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Development and Evaluation of a Carbopol 940-Based Polyherbal Gel for Potential Topical Anticancer Treatment

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ABSTRACT
The present study focuses on the development and evaluation of a Carbopol 940-based polyherbal gel (Formulation S5) incorporating extracts of Lantana camara, Aloe vera, Cordia obliqua, Ipomoea cairica, and Callicarpa macrophylla for potential topical anticancer applications. The gel formulations were prepared using Carbopol 940 (0.5–3%) and characterized for organoleptic properties, pH (6.1–6.9), viscosity (1415–1911 cps), spreadability (11.5–31.25 g·cm/sec), and extrudability (>80%, rated as good). Stability testing over three months confirmed physical and chemical stability under ICH conditions. In vitro cytotoxicity using MTT assay on A431 skin cancer cells demonstrated dose-dependent inhibition, with an IC_{50} of 36.64 µg/mL. In vivo anticancer efficacy was evaluated in a DMBA-induced skin carcinogenesis model in Swiss albino mice. Formulation S5 significantly reduced tumor incidence, tumor burden, and tumor yield compared to the carcinogen control group, with improved body weight maintenance. Histopathological analysis confirmed the protective effect of the formulation. The findings indicate that Formulation S5 exhibits promising anticancer activity and warrants further molecular investigations.
Keywords: *Polyherbal gel, Formulation, Stability study, Anticancer efficacy, DMBA-induced, Histopathological analysis etc*

1. INTRODUCTION

Skin cancer is one of the most active types of cancer in the present decade **(Kachuri et al., 2013).** Given that the skin is the body's largest organ; it's natural that skin cancer is the most common type of cancer in humans **(Erb et al., 2005).** It is generally classified into two major categories: melanoma and non-melanoma skin cancer **(Chinembiri et al., 2014).**Melanoma is a dangerous, rare, and fatal form of skin cancer. According to statistics from the American Cancer Society, melanoma skin cancer patients make up only 1% of total instances, but they have a higher fatality rate **(Madan et al., 2010).** Melanoma develops in melanocytes. It begins when healthy melanocytes proliferate out of control, resulting in a malignant tumor. It can affect any part of the human body. It commonly arises in places that are exposed to sunlight, such as the hands, face, neck, and lips. Melanoma malignancies can only be healed if detected early; otherwise, they spread to other body parts and cause the victim's terrible death **(Freedman et al., 2007).** There are several kinds of melanoma skin cancer, including nodular melanoma, superficial spreading melanoma, acral lentiginous, and lentigo maligna **(Chinembiri et al., 2014).**
Several attempts have been made to overcome drug resistance, including the use of nanoparticles, liposomes, and micellar drug delivery vehicles, with some reported successes **(Kunjachan et al., 2013).** The side effects of cancer chemotherapy can be treated symptomatically, but in some cases, such secondary treatments may be exceedingly toxic, which is unacceptable to some cancer patients **(Alifrangis et al., 2011; Slevin et al., 1990).** Skin cancer treatment relies heavily on local therapy strategies, and hydrogels can serve as ideal substrates **(Caló and Khutoryanskiy et al., 2015).** Hydrogels have recently been researched in medicine and pharmacy because of their desired biocompatibility and physicochemical features, such as softness, high water content, and flexibility **(Nair et al., 2021).** Hydrogelscan be formed from synthetic, semi-, and natural polymers chemically or physically cross-linked. Their resemblance to living tissue can find enormous biomedical applications. The principal problem with common melanoma chemotherapy is the strong side effects, because neoplastic factors do not recognize cancer cells from healthy cells. For example, some of the side effects of treating melanoma cancer with chemotherapy and immunotherapy are nausea, vomiting, kidney damage, lethargy, cellular depression, stomach discomfort, dermatitis, hepatitis, and infection **(Huang et al., 2020).** The side effects of conventional therapies encourage the search for novel therapies for cancer cells. Recently, hydrogel has been applied for tissue engineering scaffolds, wound dressings, and drug delivery systems. These percutaneous drug delivery systems are emerging as a promising alternative strategy for carrying anti-neoplastic agents to prevent side effects. The aim of this research is to create an effective and stable topical gel that can inhibit skin cancer

growth and reduce tumor incidence, burden, and yield in a DMBA-induced skin carcinogenesis model **(Valizadehm et al., 2021).**

