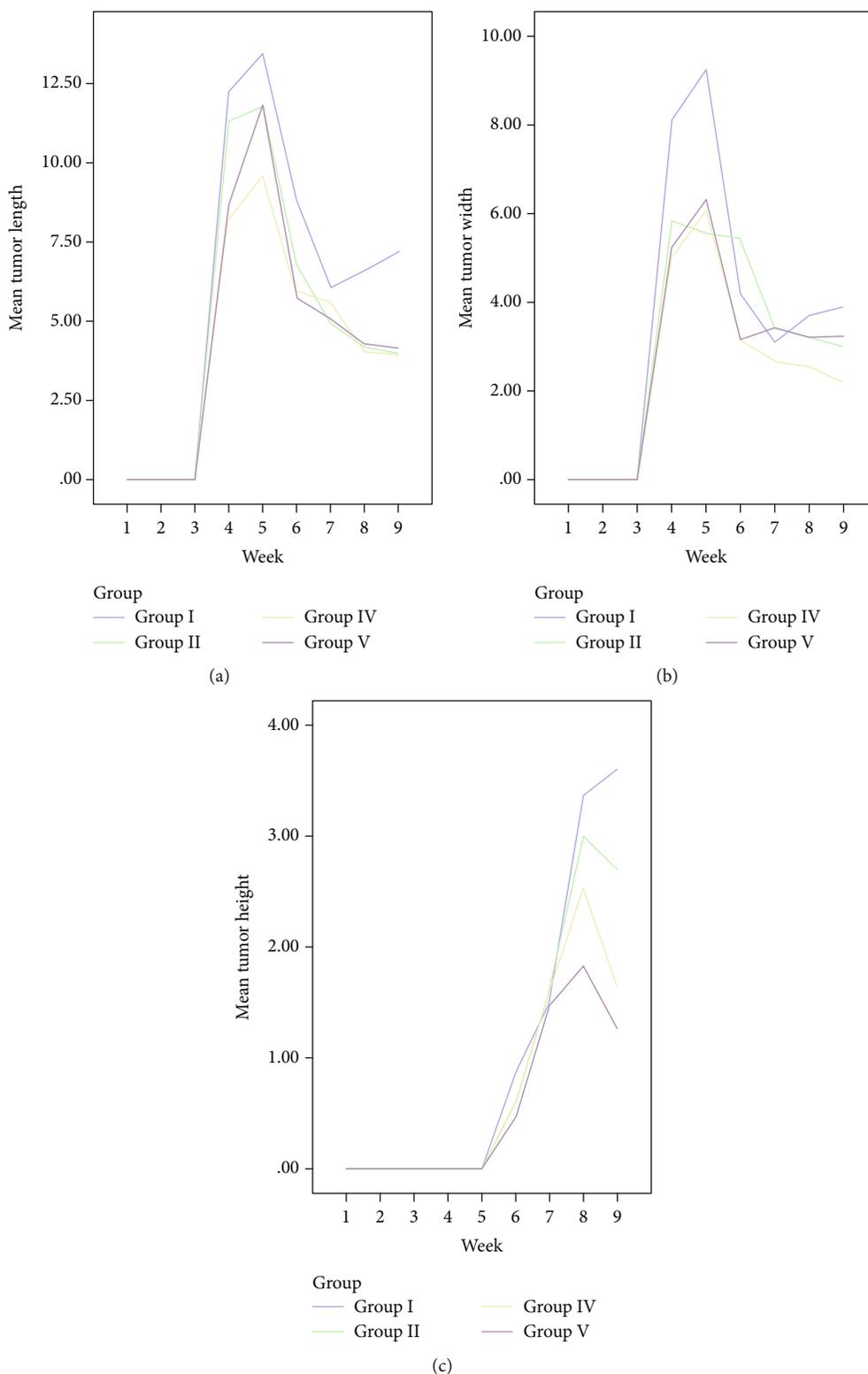


FIGURE 6: (a–c) Tumor measurement of experimental mice (DMBA-induced mice significantly ( $p < 0.05$ ) increased the tumor size; FA-treated mice significantly ( $p < 0.05$ ) decreased tumor size).

