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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF IN VIVO WOUND HEALING ACTIVITY OF BIOEPOXY RESINS OF COTTON SEED OIL

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ABSTRACT
Bio-based monomers and polymers have been of interest for the synthesis of new biomaterials owing to their low carbon footprint and wide availability. Among the vegetable oil-based polymers, Bio-epoxy resins obtained from vegetable oils are the most important polymer. In the present study, bioepoxy resin was synthesized from epoxidized cottonseed oil (ECO) and evaluated for potential wound-healing activity. The cottonseed oil was subjected to epoxidation using hydrogen peroxide and acetic acid. Bioepoxy resins were prepared using citric acid (CA) and tartaric acid (TA) acting as biobased curing agents. Four bioepoxy resin films (A-D) were prepared using ECO, CA, TA and ofloxacin (OFL). Evaluation of various physicochemical parameters i.e. folding endurance, surface pH, swelling index, moisture absorption, water permeation and mechanical properties showed that the bioepoxy resins are suitable for use as wound healing dressing. The Wound healing potential was screened using excision wound model on Wistar albino rats. All the animal groups treated with bioepoxy resins (A-D) showed significant improvement ($p<0.05$) in the percentage wound contraction rate and time of epithelisation as compared to control. Group III treated with film D (ECO + TA + OFL) demonstrated highest rate of wound contraction (7.4% to 53.72%) and shortest time for epithelization (5.66 + 0.22 days). Histopathological investigation of wound tissues samples revealed that groups treated bioepoxy resins had significant granulation, remodelling and contraction with horizontally arranged fibrous connective tissue. Thus, bioepoxy resin film dressings of prepared from epoxidized cottonseed oil can serve as an alternative for wound dressing films in the treatment of excision wounds.

Keywords: cottonseed oil, epoxidation, bioepoxy resin, wound healing,

INTRODUCTION
The market's rising demand for new materials is what propels modern technological advancements in the search for novel properties and functions of materials[1]. Due to their low carbon footprint and widespread availability, bio-based monomers and polymers are an intriguing area for chemists to explore when creating new biomaterials[2]. Due to their accessibility, affordability, and sustainability, vegetable oils derived from various natural

resources, such as soybean, castor, or canola crops, proved to be promising raw materials for the production of both chemicals and bio-based polymers. Among the vegetable oil-based polymers, Bio-epoxy resins obtained from vegetable oils are the most important polymer[3,4].

Epoxidation is one of the most significant reactions used for the chemical modification of triglycerides present in vegetable oils. Such bio-based epoxy resins are being explored for biomedical applications owing to their high and low water uptake properties, excellent mechanical properties, and biodegradation rate.

In order to approximate pre-wound characteristics, wound healing involves restoring the structure and function of an injured tissue. The number of complications and speedy return to normal function will be reduced by effective wound management[5]. In some industrialized countries, 70% to 90% of people, and in the majority of developing nations, 70% to 95% of the population, use traditional medicine as their primary form of healthcare to meet their healthcare requirements[6]. The Ethiopian folk medicine for wound management mentions a few medicinal plants that have been scientifically proven to be used to treat wounds[7].

With technological advancement, various types of wound dressings are available such as tissue-engineered substitutes, medicated moist dressings, biomaterials-based biological dressings, and medicated sutures, biological and naturally derived dressings, etc[8-11]. However, not all meet the requirements of ideal wound dressing material. Considering the limitations of the various types of wound dressing, there is a need for the development of more effective as well as cost-effective dressings.

Gossypium herbaceum (cotton) Linn, belongs to the Malvaceae family, cultivated in India and other parts of the world. Cottonseed oil is extracted from the seeds after the removal of cotton lintiles. Cottonseed oil is used in the industries for soap, and glycol as edible oil. Also, cottonseed oil is suitable as a raw material for biodiesel production.[12] The plant has been evaluated and found to be effective as diuretic, haematinicc, astringent, drug to treat bronchitis, antidysenteric, expectorant, wound healing agent, and agent for prevention of excessive bleeding during menstruation, and anti-inflammatory. The principal constituent of the plant is gossypol.[13] Cottonseed oil is used in the manufacture of margarine, salad oil and dressing, and blended oil and hydrogenated fat. It is used as a frying oil for frying potato chips and other snacks and in the formulations of bakery products. The oil has a relatively high smoke point to be suitable as a frying medium. Almost 20 percent of oil from cottonseeds contains oleic acid, an omega-9 fatty acid that's found naturally in vegetable fats. Oleic acid is known for its ability to reduce blood pressure and cholesterol. It may also help prevent type 2 diabetes, fight infections, and promote brain function.