2. MATERIALS AND METHODS

1.1 Plant authentification and extraction techniques

All the studies related to the photochemistry, the plant authentification and the extraction method is already published in the another paper **(Ojha and Ghule, 2024).** This is the next portion of the studies to be continued.

1.2 Formulation of Gel containing extract of Lantana camara, Aloevera, Cordia obliqua, Ipomoea cairica, and Callicarpa macrophylla

Carbopol 940-based hydrogels were made using herbal extracts of Lantana camara, Aloevera, Cordiaobliqua, Ipomoea cairica, and Callicarpa macrophylla. The current hydrogels were created by adding Carbopol 940 at a concentration of 0.5%-3%. Triethanolamine and propylene glycol were employed as pH neutralizers and wetting agents, respectively. First, distilled water was slowly added to Carbopol 940. Later, predetermined volumes of extract were mixed separately with propylene glycol. Finally, Carbopol 940 solution was combined with a solution containing herbal extracts, and 3-4 drops of tri-ethanolamine were added while stirring continuously until the hydrogel was homogeneous **(Nandgude et al., 2008).**

Table1 : Composition table Formulation of gel

Ingredients	G1	G2	G3	G4	G5	G6
Lantana camara; Aloevera; Cordiaobliqua; Ipomoea cairica and Callicarpa macrophylla extracts	1:2:1:1:1 (100 mg each extract)	2:1:1:1:1 (100 mg each extract)	1:1:2:1:1 (100 mg each extract)	1:1:1:2:1 (100 mg each extract)	1:1:1:1:2 (100 mg each extract)	2:1:1:1:2 (100 mg each extract)
Carbopol 940	0.5%	1%	1.5 %	2%	2.5%	3%
Propylene glycol	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
Methyl paraben	0.15 g	0.15 g	0.15 g	0.15 g	0.15 g	0.15 g
Propyl paraben	0.30 g	0.30 g	0.30 g	0.30 g	0.30 g	0.30 g
Triethanolamine	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Water	q. s up to 100ml	q. s up to 100ml	q. s up to 100ml	q. s up to 100ml	q. s up to 100ml	q. s up to 100ml

1.3 Characterization of hydrogel

The physical and rheological properties of the produced gels and control were evaluated using several tests. The organoleptic test assessed the physical appearance and homogeneity visually by dividing tiny amounts of hydrogel and massaging small quantities on the hand's back **(Nandgude et al., 2008).** The pH was measured using a digital pH meter to ensure it ranged between 6.1 and 6.9 for skin compatibility **(Nappinnai et al., 2006).** Viscosity was determined using a Brookfield viscometer at 20 rpm with spindle 60 in a 24.8°C water bath, with viscosity levels below 15,000 cps indicating better cosmetic appeal and ease of application **(Panigrahi et al., 2006).** Spreadability was evaluated using two glass slides with a 100 g weight, and the time to separate the slides was measured to calculate spreadability using the formula $S = m \times L / T$ **(Blonco-flonte et al., 1996).** Extrudability was assessed by filling gels into collapsible aluminum tubes, applying 500 g, and rating extrudability as Excellent (>90%), Good (>80%), and Fair (>70%) **(Bhanja et al., 2013).**

1.4 Stability testing

The stability test was performed in accordance with the ICH requirements. The gels were packaged in airtight containers and maintained in a stability chamber for three months at 40°C and 75% RH. After one month, the samples were extracted and tested for pH, appearance, nature, grittiness, spreadability, viscosity, odor, and extrudability **(Rathodet al., 2015).**

