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DMBA-TPA-INDUCED MELANOMA CANCER AND ASSESSMENT OF THE PLANT EXTRACT OF BOSCIA SENEGALENSIS LEAF EXTRACT IN VIVO ANTI-MELANOMA ACTIVITY IN ANIMAL MODELS

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ABSTRACT

The potential of plant-based anti-cancer drugs targeting tumor locations has attracted considerable attention due to their potential advantages compared to conventional approaches. In particular, the BSL ointment formulation has shown promising results in decreasing the number of papilloma in vivo models. Application of Fu-5 to the study group of mice led to a decrease in the number of papillomas, and tumor promotion was observed upon the administration of DMBA-TPA to the skin of mice. Notably, the BSL group exhibited a significant decrease in papilloma, with the treatment group showing a substantial response compared to the control group. The application of BSL ointment did not significantly harm the liver, as indicated by the unchanged levels of ALT, AST, and ALP. The DMBA-TPA group exhibited an increase in liver parameters. $P < 0.001$, $P < 0.004$, $P < 0.007$, $P < 0.004$. The statistical data for the BSL extract treatment group further supports these findings, with significance indicators at $P < 0.001$, $P < 0.002$, and $P < 0.006$. These results highlight the promising potential of plant-based anti-cancer drugs, emphasizing the need for further research and development in this area.

KEYWORDS: Skin cancer, DMBA-TPA, Boscia senegalensis.

INTRODUCTION

Skin cancer is a significant public health concern, with melanoma being one of the most aggressive and treatment-resistant forms of the disease.[1] Understanding the mechanisms of melanoma development and identifying effective treatment strategies are crucial to combating this severe health issue.[2] This research paper aims to investigate the DMBA-TPA-induced melanoma cancer model and assess the in vivo anti-melanoma activity of Boscia senegalensis leaf extract in animal models. By studying the potential of plant extracts in combating melanoma, this research contributes to the ongoing efforts to find innovative and practical approaches for managing skin cancer.[3] DMBA-TPA-induced melanoma cancer and assessment of the plant extract of Boscia senegalensis leaf extract in vivo anti-melanoma activity in animal models.[4]

1. MATERIALS AND METHOD

MATERIALS: The DMPA-TPA 7, 12-Dimethylbenz [a] anthracene, were obtained from Everon Life Sciences - New Delhi. Boscia senegalensis leaf were purchased from ARC-Sudan. Stander drug (Fluorouracil 5%, were purchased from Unique Health Pharmacy-Delhi. Male albino mice weighing 18-20g were obtained from Sri Venkateshwara Enterprises, Bangalore. The Institution Animals Ethics Committee, Nargund College of Pharmacy, Bangalore. Has approved the experimental protocol (IAEC/NCP/136/2024). All the procedures were performed following the Committee for Control and Supervision of Experiments on Animals (CPCSEA). The biomedical disposal was sent to Mardi Bio Industries Pvt, Ltd, Ref/No/568403, Bengaluru.

METHODS

Extraction of Boscia senegalensis by ethanol: The Leaf of Boscia seneglensis was subjected to extraction by Soxhlet using ethanol 95%. The dried leaf (5kg) was extracted using Soxhlet apparatus, followed by solvent removal using Rotary evaporation. Then the filtrate was evaporated. The residue obtained was dried and the percentage yield was calculated.

Figure 1: Boscia Seneglensis Leaf ointment.

QUALITATIVE PHYTOCHEMICAL TESTS

Qualitative phytochemical tests for the ethanol extract of Boscia Seneglensis Leaf were subjected to qualitative tests for tannins, saponins, flavonoids, terpenoids, phenolic compounds, alkaloids, and anthraquinones.

Formulation of Boscia seneglensis ointment: To prepare 100 gm of simple ointment, accurately measure the required quantity of the ingredients. Melt hard paraffin and cetostearyl alcohol in water. To the melted mixture, add soft paraffin and zinc oxide while stirring. Continue the stirring until all the ingredients are melted. Strain the melted mixture to remove any foreign particles, and add the prepared leaf extract of Boscia sengelensis with the first preparation; stir the mixture thoroughly until it is cooled. Pack the ointment in an ointment jar.

Table 1: compassion of ointment of Boscia sengelensis.