3.5.1. Analytical Method. The FA was dissolved in MilliQ water to bring about a homogeneous solution for scanning in a range of wavelengths between 200 and 700 nm, and it showed maximum absorbance at 280 nm. The UV absor-

bance of the present study was similar to the result of Azizi et al. [26], who have reported the aqueous extract polysaccharide from *S. muticum* recorded the same UV absorbance at 280 nm. UV-visible spectroscopy is widely used for the

investigation of chromophore associations of atoms identified by energetically absorbing electronic transitions. The UV-visible absorption spectrum of the FA calculated in the present study is displayed in Figure 1. Our data indicate that there was a significant amount of absorption in the UV region, particularly, the maximum absorption ranged from 280 to 350 nm. Further, there was a shoulder detected around 280 nm.

**3.5.2. FT-IR Spectroscopy of FA.** FT-IR spectroscopy is a promising method for the biochemical analysis of FA and other cellular materials. It affords objective assessment on the holistic biochemistry of a cell or formulated sample and has been practiced in many areas of biomedical research. The FT-IR spectrum of FA in the range  $4000\text{--}500\text{ cm}^{-1}$  is depicted in Figure 2. Analysis of the given spectra, the peaks obtained were identified as C-H stretch (alkanes) that appeared in the range of  $3000\text{--}2850\text{ cm}^{-1}$ , -C=C- stretch (alkenes) in the range of  $1680\text{--}1640\text{ cm}^{-1}$ , C-N stretch in the range of  $1250\text{--}1020\text{ cm}^{-1}$  that contributes aliphatic amines, and C=H bend (alkenes) in the range of  $1000\text{--}650\text{ cm}^{-1}$ , when compared with standard agar. Sankar and Rhim et al. [27] reported similar types of FT-IR analyses conducted on both brown algae *S. wightii* and red algae *G. corticata* in between the ranges  $4000$  and  $500\text{ cm}^{-1}$ .

**3.5.3. SEM Analysis.** SEM offers surface morphology evolution information due to 2D island nucleation and amalgam in FA and adsorbents. SEM images affirm various amount of layer-by-layer arrangement of FA. The step proliferation acceleration can be examined from these images. The vertical verdict was highly adequate to distinguish an atomic layer, while the lateral resolution was  $2\text{--}100\text{ nm}$ , depending on the sheet appearance and dispatch intensity. The biological and natural source of a formulated material serves as a major determining factor on the basis of granule shape, morphology size, and morphology. The SEM of FA is shown in Figure 3; it exhibits regular, layered, patched, and smooth, flat continuous sheet appearance. It has an irregular geometrical shape with certain gaps on the surface and loosely superimposed. These properties could give significance when considering applications based on surface characteristics. These SEM pictures revealed the structure of the FA network. This intimate connection could also assist the physical stability of the formulated product. On comparison with a recent study by Kim et al. [7], fucoidan present in brown algae induces apoptosis of human colon cancer. In this study, the FA from brown seaweed was fractioned and evaluated for surface features and structural forms compound with smooth, flat continuous sheet appearance.

**3.6. In Vitro Cell Viability Study (MTT Assay).** The *in vitro* cell viability effects of FA on NIT 3T3 mouse fibroblast cells were assessed. The set of cells that were administrated with concentrations above  $300\text{ }\mu\text{g/ml}$  exhibited significant death when compared to control group Figures 3(a) and 3(b). From this result, the  $\text{LD}_{50}$  of FA was calculated as  $300\text{ }\mu\text{g/ml}$ . Fonseca et al. [28], reported the cytotoxicity effect of *Calendula officinalis* extract against UV-B-induced oxidative stress in

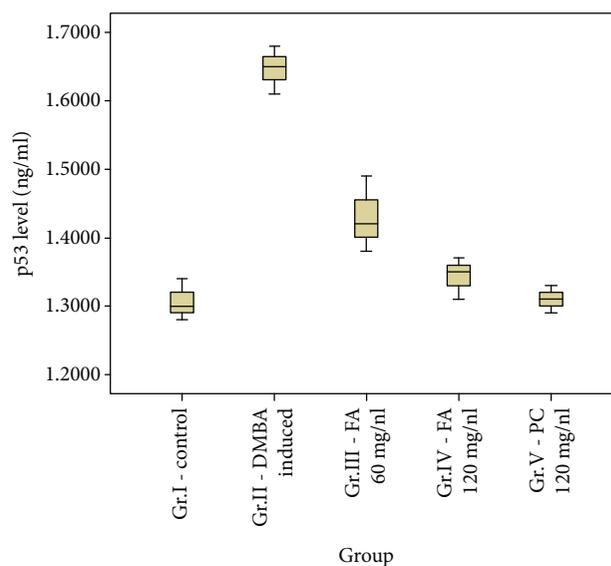


FIGURE 7: p53 level in skin of experimental mice (DMBA significantly ( $p < 0.05$ ) increased the p53 level; FA-treated mice significantly ( $p < 0.001$ ) reduced the p53 level).