1.5 MTT assay

1.5.1 Assay controls:

The MTT assay was conducted to evaluate cell viability. Cells were seeded at 20,000 cells per well in a 96-well plate and incubated for 24 hours without the test agent. Significant concentrations of the test agent were then added, and the plate was incubated for another 24 hours at 37°C in a 5% CO2 atmosphere. Spent media were removed, and MTT reagent (0.5 mg/mL) was added. The plates were wrapped in aluminum foil and incubated for 3 hours. MTT reagent was removed, and 100 µL of DMSO was added to dissolve the formazan crystals, which were gently stirred or pipetted if necessary. Absorbance was measured at 570 nm using a spectrophotometer or ELISA reader. Controls included a medium control (no cells) and a negative control (cells without the test agent) to account for background MTT reduction by extracellular reducing agents.

1.6 In vivo Anticancer Activity

2.5.1 Animals and treatment

Swiss albino mice of weighing 25±5g were selected and procured from PBRI animal house. The animals were maintained under standard conditions of humidity, temperature (25±2 ◦C) and light (12 h light/dark). They were fed with standard pellet diet and water ad libitum.

2.5.2Induction of tumor

Swiss albino mice were divided into three groups, with six animals in each group. All animals were treated with a single dose of DMBA (100 µg/100 µL of acetone) over the shaven area of the skin. Two weeks later, croton oil (1% in 100 µL of acetone) was applied as a promoter three times per week until the end of the experiment (16 weeks). Group I (Normal Control) received vehicle (acetone) over the shaven area of the skin. Group II (DMBA + Croton Oil) was treated with a single dose of 100 µg of DMBA in 100 µL of acetone, followed by the application of croton oil (1% in acetone) three times per week for 16 weeks. Group III (Formulation S5 + DMBA + Croton Oil) was topically applied with Formulation S5 four hours before DMBA application and continued to receive the formulation until the end of the experiment (16 weeks). The investigation parameters included tumor incidence, tumor yield, tumor burden, and body weight.

2.6 Histopathology

Skin tissue was immersed in 10% formalin for histopathological examination. The tissues were processed, dehydrated in graded alcohols, cleared in toluidine, and impregnated in paraffin wax. They were embedded in fresh paraffin wax, sectioned at 3 µm, dried on a hot plate for 15 minutes, and stained with hematoxylin and eosin to highlight tissue structure. Stained slides were dehydrated in ascending alcohols, cleared in xylene, and mounted in Canada balsam. Sections were viewed microscopically using ×10 objective lense **(Yang et al., 2014).**

2. RESULTS

2.1 Characterization of hydrogel

2.1.1 Homogeneity and appearance:

Table 2: Physical characterization of formulations

S. N	Parameters	G1	G2	G3	G4	G5	G6
1.	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
2.	Grittiness	Non gritty	Non gritty	Non gritty	Non gritty	Non gritty	Non gritty
3.	Appearance	Translucent	Translucent	Translucent	Translucent	Translucent	Non gritty
4.	Color	Light brown	Dark brown	Slightly brownish	Brownish	Brownish	Brownish
5.	Odor	Stringent	Stringent	Stringent	Stringent	Stringent	Stringent

It was observed that the freshly prepared formulations were light brown to dark brown in color . All the formulation possesses stringent odor with translucent in appearance.

On careful visual inspection against dark and white background, all the prepared dermal gel formulations were found to be free from any suspended particulate matter. All the formulations were found to be translucent gel. The texture was non greasy and smooth in touch.

2.1.2 pH

Table 3: pH of formulations

S. N	Parameters	G1	G2	G3	G4	G5	G6
1.	pH	6.1±0.021	6.2±0.42	6.4±0.11	6.6±0.31	6.7±0.22	6.9±0.25

The pH of the optimised formulations was found to be in the range of 6 to 7 ideally, the dermal gel should possess pH in the range of 6-7.The pH of the formulation ranges from 6.1±0.021 to 6.9±0.25

2.1.3 Viscosity determination

Table 4: Rheological study of formulations

S. N	Parameters	G1	G2	G3	G4	G5	G6
1.	Viscosity	1415±0.85	1597±0.74	1688±0.44	1758±0.61	1847±0.45	1911±0.23

Viscosity and Rheological properties of the formulations were found to be 1415±0.85 to 1911±0.23 as the carbopol concentration increases the viscosity of the gel was increase.

