Title: "Fabrication of Novel Biopolymer-Based 5- Fluorouracil Transdermal Biodegradable Films Against Skin Carcinoma"

Introduction:

Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and multiply (through a process called cell division) to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. Skin cancer is the common form of cancer mostly develops on the skin exposed to the sun. It has the rate of 2.6 percent for whites and 0.1 percent for blacks. Problems due to cancer is faced by all over the world especially in the United States and can be threatening and if not treated on time, can be fatal.

Anticancer drug, also called antineoplastic drug, any drug that is effective in the treatment of malignant, or cancerous, disease. Novel delivery systems have made this possible by the clinical use of new therapeutics, have permitted cancer treatments that has increased the efficacy and significantly reduced side effects, and have enabled new and better chemotherapeutic regimens using existing pharmaceuticals.

Objectives:

- Preparation of transdermal film by solvent evaporation process using biopolymer (Manihot esculenta) polymers so as to enhance the bioavailability of 5 – Fluorouracil and reducing the side effects.
- Subjected to the analysis of the physiochemical properties such as FTIR, SEM, DSC, etc.
- To investigate any potential skin irritation caused by the formulation in animals (Wistar rats).
- In-vitro diffusion study of the selected formulation.

Materials and Methods:

Materials:

Drug – Fluorouracil

Other Excipients :Eudragit RL 100, Eudragit RS 100, Methanol, Acetone, Ethanol, HPMC 15cps, HPMC K100, HPMC K4M

Method of Preparation:

Film was prepared by the Solvent casting method

Preparation of 5-Fluorouracil anticancer transdermal films

- A solvent evaporation process was used to create the transdermal film. Polymer(biopolymer,) plasticizer (Sorbitol, PEG 400), and a penetration enhancer (Dextrose), were dissolved in 6 ml of water to form the film-casting solution.
- 1% w/w of the drug was dissolved in the 8ml of water and sonication for 10-15 minutes.
- The resulting uniform solution is poured into a Petri dish with a diameter of 6cm.

- The solvent was allowed to evaporate and kept in a dark place (drug is light-sensitive) at room temperature. The films took approximately 48 hours to completely dry.
- After 48 hours, all of the dried films were hauled out and wrapped in aluminium foil before being kept in a desiccator at room temperature. As a result, the produced films adhered to the bandage's adhesive layer.

Formulation table of transdermal films

Ingredient	F1	F2	F3	F4	F5	F6
5-FLUOROURACIL (%w/w)	1	1	1	1	1	1
BIO-S (mg)	500	500	500	500	500	500
DEXTROSE (mg)	100	150	200	200	200	200
SORBITOL (ml)	0.4	0.4	0.4	0.4	0.4	0.4
SLS (sodium lauryl sulfate) (%w/w)	0.1	0.1	0.1	0.2	0.3	0.4
WATER (ml)	10	10	10	10	10	10

Treatment groups :

Group Number	Group Name	Treatment
1	Vehicle Treated Group	Croton oil
2	Induced Group	DMBA+ NDEA+ UV+ Formaldehyde +Benzoyl Peroxide
3	Blank Patch Group	DMBA+ NDEA+ UV+ Formaldehyde +Benzoyl Peroxide + Blank Transdermal Patch for 3 weeks
4	Treatment Group	DMBA +NDEA +UV +Formaldehyde +Benzoyl Peroxide + 5 FU Transdermal Patch for 3 weeks
5	5 FU Treated Group Topically	DMBA+ NDEA+ UV+ Formaldehyde +Benzoyl Peroxide + 5 FU for 3 weeks

Preparations for the Stock Solution: -

DMBA: - 1gm vail containing DMBA powdered form were mixed with croton oil 10ml solution marked up to 10ml mixed properly using the vortex. Kumar et al.[27]

Formaldehyde: - 37% Formalin solution was taken. 10% formaldehyde solution was prepared by taking formalin solution around 10% of the volume and the rest 90% was taken is distilled water. Iversen[21]

Benzoyl Peroxide: - Benzoyl peroxide solution was prepared by using 70% ethanol solution in distilled water. Benzoyl peroxide is soluble in ethanol. Benzoyl peroxide was in the pellet form and dissolved in the 70% ethanol solution. Ethanol solutions were prepared by taking the ethanol and distilled water ratio around 70:30. Around 2mg/ml was the ratio taken for preparing the solution. Mathur et al.[30]

NDEA: - 200ng/ml of the NDEA solution is prepared by diluting the NDEA in the distilled water as per the calculation. Zhang et al.[62]

UV (TYPE B): - UV- B exposure is given through the UV lamp range of 320nm.