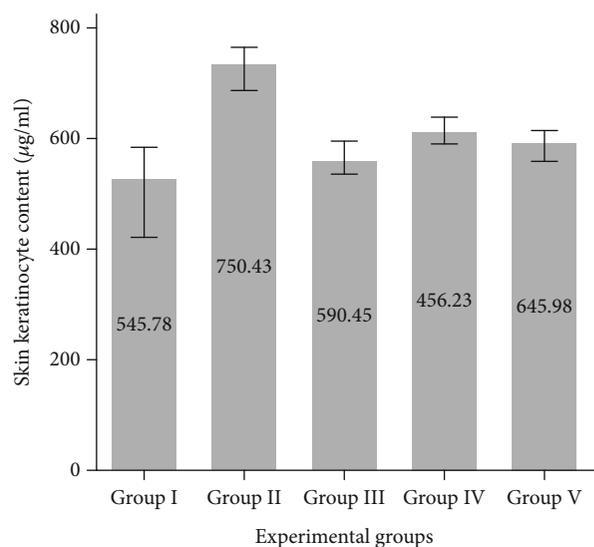


FIGURE 8: Keratinocyte contents in skin of experimental mice (DMBA-induced mice significantly ( $p < 0.05$ ) lowers the keratinocytes, but it was increased in FA-treated groups).

the skin. The result indicated that above  $500\text{ }\mu\text{g/ml}$  concentration, significant cell death was observed. UVA and UVB influence the skin surface and are moderately rejected by the stratum corneum of the epidermis and engrossed by epidermal melanin. Conversely, significant volumes run off the cell walls can activate DNA and cause damage to skin cells, inducing skin cancer.

**3.7. Skin Irritation Test.** The FA was evaluated for its skin irritant effect. No skin irritation was observed even after 10 days of study, demonstrating that the FA could be a safe material. Daniel et al. [29] reported that the UV-A sunscreen