The present investigation aims to develop a formulation of bio-based epoxy resins synthesized from epoxidized cottonseed oil as potential wound-healing dressing material. The bio-based epoxy resins were prepared using citric acid (CA) and tartaric acid (TA) acting as biobased curing agents. The prepared bio epoxy resins were then characterized and evaluated for in vivo wound healing activity on Wistar albino rats using an excision wound model.

MATERIAL AND METHODS

Materials

The chemicals/reagents were purchased from SD Fine Chemicals and Spectrochem, India.

Trimethylolpropane triglycidylether was procured from Sigma Aldrich, Canada. The cottonseed oil was procured from Vijaya Agro Chemicals, Sangamner, India and used as received.

Synthesis of Bioepoxy Resins

Epoxidation of cottonseed oil

Epoxidation of cottonseed oil was performed by using 12 g cottonseed oil, 5.28 mL toluene, 2.64 g amberlite 15 (strongly acidic, polymeric catalyst) 1.58 ml of glacial acetic acid were added taken into a round bottom flask. The apparatus was maintained at 80^0C in a water bath. 8.78ml of 30% hydrogen peroxide was added slowly with constant agitation. The reaction was continued further for the desired time duration. The reaction mixture was dissolved in ethyl acetate and then amberlite was filtered off. The filtrate was poured into a separatory funnel and washed repeatedly with warm water until the pH was neutral. The oil phase was further dried over above anhydrous sodium sulfate and was then filtered. The solvent was removed using a rotary evaporator.

Synthesis of bioepoxy resins from epoxidized Cottonseed oil

The solution polymerization method was used to synthesize four bioepoxy resin films (Table 1). 1.55 g citric acid or 1.20 g tartaric acid (epoxy equivalent weight: acid equivalent weight ratio of 1:1) and 10-15 mL of THF were added in a three-necked round bottom flask fitted with a reflux condenser. The reaction mixture was stirred continuously to dissolve citric or tartaric acid. 10 g epoxidized Cottonseed oil (epoxy equivalent weight 310 g/mol) was added. For films B and D, 1 g ofloxacin was added and then all the mixture was stirred under a nitrogen atmosphere at 60-80 °C for 30 min. The solution becomes viscous after 30 min. The reaction mixture was cooled up to room temperature and removed from the flask. The thin films were casted pre- heated glass plates (10x10 cm^2) to avoid thermal shock at what time when the liquid resin was poured on it. The oven temperature was kept further at 80°C for 6 h. The glass plates were taken out and cooled to room temperature (28 °C) and the films were peeled out from the glass plates by dipping them into water. Residual solvent and moisture from the films were removed by drying them on a vacuum oven until constant weight[14].

Table 1. Synthesis of bioepoxy resin films (A-D)

Bioepoxy resin film	Contents
A	Bioepoxy resin film (ECO + CA)
B	Bioepoxy resin film (ECO + CA + OFL)
C	Bioepoxy resin film (ECO + TA)
D	Bioepoxy resin film (ECO + TA + OFL)

ECO: Epoxidized cottonseed oil; CA: Citric acid; OFl: ofloxacin; TA: Tartaric acid

Evaluation of Bioepoxy Resin Films of Cottonseed Oil

The synthesized bioepoxy resin wound dressing films (A-D) were evaluated for various parameters e.g. physical appearance, surface texture, thickness, folding endurance, surface pH, swelling index, Invitro adhesion test, In vitro drug release evaluation, moisture absorption, water permeation, mechanical properties, microscopic study and wound healing activity.

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Physical Appearance and Surface Texture

The prepared wound dressing films were evaluated visually for their physical appearance such as colour and transparency. The surface textures of the films were evaluated by pressing the film with a finger.

Thickness

The thickness of prepared samples of each film was measured using a screw gauge at different places. The average film thickness was computed [15].

Folding Endurance

The folding endurance was measured manually. A small strip of film 2 cm^2 of each formulation was taken and folded at the same place till it breaks. The number of times a film could be folded at the same place gave the value of folding endurance. The average of three determinations was calculated and the standard deviation was computed[15].