Results:

Spectrophotometric scanning of 5-Fluorouracil - By scanning 10 µg/ml concentration solution in the range of 200-800nm, λmax of 5- Fluorouracil in pH 7.2 phosphate buffer was found to be 265nm

FT-IR spectroscopy - The spectra were taken of pure drug 5- Fluorouracil, extracted biopolymer(sago), polymers were done and the spectra reveal that there is no interaction between the 5- Fluorouracil and the polymer used or that the drug and the polymers are compatible with each other.

DSC studies – The report demonstrates that 5- Fluorouracil signifies a single and sharp endothermic peak at 291.73°C which is comparable to its melting point or transition temperature. DSC analysis is essential for subsequent drug excipient interaction studies to investigate polymer compatibility.

Solubility studies- The solubility study data of 5-Fluorouracil are as follows:

S. No	Medium	Solubility(mg/ml)
1.	Water	0.21±0.33
2.	NH_4OH	36±0.19
3.	DMSO	47±0.23
4.	Phosphate buffer 7.2	38±0.55
5.	Phosphate buffer 6.8	19±0.39
6.	Ethanol	0.8±0.73
7.	Methanol	0.9±0.88

#Each value has been displayed in mean ± SD(n=3)

EVALUATION PARAMETERS

The results of the characterization of the transdermal films given below in the Table:

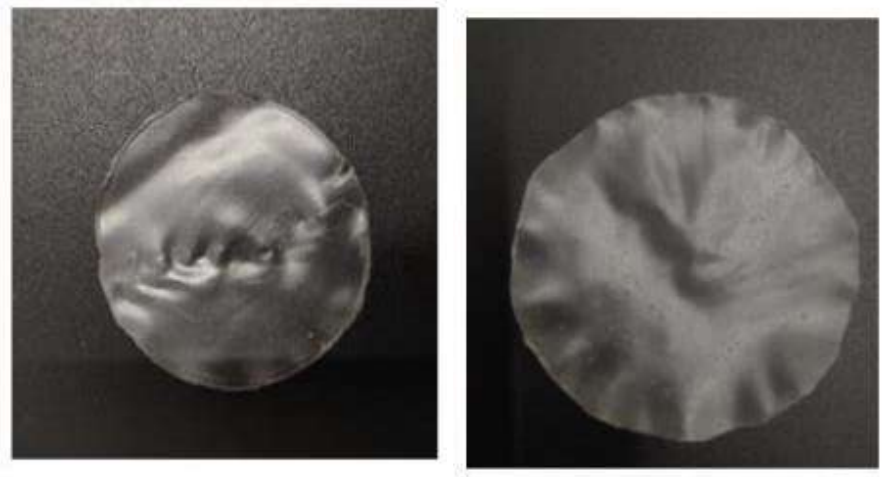
Formulation code	Thickness (mm) (mean ± SD) (n=3)	FE (mean ± S.D), n=3	Weight (mg) (mean ± S.D), n=3	PMC (%) (mean ± S.D), n=3	PMA (%) (mean ± SD.), n=3	Tensile strength (kg/cm^2) (mean ± S.D)	Drug content (%) S.D (n=3)	pH ± S.D (n=3)
F1	0.21±0.052	98 ±8	156.3±0.74	1.03±0.42	2.22±0.11	0.33 ± 0.013	99.15±3	6.24±0.09
F2	0.28±0.064	104 ±5	143.2±0.66	1.44±0.34	2.53±0.32	0.44 ± 0.015	99.77±5	6.40±0.07
F3	0.24±0.019	113 ±3	164.4±0.81	1.23±0.21	3.29±0.25	0.96 ± 0.016	99.22±2	6.32±0.05
F4	0.26±0.032	101 ±3	161.3±0.55	1.96±0.14	2.77±0.45	0.33 ±0.014	98.61±7	5.94±0.10
F5	0.22±0.049	112 ±7	153.8±0.93	1.87±0.56	2.65±0.56	0.22 ± 0.023	98.92±4	6.62±0.09
F6	0.25±0.055	105 ±9	166.1±0.29	1.74±0.22	2.84±0.87	0.66 ± 0.016	99.04±1	6.48±0.07