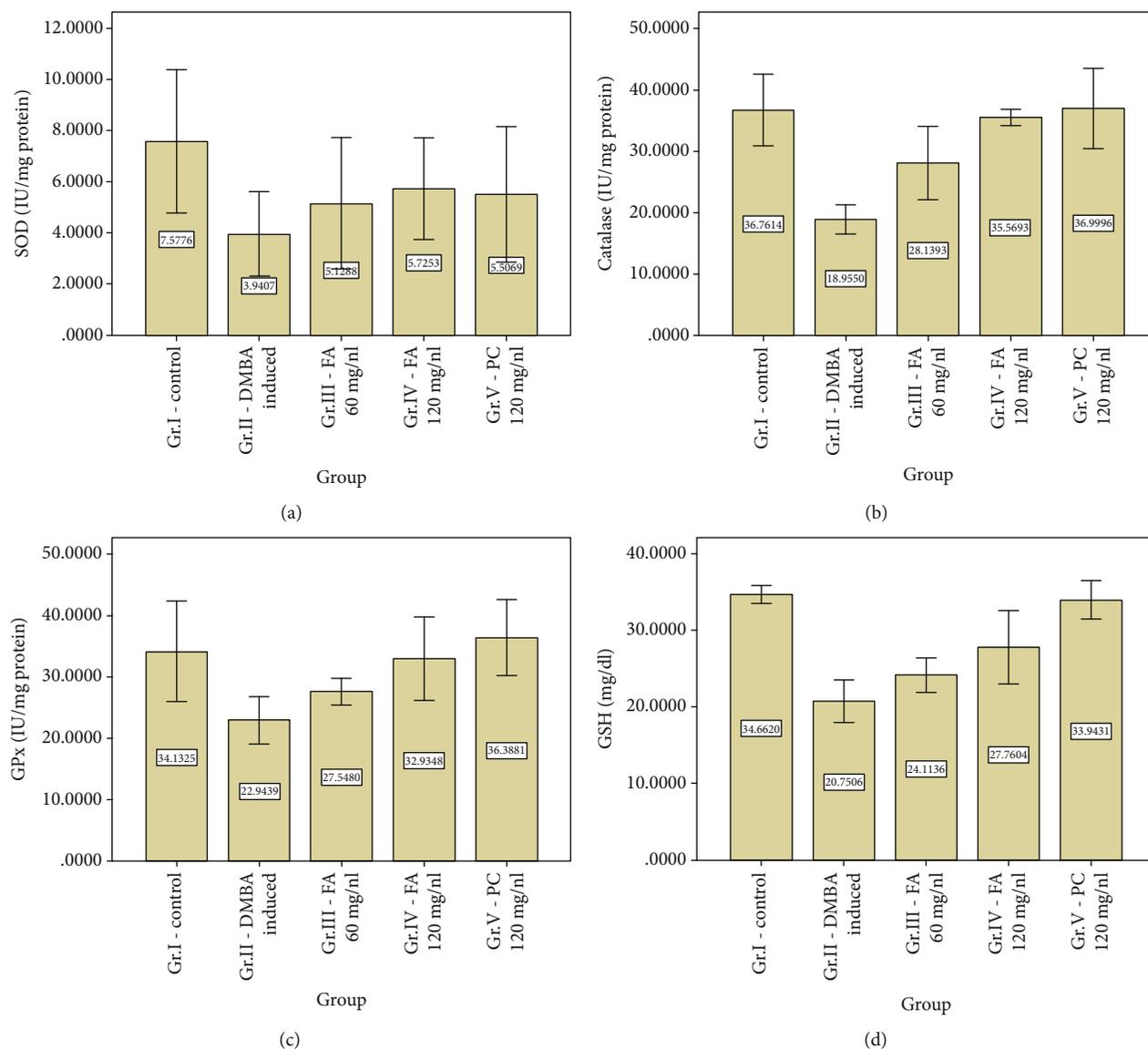


FIGURE 9: (a–d) *In vivo* antioxidant enzymes in skin tissues of experimental mice (The DMBA significantly ( $p < 0.05$ ) decreased levels of SOD, CAT, GPx, and GSH, whereas, the FA enhanced the antioxidant enzymes activity of SOD, GSH, GPx, and CAT).

gel from red algae-formulated product did not show any skin irritation which corroborates our current data.

**3.8. Physiological Observation Tumour Measurement.** Experimental mice exhibited significant variations ( $p < 0.05$ ) in food and water intake Figures 4(a) and 4(b). The water intake was significantly increased ( $p < 0.05$ ) after DMBA administered mice (group II, (from weeks 3 to 9)) on association with group I, II, and III mice. Later, the water intake becomes near normal after treatment with FA to mice groups III and IV Figure 4(b). Group II mice food intake is significantly reduced ( $p < 0.05$ ) after induction of DMBA (weeks 3 to 9), and the food intake becomes near normal after topical treatment of FA to mice groups III and IV Figure 4(a).

Before animals were sacrificed, no mice died during the full experimental course. From the third week onwards, mice induced with DMBA (group II) presented evident cancer effects such as listlessness, lag in response, and slow move-

ment, which continued up to nine weeks. The body weights of DMBA-induced mice were significantly decreased ( $p < 0.05$ ) in group II when compared with control group (group I) and FA-treated groups (group III (60 mg/ml) and group IV (120 mg/ml) mice Figure 5. Additionally, body weights of group II mice are significantly decreased ( $p < 0.001$ ) when compared to other groups.

Measurements of tumor length, width, and height in group II mice were significantly maximum ( $p < 0.001$ ) on comparison with groups III and IV (Figure 6). The present results confirm Swallow et al. [30] study, which stated that food intake, water intake, body weight measurement, lack of physical activities, physiological changes, and tumor measurement potentially play a major role in body composition. This study results suggested that all of the above-mentioned behaviors and detected changes were due to DMBA-induced mice skin cancer, whereas this was normalized in the FA topically applied mice.