S. No	Name of ingredient	Quantity to be taken
1	White soft paraffin	8.5g
2	Cetostearyl alcohol	0.5g
3	Zinc oxide	0.5g
4	Rose water	0.2g

Evaluation of ointment

Physicochemical parameters

Colour & odour: Physical characteristics, including colour and smell, were assessed visually.

Consistency: It was smooth and showed no signs of greed**.**

pH: Using a digital pH meter, the prepared herbal ointment's pH was determined. After making the ointment solution with 100 milliliters of distilled water, it was left for two hours. The solution's pH was measured three times, and the average value was computed.

Spreadability: By sandwiching an extra sample between two slides and compressing it to a consistent thickness with a fixed weight for a predetermined amount of time, the spread ability was ascertained. The spreadability was defined as the amount of time needed to separate the two slides. Better spreadability is the outcome of the time required to separate two slides. Spreadability was determined using the formula below.

$$S = M \times \frac{L}{T}$$

Were,

S = Spreadability

M = Weight tide to the upper slide

L = Length of glass slide

T = Time taken to separate the slides. It was found to be 5 seconds.

Extrudability: The formulation was filled in a collapsible tube container. The extrudability was determined in terms of the weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

Solubility: Soluble in water, alcohol, and chloroform.

Washability: The formulation was applied on the skin and the extent of washing with water was checked.

Non-irritancy Test: Prepared herbal ointment was applied to the skin of human beings and observed for the effect.

Stability study: A physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions such as 20C, 250C.

IN VIVO STUDIES

Table 2: Animals grouping.[5]

Group 1 (Normal control)	Mice receive a normal diet
Group 2 (Tumors -bearing mice)	Mice receive 50µg of DMBA+5 µg of TPA in 0.2ml of acetone twice weekly (topically).
Group 3 (Tumors-bearing mice+ standard drugs (Fluorouracil).	Mice receive 50µg of DMBA+5 µg of TPA in 0.2ml of acetone twice weekly (topically) + 5% Fluorouracil Topically.
Group 4 (Tumor-bearing mice + extract).	Mice receive 50µg of DMBA+5 µg of TPA in 0.2ml of acetone twice weekly (Topically).dose of Boscia-Senegalensis extract **(**Topically).

The 10% of the skin is removed, and after three days[6], 50µg DMBA and 5µg TPA are applied topically to mice's skin with the help of acetone 0.2 ml for 14 weeks[7], the DMBA randomly mutates DNA by forming covalent adducts with primary keratinocyte stem cells and epidermal cells' DNA.[8] After a week of DMBA treatment, the third and fourth groups received five formulations of Boscia senegalensis leaf extract ointment represented by F1, F2, F3, F4, and F5.

Blood parameters: At the end of the experiment, blood samples were collected in blood collection tubes from all fasted group animals by cardiac puncture under anesthesia with pentobarbitone 100mg/kg[9] for after blood was collected, all the animals were sacrificed by overdose of anesthesia.[10] The isolated skin was washed with ice-cold saline and kept in a 10% formalin solution for histopathology studies.[11]

All the data were subjected to column statistical analysis to obtain the Mean ± S.E.M values for the group.[12] These values were used to assess whether the treatments are significant using one-way analysis of variance (ANOVA) followed by Dennett's test (Graph Pad Prism 9 for Windows, Version 9.1.2(226). The P value ($P<0.05$) and ($P<0.01$) were considered as significant.[13]

RESULT

Table 3: Preliminary evaluation of Boscia Seneglensis Leaf ointment.

Ointment formulation	pH	Viscosity	Spread-ability	Extrudability	Drug release
F-1	5.5±0.05	14428±7.26	21.3±1.25	72.2 ± 0.55	77.2±0.12
F-2	5.2±0.05	15481±18.3	21.5±1.25	61.1±1.22	74.1±0.23
F-3	5.7±0.05	16604±18.4	21.9±1.78	59.2±1.70	72.2±0.18
F-4	5.9±0.05	16802±18.4	22.8±1.90	59.3±2.10	65.3±0.91
F-5	5.8±0.05	16810±18.4	23.2±1.98	62.1±2.5	72.1±0.25

± Mean value with a standard deviation of three replicates.

a. Dermal toxicity studies OECD 402[14]