*FC=Formulation Code, ** Folding Endurance = FE, *** Percentage Moisture content=PMC, **** Percentage Moisture Absorption=PMA

EVALUATION PARAMETERS

Fabricated 5-Fluorouracil transdermal biofilms

All the films were visually assessed for their uniformity, surface texture, flexibility, clarity, and smoothness, and free from the entrapment of air bubbles. as illustrated in Fig. below



Skin irritation test:

The skin irritation test was evaluated visually. It was discovered that erythema in group II (Transdermal patch applied group) was insignificant as compared to the group I (Normal). The

transdermal film was applied, and the application site was covered with non-sensitizing microporous tapes. The film was removed after 24 hours, and the erythema score was recorded and compared to the Normal. No significant changes were observed between both the group. As a result, it may be stated that the formulation causes no skin irritation.

Fig. 2. Image of Wistar Rat skin after testing skin irritation test

In-vivo studies

Anticancer evaluation of transdermal patch

- Evaluation of transdermal patch is done through the parameters such as to evaluating the efficacy of drug loaded transdermal film, we used our developed skin carcinogenesis model for evaluation of the efficacy of transdermal patch we used some parameters such as biochemicals, Morphological, histopathological assessments were done.
- The drug loaded transdermal patch (5-FU) were applied on the animals of Group IV for the treatment. The patch was applied once a day for period of week 3 consecutively for treatment. Similarly, in the Group Unloaded Transdermal patch is applied for the duration of 4 Weeks. The Group III does not show the normalization in comparison to the Group IV.

ANIMALS
Morphology

Weight variations:

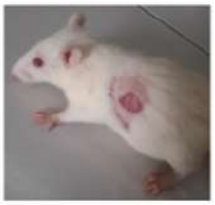
Weight of Animals

Experimental Groups	Initial Weights	Final Weight
Vehicle treated Group	157.5 ± 3	212.7 ± 5
Positive Control Group	161.1 ± 4	214.2 ± 3
Blank Patch Group	168.2 ± 5.2	221.5 ± 2
5FU Patch Treated Group	165.81 ± 8	224.12 ± 6
5FU Treated Topically Group	169.54 ± 2.5	225 ± 3.5

- In the skin cancer studies there were no significant variations observed in the weight of animals.
- After the completion of studies, it was observed that there were no major variations in weight among all 5 groups of the rats. The model used in this study is a rapid carcinoma development model which does not reveal any significant change at the cancer progression stage after 4-6 weeks of induction.

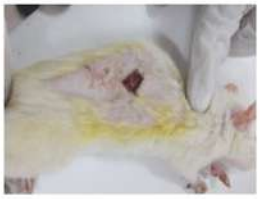
Developed Cancer Model

Tumor Model



Week 1

Week 4



Week 5

Histopathological Analysis

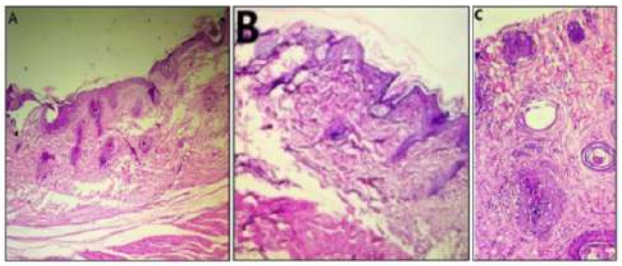


Fig 6.3 (a)