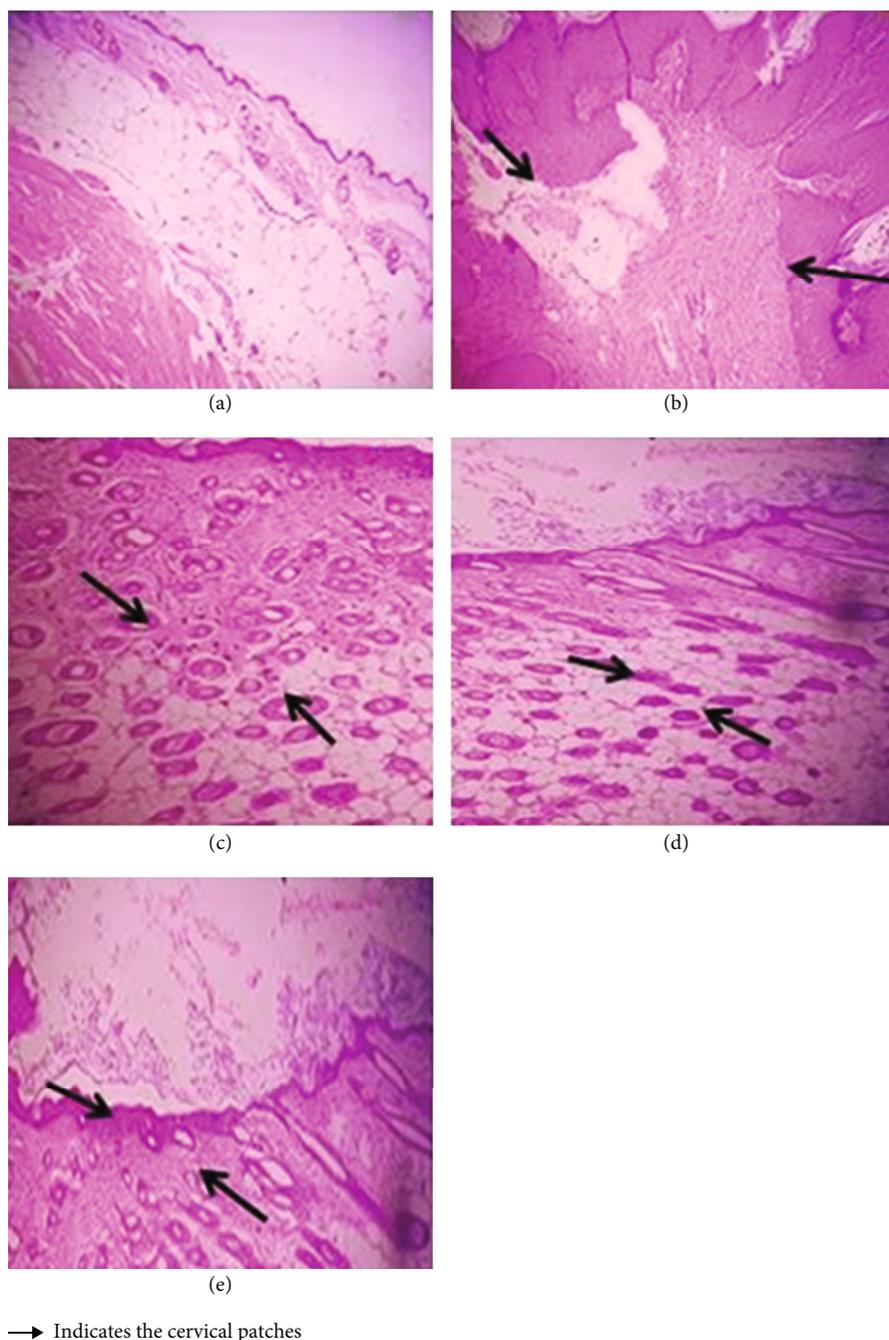


FIGURE 10: (a–e) Histological evaluation of skin tissue corresponding untreated, DMBA-induced group, FA-treated group, and positive control (The DMBA induced the degenerated cervical patches of the skin of mice; it was brought to normal by FA-treated mice).

**3.9. p53 Level Analysis.** The p53 protein level in group II mice showed significant elevation ( $p < 0.001$ ) when compared with control and which was significantly reduced ( $p < 0.001$ ) in other treated groups Figure 7. High expression of p53 protein and p53 alterations have been noticed in a great proportion of SCC and premalignant abrasions in renal allograft recipient cases. The p53 tumor suppressor gene was reported to have association with the cell cycle capture and inauguration of programmed cell death [31].