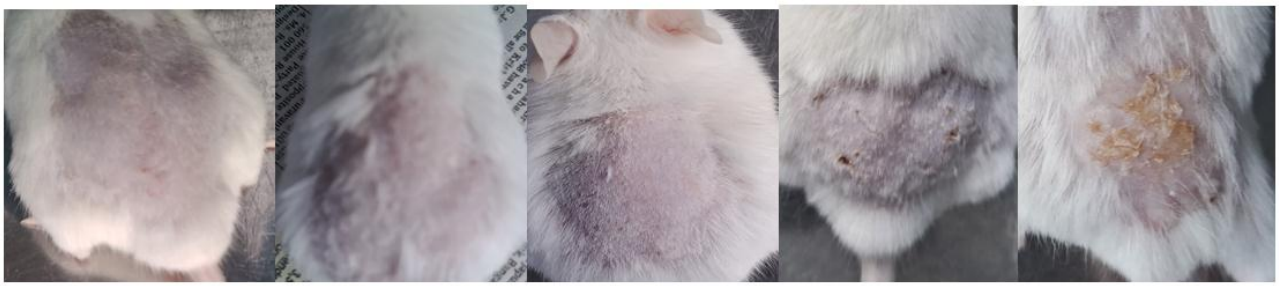
Acute dermal toxicity study of extracts. The extracts were evaluated for their acute dermal toxicity, and no erythema or edema was observed in any of the extracts. All the extracts were found to be safe.[15]

Table 4: Observation upon administration of Boscia Seneglensis Leaf ointment. To study dermal toxicity in mice.

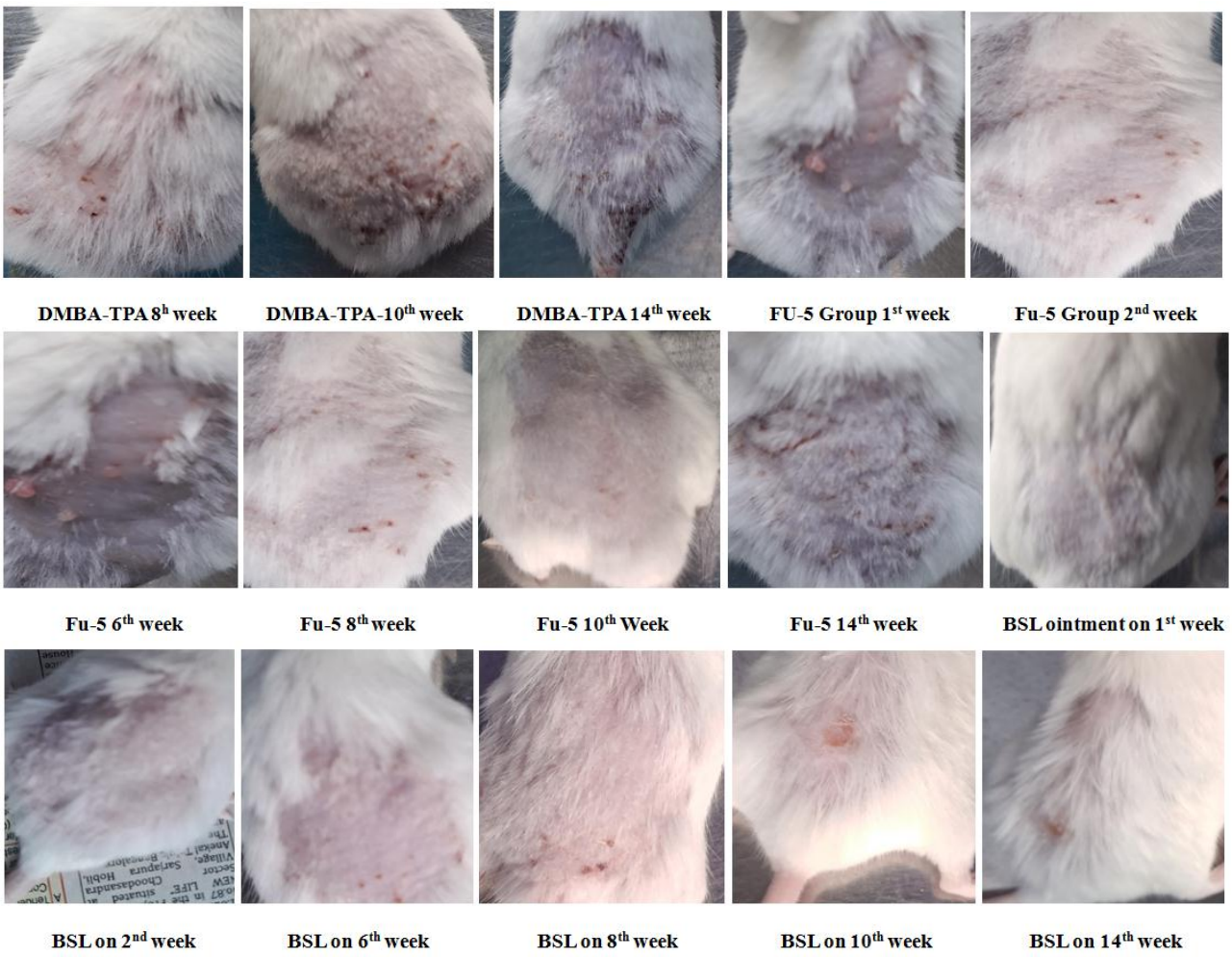
Observations	Changes	1000mg/Kg	2000mg/Kg
Fur	Norma	---	---
Eyes	Red	---	---
Mucous membrane	Normal	---	---
Respiratory	Normal	---	---
CNS	Normal	---	---