Surface pH

For the determination of the surface pH, keeping the film in touch with 0.5 ml of distilled water in a 50 ml glass beaker for an hour was done. The water used for this endeavor had a pH of 6.5±0.05. To determine the surface pH, a pH meter was employed by bringing a combined glass electrode near the surface of the film for 1 minute. The average of three determinations was calculated[16].

Swelling index

While studying wound healing dressings, swelling ability is a significant parameter. A 2cm^2 strip of each film was accurately weighed by using a single pan balance (W_1) and placed in a petri dish containing 50 ml distilled water. After the interval 90 min, the film was removed, blotted, and weighed again (W_2) and the swelling index was calculated [15].

***In vitro* adhesio test:**

For testing bioadhesion double pan physical balance was used. Then both the pans were removed and the left pan was replaced with a brass wire and the right pan with a lighter pan. Polypropylene block was placed in the left pan. The goat cheek pouch was carefully excised without removing connective and adipose tissue and stored in a saline solution. The left side pan was placed in the beaker containing phosphate buffer of pH 6.6 and kept at 37^0 C. The film was taken and attached to the upper polypropylene cylinder and the goat cheek pouch was attached to the lower polypropylene block. A preload weight of (30gms) was placed on the left pan of the balance for 10 min. The weights were then removed slowly and weights were added slowly in increasing order to the right pan till the patch separated from the mucosal surface. The weights required for complete detachment of the film from the mucosal surface were noted. The average of three determinations was calculated[17-19].

***In vitro* drug release evaluation**:

In vitro, diffusion studies were carried out in a fabricated diffusion tube of a surface area of 1.5 cm^2 through Sigma dialysis membrane. The sigma dialysis membrane was hydrated by the addition of distilled water and fixed to one end of the tube which acts as a donor compartment. The assembly was placed in the beaker containing 50 ml of phosphate buffer of pH 6.6. The Teflon-coated magnetic bead was placed in the beaker and rotated at 100 rpm using a magnetic stirrer and the temperature was maintained at 37^0 C. Samples of 1 ml were withdrawn at regular intervals and replaced the volume with the same buffer and maintained

sink condition through the studies. The absorbance was measured at 235nm for quantifying the drug released. The same study was conducted for drug devoid film as control[17-19].

Moisture Absorption[20]

Each film was cut in a circular shape with a diameter of 2.8 cm and weighed accurately. The films were kept in desiccators having silica beads at room temperature for 24 h. The films were then placed in desiccators having saturated potassium chloride solution (75% R.H.). After 24 h, the films were removed and accurately weighed. The percentage of moisture absorption was then calculated as the difference between the initial and final weights with respect to the initial weight.

Water vapour transmission rate

The Water vapour transmission (WVT) study was carried out in accordance with the "B" method of the ASTM (American Society for Testing and Materials), guidelines E96- 66. 10.0 mL of distilled water was taken in each permeability cup (Payne permeability cup model, Belgium). The sample films (9.62 cm^2) were attached on the cups appropriately. The cups were weighed at time 0, 24, 48, 72, 96, and 120 h and stored in desiccators containing silica gel in order to determine the permeated amount of water(mass loss %). Different values of the mass of the cups were recorded and the rate of water vapour transmission through free films was recorded.

Mechanical properties

The films were analyzed for their mechanical properties such as tensile strength, tensile strain, and % elongation[22].

Stability Study

The stability studies were conducted for all the films at 40^oC and 75% RH to investigate the effect oftemperature on different film formulations[16].

Microscopic Study

The films are studied under the digital microscope at 5x and 10x magnification power.

Scanning electron microscopy (SEM):

The surface morphology of the selected film (A) was characterized by scanning electron microscope before and after the diffusion study and determined the drug distribution and the drug remained in the film after diffusion at 2.51 Kx. Magnification respectively[23].

Wound healing Activity

The excision wound healing model was used to evaluate the wound healing activity of bioepoxy resins (A-D). Wistar albino rats of either sexes weighing 150-200 g were randomly divided into five groups of six animals (n = 6) each (Table 2). The rats were kept under-maintained conditions (25±2 °C, relative humidity 60± 5%, 12 h light-dark cycles). The study protocol was approved by the Ethics Committee of Nanded Pharmacy College, Nanded, Sangli (Approval number: 650/02/C/CPCSEA/17) as per Committee for the Purpose of Control andSupervision of Experiments on Animals (CPCSEA) guidelines, India.