**3.10. Keratinocytes Contents of the Skin.** In the present investigation, significant loss of keratinocytes was found in the DMBA-induced mice ( $p < 0.05$ ) than control mice. The FA administrated groups III and IV displayed significant improvement ( $p < 0.05$ ) in keratinocyte content when compared to induced group mice Figure 8. Epidermis said to abide of multistate constantly renovating keratinocytes most of the time, and this structure forms the epidermal barrier which need to donate the self-protective responses against

various environmental factors (for example, cold, heat, radiation, trauma, and microbial infection to name a few). Increased level of keratinocytes indicates keratinocyte-connected cutaneous disorders, covering CSC, zoster herpes, and psoriasis [32].

**3.11. In Vivo Antioxidant Enzymes Levels.** The *in vivo* antioxidant defence system like SOD, CAT, GPx, and GSH in DMBA-induced mice exhibited a significant reduction ( $p < 0.05$ ) in the skin tissues when compared to treated groups Figures 9(a) and 9(d). Cell life in an oxygenated background has necessitated the progression of effective cellular tactics to identify and detoxify metabolites of free radicals. These effects reported to disturb significantly the host of physiological practices and metabolic pathway locally bounded up with the skin cancer of the animal and skin or the change of its related disorders [33]. The results revealed significant rise ( $p < 0.05$ ) in the activities of the primary antioxidant defence enzymes SOD, CAT, GPx, and GSH in the samples of topically applied mice group (groups III and IV) suggesting that the FA could enhance the antioxidant system in the skin of experimental mice. In particular, GSH estimation is an important indicator for quantifying the level of antioxidant activity. The GSH enzyme can be as curtailed in almost every cell of the body and have tremendous antioxidant and detoxifying activity. GSH not only eradicates free radicals *in vivo* but also boosts the immunity level [34].

**3.12. Histopathological Analysis.** The paraffin sections of the skin, the liver, the heart, and the kidney of experimental mice are represented in Figure 10. From this result, the degenerated cervical patches of the skin tissue of the (DMBA) group II was more than that of groups I, III, IV, and V. The cervical patches of the skin were degenerated with skin cancer formation, whereas, these changes were regenerated in the skin cervical patches of formulated agar (group III and group IV)-treated mice. The DMBA-induced mice kidney showed oedema formation and treated mice seen as decrease of oedema. In the heart, the myofibrils were collapsed in the induced group, and in the treated group, showed the normalized myofibrils. The DMBA-induced mice showed significant liver cirrhosis, and these changes were nearly normalized in FA-treated mice.

## 4. Conclusion

Both the *in vitro* and *in vivo* findings of our present study revealed that FA from brown seaweed could act as a potent antiskin cancer agent and antioxidant. All these outcomes seem to be proposing that pretreatment of FA from *L. digitata* has a great development perspective for mitigation of the skin cancer induced by DMBA. However, there is a need to find the exact mechanism of action of FA, which warrants the need for extensive biochemical and molecular studies. The microbial load and UV-induced skin cancer activities of FA from brown seaweed are not analysed in this study, which could be tested in the future.

## Data Availability

The data used to support the findings of this study are available from the authors upon request.

## Additional Points

**Animal Welfare.** Animal use complied with institutional, national, and international guidelines including the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India guidelines. Guidelines of “*Guide for the Care and Use of Laboratory Animals*” (Institute of Laboratory Animal Resources, National Academic Press 1996; *NIH publication* number #85-23, revised 1996) were strictly followed throughout the study. Institutional Animal Ethical Committee (IAEC) approved this study. Research ethical clearance was obtained (IAECA/Proposal: 31/A.Lr:13/Dated: 20.12.18).

## Ethical Approval

This research does not involve human participants, human material, or human data. This research involves experimental animals approved by the Institutional Animal Ethics Committee, Chettinad Academy of Research and Education (Deemed to be University), Kelambakkam-603 103, Tamil Nadu, India, and adhered to the Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA), Govt. of India guidelines.

## Conflicts of Interest

All authors declare that there are no conflicts of/or competing interests.

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