IN VIVO RESULT

Papilloma started to appear after mice were treated with DMBA. The fourth group received five different concentrations of formulations of the Boscia senegalensis leaf extract ointment, and the number of papillomas decreased compared to the control group. The Fu-5 group also Demonstrated a statistically significant decrease due to the anticancer drugs. At six weeks, most mice that had received DMBA-TPA developed papilloma, as observed morphologically, while the group that received standard control showed no change. The number of papillomas was significantly lower in the group that received BSL treatment.



Normal Group | Control Group | DMBA-TPA 1st Week | DMBA-TPA 2nd Week | DMBA-TPA-6th week



a. Histopathology studies: Certain proto-oncogenes, like Hras1(mutations in Kras and Nras have also been found), are the target of some of these random mutations. Tumor formation results from proto-oncogenes changing into oncogenes in response to specific stimuli. In turn, TPA is the most widely utilized agent that promotes tumor growth. Protein kinase C (PKC) is its molecular target. -catenin Also.

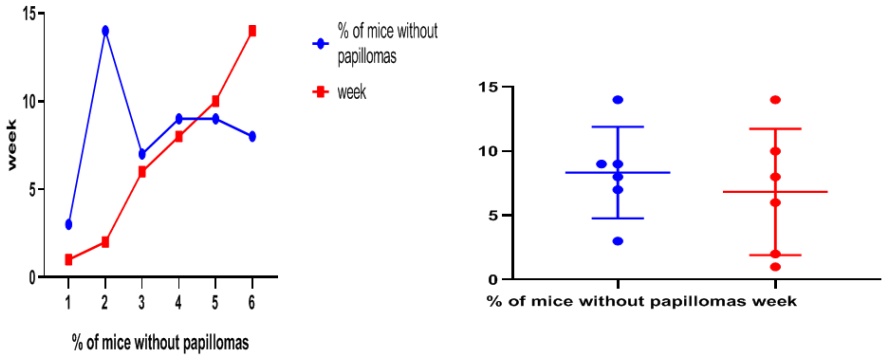


Figure 2: Animals treated with BSL ointment, Fu-5, and DMBA-TPA on their skin during the study.
Figure 2: The main outcomes of the DMBA-TPA-induced skin carcinogenesis model

The representative images of papilloma on mice skin and the two main outcomes.[16] Data were combined from four experiments. (A) a survival plot of the papilloma free time and Figure 2, represents animals after DMBA-TPA, Fu-5, and BSL ointment treatments. Fu-5: After group three received the anticancer Fu-5, the number of papilloma significantly decreased compared with the control group. When compared to the Fu-5 and DMBA-TPA groups, the number of papilloma cases decreased moderately and significantly in group 4, which received BSL ointment.