2.1.4 Spreadibility

Table 5: Spreadability test of formulations

S. N	Parameters	G1	G2	G3	G4	G5	G6
1.	Spreadability	11.5±0.02	18.74±0.11	21.35±0.18	26.32±0.14	28.99±0.21	31.25±0.24

Spread ability of the base and formulations were studied and found to in the range of 11.5±0.02 to 31.25±0.24.All the formulations and base were found to possess good spread ability

2.1.5 Extrudability

Table 6 : Extrudability test of formulations

Formulation	Weight of formulation	Weight of gel extruded	Extradibility amount (%)	Grade
G1	15.2	13.1	86.18	Good
G2	15.64	12.9	82.48	Good
G3	15.95	13.42	84.13	Good
G4	15.26	13.15	86.17	Good
G5	15.23	12.7	83.38	Good
G6	15.24	13.22	83.46	Good

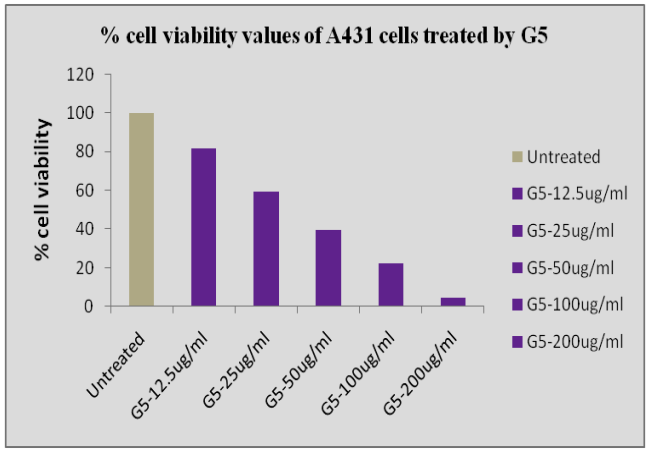
2.2 Stability testing

Table 7: Accelerated stability study

(G5) accelerated stability study			
Parameter	**1Month**	**2Month**	**3Month**
Appearance	Brownish	Brownish	Brownish
Nature	Homogeneous	Homogeneous	Homogeneous
Phase Separation	Nil	Nil	Nil
pH	6.7±0.22	6.7±0.66	6.66±0.78
Viscosity	1847±0.45	1866±0.68	1867±0.32
Spreadability	28.99**±0.21**	28.72±0.31	28.11±0.33
Extrudability	83.38	83.7	83.86

The temperature had no effect on the stability of formulations, which retained their integrity and physical properties. The pH ranged from 6 to 7. All formulation parameter value are within range n=3.By observing three-month stability study of formulation, there is no considerable change in stability of formulation throughout study, this formulation was stable for three months at 40°Cand 75%RH.

2.3 MTT test results



Graph 1 : Overlaid bar graph showed the % cell viability values of A431 cell lines cured with various concentrations of G5 after the 24hours of incubation.