Table 2. Evaluation of wound healing activity of bioepoxy resin films (A-D) on Wistar albino rats (group II-V) with the group I serving as control

Animal Group	Treatment
I	Control
II	Bioepoxy resin film A (ECO + CA)

III	Bioepoxy resin film B (ECO + CA + OFL)
IV	Bioepoxy resin film C (ECO + TA)
V	Bioepoxy resin film D (ECO + TA + OFL)

Experimental study:

An excision wound model was used for studying wound healing activity. Wistar albino rats of either sexes weighing 150-200 gm were randomly divided into 9 groups of six animals each. The animals were anesthetized by anesthetic ether prior to and during incision activity. The back of each animal was shaved and prepared after washing with spirit. An area of about 2 cm in length, 1cm in width (5mm away from ears) was defined with a marker on the shaven back of the animals. The circular marked area was excised with its full thickness using a surgical sterile blade and scissors under ether anesthesia. The formulations A to D were applied to the wounded rats of the respective groups, three times a day. The wound contractions were measured as the percentage of wound reduction in the wound area for 7, 14, 21, and 28 post-wound days by counting a number of squares of the retraced wound area on graph paper. The degree of wound healing is calculated as % closure of the wound area from the original wound using the formula:

% closure of wound = Initial wound size - Final wound size / Initial wound size x100

Time for epithelisation

The number of days required for falling of eschar without any residual raw wound was recorded to estimate the period of epithelialization.

Histopathology

On day 28, the wound area was removed from the surviving animals for histological examination and preserved in 10% buffered formalin. Thick sections stained with hematoxylin and eosin and evaluated for several histological parameters such as cellular proliferation, formation of granulation tissue, and synthesis of collagen. All the animal groups were assessed blindly by the pathologist and the results were compared with the control group.

Statistical analysis

Data are expressed as mean ± S.E.M. and were subjected to ANOVA followed by Turkey-Kramer Multiple Comparisons Test. The values of $P < 0.05$ were considered statistically significant.

RESULT AND DISCUSSION

The bioepoxy resin films of cottonseed oil (A-D) were synthesized and evaluated for their potential as wound dressings (Table 1). The synthesized films were evaluated for various parameters physical appearance, surface texture, thickness, folding endurance, surface pH, swelling index, moisture absorption, water permeation, mechanical properties, microscopic study, and wound healing activity were studied so as to evaluate their appropriateness as drug delivery system and promoter of wound healing[24].

Average weight, Thickness, and Surface Texture

The average weight of all films ranged from 0.58-0.63 g and the average thickness of all films ranged from 0.10 to 0.12 mm (Table 3). Film thickness is a key property as it affects the time required to absorb the polymer into the body[24]. The films were found to be uniform in their weight and thickness. The value of weight and thickness of the films are acceptable

for wound dressings. All the films were yellow in color, opaque, and flexible with rough surface texture.

Table 3. Evaluation of weight, thickness, folding endurance, surface pH, mechanical properties, and moistureabsorption of bioepoxy resin films (A-D)

Group	Average weight(g)	Thickness(mm)	Folding Endurance (unit)	Surface pH	Tensile Strength (MPa)	Tensile Strain	% Elongation	% Moisture absorption
A	0.62	0.10	425	3.4	18.72	0.0182	1.82	13.59
B	0.58	0.11	467	5.6	20.03	0.0093	0.93	0.4
C	0.61	0.12	442	8.9	11.25	0.0163	1.63	2.38
D	0.63	0.11	459	5.6	8.39	0.0301	3.01	1.92

Folding Endurance

It has been reported that the proper wound dressings should be elastic, to be able to skin movements and possesses adequate resistance to mechanical abrasion[25]. The folding endurance of all films ranged from 425- 467 and was approximately similar to each other (Table 3). The bioepoxy resin film B (ECO + CA + OFL) was found to have the highest folding endurance as compared to others.