Skin Histopathology studies

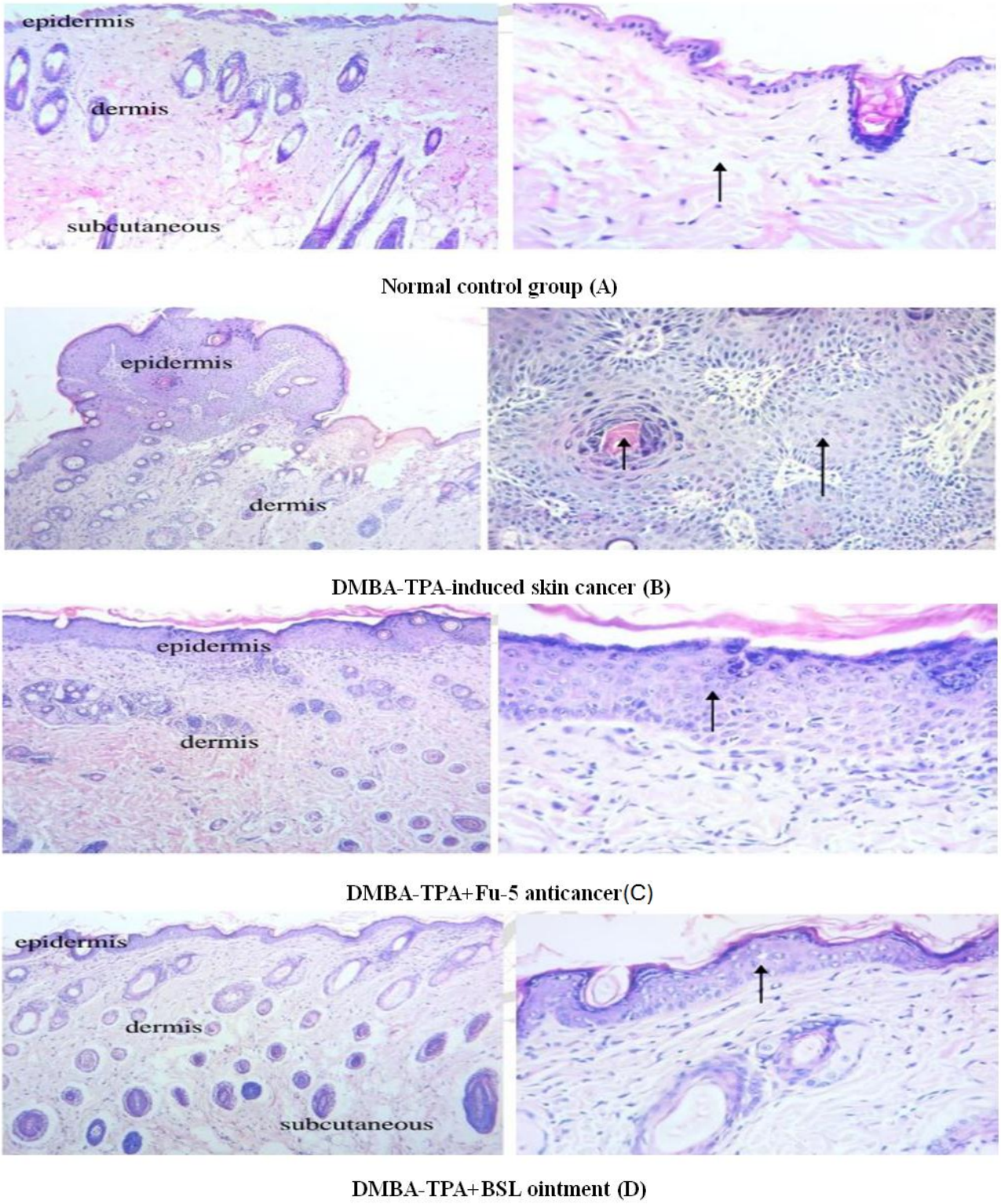


Figure 4: Report on skin histopathology following administration of DMBA-TPA, Fu-5, and BSL.

The epidermis of the average Fig.1 control group exhibits fibroblast with collagen bundles Fig.2. In group B, the epidermis has an irregular thickness of the epidermis (Tumor) with the proliferation of dysplastic cells. Group C mild chronic inflammation intact blood vessels were seen, and the application of Fu-5 significance showed a response in the treatment control group: thus, the effect of anticancer could result from the inhibition of retinoblastoma or activation of CDKN2A.[17] The D, the application of BSL ointment on skin mice affected by cancer demonstrated a noteworthy response, with animals exhibiting a decrease in the number of papilloma's Wnt/ β-catenin catenin signaling is triggered by TPA[18] and is essential for the