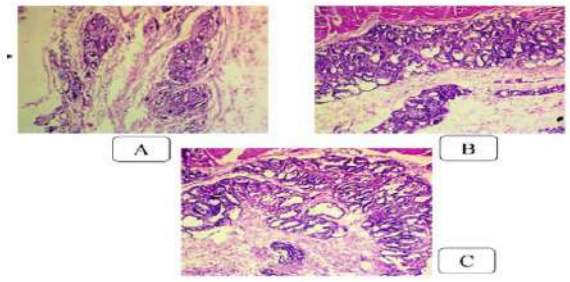


Fig6.3(b)

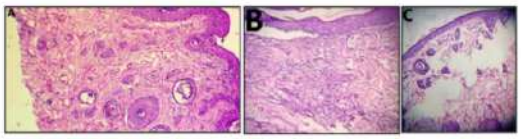


Fig6.3(c)

Histopathological Analysis

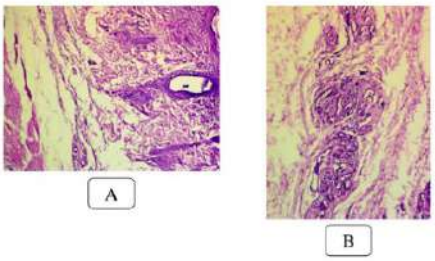


Fig 6.3(d)

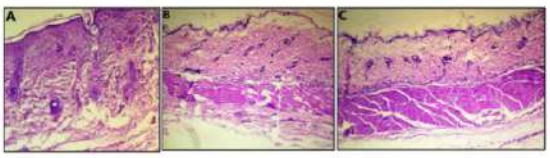


Fig 6.3 (e)

Histopathological Analysis:

- **Figure 6.3 Histopathological anlaysis (a) Vehicle treated group of animals (b) Skin cancer induced of animals (c) Skin cancer induced animals treated blank transdermal patch (d) Skin cancer bearing of animals treated with 5 FU transdermal patch (e) Skin cancer induced animals treated with 5 FU solution**
- Histopathological observations showed the initiations, growth and proliferation of the cancerous cells on the skin cells. In figure 6.6 (a) Histopathology of vehicle treated group shows normalization in cells. According to the histopathology analysis we can see the development of the SCC in figure 6.3 (b) Skin cancer Induced group and (c) Blank Transdermal patch group, shows the maximum growth and of necrotic tumor cells, hyperplasia, cell proliferation, hyperkeratosis, enlarged cells. All these changes were reduced in the (Group V) figure 6.3(e) and the maximum reductions occurs in the drug loaded patch treatment Group IV) figure 6.3 (d).
- Skin sections of all the group animals were obtained for histopathological estimations. The microscopical analysis of induced group II (wound + DMBA/croton oil-treated + NDEA + benzoyl peroxide + formaldehdye + UV) rats showed that there was a gradual

increase in cell proliferation, hyperplasia, hyperkeratosis, dysplasia, papillary pattern of projection, enlargement of cells, variability in nuclear size and shape, increased number of blood vessels, inflammatory cells of skin epidermis.

- These results are similar to previous skin carcinogenesis models in experimental . 5-FU patch treated group IV rat showed an intact epidermis, suggesting the anti-proliferative effect after the treatment with 5-FU patch. The treatment with transdermal patch in group IV revealed more restorative changes as compared to group V animals.
- The treatment with blank transdermal patch revealed that biopolymer based transdermal patch does not have any anticancer property.

Future prospective:

Cancer is a wide word that encompasses numerous forms of malignancies that can affect any region of the body. Cancer treatment must incorporate targeted chemotherapeutic drugs to the specific target cell while minimizing adverse effects and preventing chemotherapeutic agent resistance. Transdermal biofilm has overcome these challenges.

• More polymer research with therapeutic techniques will lead to more application in cancer therapy in the future.

• Possibilities of the target treatment can be enhanced as biopolymer-based drug loaded transdermal patches are more efficacious, bio compatible, biodegradable and more improved treatment.

• As in this study in-vivo and invitro studies were performed. This research can proceed for further animal studies for a large group and may be subjected to human clinical trials.

• After clinical trials, this isolated biopolymers transdermal films can go for patent and market formulation.

• In the future, treatment can become more reachable, and cost-effective and most populations can avail the treatment of skin cancer presently not possible.