Surface pH

Ideally, wound dressings should maintain a slightly acidic environment on the wound surface (normal human skin ranges between 4.0 and 6.8), thereby accelerating the wound healing process compared to neutral and alkaline environments. The surface pH of all films was found to range from 3.4 to 8.9 owing to the presence of citric acid/tartaric acid (Table 3). This mildly acidic environment provided by films helps in wound healing by controlling wound infection, altering protease activity, increasing antimicrobial activity, reducing toxicity of bacterial products, releasing oxygen and end-enhancing epithelisation and angiogenesis[26]. The acidic environment also enhances cell proliferation and fibroblast formation[27].

Mechanical properties

The bioepoxy resin films were evaluated for their mechanical properties such as tensile strength, tensile strain, and elongation in order to analyze their strength and elasticity. A film with a higher tensile strength corresponds to a stronger film because the tensile strength of a film is the maximum stress it can withstand when stretched before necking or tearing occurs[24]. The usual range of the tensile strength of skin is reported to be 2.5-16 MPa[22]. The tensile strength of films A-D ranged from 8.39 to 20.03 MPa (Table 3). Film B (ECO + CA + OFL) was found to have the highest tensile strength i.e. 20.03 MPa, followed by films A > C > D. All the films showed percentage elongation in the range of 0.93 to 3.01 %, with film D (ECO + TA + OFL) having highest percentage elongation of 3.01% followed by films A > C > B. Films presented adequate properties for skin applications.

Moisture Absorption

The ability to absorb moisture in a wound dressing is a significant factor for wound healing. Table 3 shows the percentage moisture absorption of all bio epoxy resin films after 24 h. The percentage of moisture absorption of film A (13.59 %) and C (2.38 %) was found to be higher than that of film B (0.4 %) and D (1.92 %). Thus, it can be concluded that bioepoxy resin films are suitable for the treatment of light to medium-suppurating wounds.

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Swelling index

Swelling test helps identify in advance the potential for deterioration related to system hydration levels. It is used to verify that the material is structurally stable for the time required for the formation of new regenerative tissue. All bioepoxy resin films exhibited low swelling index i.e. 0 to 1.51 and the values remained unchanged for more than 90 min at pH 5.5, 6.8, and 7.0 (Table 4). This showed that there was a low degradation of the films[28].

Table 4. Swelling indices of bioepoxy resin films (A-D) at pH 5.5, 6.8 and 7.0

Group	Swelling Index (%) at variable pH		
	pH 5.5	pH 6.8	pH 7.0
A	0.10	0.10	0
B	1.51	0.23	0.52
C	0.46	1.49	0.25
D	0.83	1.24	1.49

Water vapour Transmission Rate

Dressings used for wound healing should optimally maintain moisture loss from the wound surface. The moisture vapor permeability of wound dressings is designed to prevent over-drying and the formation of exudate. Excessive water vapor loss can cause the wound to dry out and the dressing to stick to the wound surface. The rate of water loss at a surface temperature of 35^0C from normal skin is 8.5 g/m^2 /h, whereas those from wounded skin can range from 11.6 to 214.1 g/m^2 /h[18]. The bioepoxy resin films showed WVT rate lower than the usual range of WVT rates in normal and wounded skin (Table 5). The comparative WVT rates show that film A has the lowest WVT rate followed by C<D<B (Figure 1). These findings suggest that bioepoxy resin film is more appropriate for use in a low-suppurating wound.

Table 5. Water vapor transmission (WVT) rates of bioepoxy resin films (A-D) at 24, 48, 72, 96, and 120 h

Sample	Water vapour permeation in hr				
	24	48	72	96	120
A	0.016	0.012	0.010	0.007	0.005
B	0.017	0.015	0.012	0.010	0.007
C	0.012	0.009	0.009	0.008	0.006
D	0.011	0.008	0.007	0.006	0.006

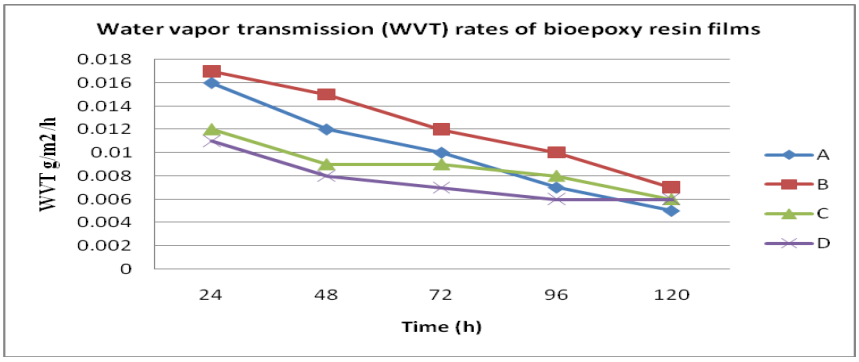

Figure 1. Comparative water vapour transmission (WVT) rates of bioepoxy resin films

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(A-D)

Stability Studies

The stability studies of all formulations were carried at 40°C and 75% RH. All the formulations were stable with respect to the physical parameters. There are no changes in visual appearance and clarity.