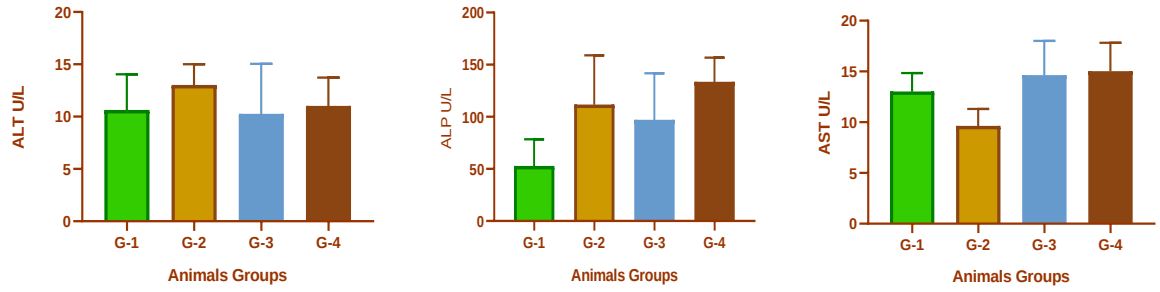
development of tumors in the model.[19] Improved cell signaling is the outcome of repeated, prolonged exposure to the promoting substance. In the promotion phase, the mutated cells' cell proliferation is enhanced, and the epidermis's sustained hyperplasia is maintained.[20] This results in the development of papilloma in the skin within 8 to 14 weeks, after which the papilloma begins to transform into squamous cell carcinomas, which are malignant tumors.[21] The genetic background also plays a crucial role in the development of papilloma.[22]

Table 5: liver parameters in animals studies.

ALP U/L				AST U/L				ALT U/L			
G-1	**G-2**	**G-3**	**G-4**	**G-1**	**G-2**	**G-3**	**G-4**	**G-1**	**G-2**	**G-3**	**G-4**
65±8.1	71±8.8	45±5.6	145±18.1	12±1.5	11±1.3	10±1.2	14±1.7	11±1.3	13±1.6	11±1.3	9±1.1
45±5.6	120±15	65±8.1	125±15.6	14±1.7	9±1.1	11±1.3	15±1.8	7±.8	14±1.7	10±1.2	12±1.5
51±6.3	131±16.3	71±8.8	124±15.5	11±1.3	12±1.5	13±1.6	17±2.1	8±1	11±1.3	7±0.8	13±1.6
63±7.8	171±19	61±7.6	160±20	13±1.6	10±1.2	14±1.7	18±2.2	9±1.1	12±1.5	8±1	14±1.7
74±8.2	121±13.4	96±12	171±21.3	17±2.1	11±1.3	15±1.8	19±2.3	8±1	17±2.1	11±1.3	7±0.8
89±9.8	141±15.6	171±21.3	120±15	12±1.5	9±1.1	16±2	12±1.5	11±1.3	14±1.7	9±1.1	8±1
16±1.7	121±13.4	121±15.1	121±15.1	13±1.6	8±1	18±2.2	14±1.7	17±2.1	12±1.5	5±0.6	11±
18±2	16±1.7	145±18.1	101±12.6	12±1.5	7±0.8	20±2.5	11±1.3	14±1.7	11±1.3	21±2.6	14±1.7

G-1 represents the standard control group. G-2 represents the DMPA-TPA group. G-3 represents the Fu-5 Group, and G-4 represents the BSL ointment. P<0.001, P<0.004, P<0.007, P<0.004.

ALT and AST are associated with not only the liver cirrhosis development but also the prognosis of hepatocellular carcinoma and liver metastasis but also some type of cancer while 4–11% of cancers use a homologous recombination-based pathway called alternative lengthening of telomerase. Treatment with DMPA-TPA significantly increased the levels of ALP, AST, and ALT in animals that received a carcinogenic substance. However, in Fu-5, the level of AST remained unchanged, with no significant change in ALT Levels compared to the standard group. ALT, AST, and ALP levels remained unchanged in the BSL group, with no remarkable effect of BSL on liver enzymes.



DISCUSSION AND CONCLUSION

Based on the findings, it can be concluded that the BSL plant extract, particularly in the form of an ointment, has shown significant potential in the treatment of melanoma cancer. The unique composition of the BSL extract, rich in phenolics and flavonoids, is believed to contribute to its potent antioxidant properties, offering promise in the fight against skin cancer.

The results from in vivo models support the potential of the BSL ointment in reducing the number of papilloma in mice. The statistical data for the BSL extract treatment group also indicates a significant reduction in toxicity outcomes over time. Further studies on the ointment of BSL extract are recommended to validate its safety and efficacy for potential use in cancer treatments. The reported number of mortalities in research in mice is statistically insignificant, indicating a promising safety profile.

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