2.4 Invivo Anticancer Activity

3.4.1 Body weight changes

Table 9: 1 Variations in body weight in Control and Sample-Formulation S5treated groups

Group No.	Treatment	0wk	2wk	4wk	6wk	8wk	10wk	12 wk	14 wk	16 wk
I.	Normal Control	25.05 ±0.432	26.63± 0.385	28.62± 0.339	29.97± 0.349	31.68± 0.291	33.10± 0.340	34.72± 0.442	36.18± 0.553	38.50± 0.716
I.	DMBA	25.53 ±0.572	27.22± 0.641	28.37± 0.681	29.40± 0.690	27.55± 0.612	25.55± 0.391	23.96± 0.353	22.29± 0.384	19.42± 0.568
I.	Formulation S5	26.59 ±0.896*	27.31± 0.675NS	27.77± 0.852NS	28.93± 1.007NS	30.37± 1.077**	31.57± 1.155**	33.54± 1.263**	35.32± 1.443**	36.28± 1.244**

Values are expressed as MEAN±SE at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ^{NS}P>0.001 compared to the tumor control DMBA treated

The body weight changes during the study displayed in Table: 9. No significant difference could be detected between tumor control group and test sample treated group.

3.4.2 Tumor Parameter

3.4.2.1 Tumor Incidence (%)

Table 10: Variations in tumor incidence (%) after DMBA induced Skin carcinogenesis

Group No.	Treatment	2wk	4wk	6wk	8wk	10wk	12 wk	14 wk	16 wk
I.	Normal Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
II.	DMBA	0.00	0.00	83.33	100.00	100.00	100.00	100.00	100.00
III.	Formulation S5	0.00	0.00	0.00	50.00	100.00	100.00	100.00	100.00

III.4.2.2 Tumor yield

Table 11: Variations in tumor yield after DMBA induced Skin carcinogenesis

Group No.	Treatment	2wk	4wk	6wk	8wk	10wk	12 wk	14 wk	16 wk
I.	Normal Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
II.	DMBA	0.00	0.00	0.83	2.00	3.33	4.50	5.33	6.67
III.	Formulation S5	0.00	0.00	0.00	0.50	1.17	1.83	3.00	3.00

III.4.2.3 Tumor burden

Table 12: Variations in tumor burden after DMBA induced Skin carcinogenesis

Group No.	Treatment	2wk	4wk	6wk	8wk	10wk	12 wk	14 wk	16 wk
I.	Normal Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
II.	DMBA	0.00	0.00	1.00	2.00	3.33	4.50	5.33	6.67
III.	Formulation S5	0.00	0.00	0.00	1.00	1.17	1.83	3.00	3.00

In this two-stage skin carcinogenesis study, the tumor yield was significantly reduced in mice treated with Formulation S5 compared to the DMBA-treated control group ($p<0.001$). Formulation S5 showed the most potent anticancer activity against the carcinogen. The body weight of mice in the carcinogen group decreased significantly. Single topical application of DMBA followed by croton oil induced skin papillomas starting from the sixth week, reaching 100% tumor incidence by the end of the 16-week experiment. Formulation S5 treatment reduced the cumulative number of papillomas, tumor burden, and tumor yield compared to the carcinogen control group. A significant reduction in tumor incidence, tumor burden, and tumor yield was observed in the Formulation S5-treated groups. These results suggest that Formulation S5 has anticancer activity in a skin carcinogenesis model, and further investigation into the underlying molecular mechanisms is warranted.

CONCLUSION

The study successfully developed and characterized a Carbopol 940-based polyherbal gel formulation incorporating extracts of Lantana camara, Aloe vera, Cordia obliqua, Ipomoea cairica, and Callicarpa macrophylla. The formulated gel demonstrated desirable physicochemical properties, including optimal pH, viscosity, spreadability, and extrudability, ensuring suitability for topical application. Stability testing confirmed the formulation's physical and chemical stability over three months under accelerated conditions. In vitro cytotoxicity evaluation via MTT assay on A431 human skin carcinoma cells revealed a significant dose-dependent reduction in cell viability, indicating potent anticancer potential. Furthermore, in vivo studies using a DMBA-induced skin carcinogenesis model in Swiss albino mice showed that the S5 gel formulation significantly reduced tumor incidence, yield, and burden compared to the carcinogen control group. These findings suggest that the S5 polyherbal gel possesses promising anticancer activity, making it a potential therapeutic candidate for skin cancer management. Further molecular investigations are warranted to elucidate the underlying mechanisms of its anticancer efficacy.

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