Microscopic Study

The films are studied under digital microscope at 10x magnification and air droplets were observed in film with non-uniform texture of bioepoxy resin films.

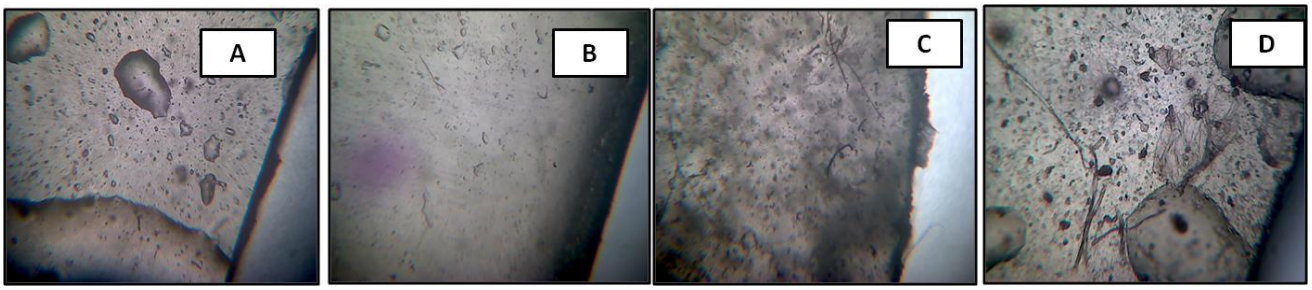

Figure 2. Bioepoxy resin films (A-D) as observed under digital microscope at 10x magnification

Wound Healing Activity

Wound healing is the process of restoring damaged tissue to a normal state as much as possible, while wound shrinkage is the process of reducing the wound area. Quantitative measurements of wound size are commonly used to assess initial wound size before and after debridement and the progression of wound closure[29].

Percentage Closure of Original Wound Area

A significant increase in wound healing was observed in groups treated with bioepoxy resin films (A-D) as compared to the control group. The mean percentage closure of the wound area was calculated on 0, 7^{th}, 14^{th}, 21^{st}, and 28^{th} post-wounding days as shown in Table 6. In comparison with the control, group V treated with film D (ECO + TA + OFL) demonstrated the highest rate of wound contraction (7.4% to 53.72%) from day 7 to 28 (Figure 1). Group IV treated with film C (ECO + TA) closely followed Group III with an increase in wound contraction from 13% to 42.61%. The percentage of wound contraction in Group III treated with film C (ECO + TA) was found to increase from 20.38% to 48.1% and that of Group II treated with film A (ECO + CA + OFL) from 7.4% to 40.77%. All the groups treated with bioepoxy resin films (A-D) showed a higher rate of wound contraction than that shown by control group I (7.4% to 33.3%). There was only a slight increase in the rate of wound contraction with the use of tartaric acid in films as compared to citric acid. There was significant improvement ($p<0.05$) in the percent wound contraction rate in treatment groups as compared to the control group. These observations indicate that epoxidized cottonseed oil film has the potential to improve wound healing rate.

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Table 6. Effect of bioepoxy resin films (A-D) on percentage wound contraction by excision wound model

Animal Group	Treatment	Wound area (mm^2) (mean±S.E.) and percentage of wound contraction on post-wounding day					Time for epithelization in days (Mean±S.E.)
		0	7	14	21	28	
I (Control)	–	18 ±0.00	16.33±0.33 (9.27%)	15.33±0.33 (14.83%)	13.33±0.33 (25.94%)	12±0.66 (33.3%)	12.33+0.33
II	A	18 ±0.00	16.66± 0.24 (7.44%)	15.33±0.72 (14.83%)	12.33±0.32 (31.5%)	10.66± 0.66 (40.77%)	12.33+0.22
III	B	18 ±0.00	14.33±0.68 (20.38%)	12.33±0.27 (31.5%)	10.66±1.15 (40.77%)	9.33 ±0.57 (48.1%)	11 +0.22
IV	C	18 ±0.00	15.66±0.88 (13%)	13.33±0.43 (25.94%)	11.66±1.06 (35.22%)	10.33± 0.65 (42.61%)	11.66+0.22
V	D	18 ±0.00	16.66±0.33 (7.4%)	13.33±0.27 (25.94%)	10.33±0.97 (42.61%)	08.33± 0.33 (53.72%)	6.33+0.22

$p < 0.05$. The statistically significant difference in comparison with the control group

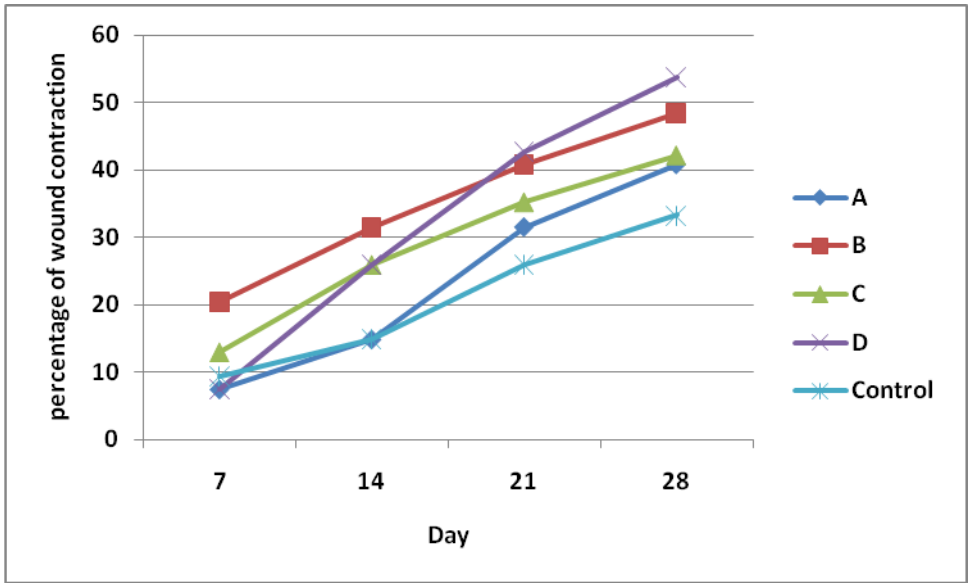

Figure 3. Comparison of wound contraction rates of bioepoxy resin films (A-D) with control.

Time for Epithelization

Group V treated with film D showed epithelization in the shortest period (6.33 + 0.22 days) as compared to 12.33 +0.33 days shown by control i.e. approximately six days faster (Table 6). It was followed by Group III, IV, and II which showed epithelization in 11 + 0.22, 11.66 + 0.22, and 12.33 + 0.22 days respectively. All the groups treated with bioepoxy resin films (A-D) showed a significant decrease in epithelization time ($p<0.05$) as compared to that shown by the control group (Table 6).

Histopathology

The wound tissue samples of Group I-V were evaluated for the cellular proliferation,

formation of granulation tissue, and synthesis of collagen (Figure 4). Homeostasis or inflammatory, proliferative, and remodeling phases are the three phases of normal wound healing that occur in succession but overlap[30]. One of the signs of wound healing is the regular growth of granulation tissue[31]. The collagen is initially thin and parallel to the skin during the early stages of wound healing. Over time, thicker collagen fibrils that are aligned with stress lines resorb and take their place[32].

In control group animals (Group I), poorly formed granulation tissue with abundant mononuclear inflammatory cells can be observed (Figure 4). Whereas, in groups treated with bioepoxy resin films (II-V), granulation, remodeling, and contraction with horizontally arranged fibrous connective tissue were observed, all of which are indicative of wound healing. The presence of collagen fibers was found to be less in all groups, however, they were least observed in Group II (film A) as compared to other groups. These observations indicate that bioepoxy resin films (A-D) promote significant wound healing activity by increasing cellular proliferation, formation of granulation tissue, synthesis of collagen, and increase in the rate of wound contraction as compared to the control animals.

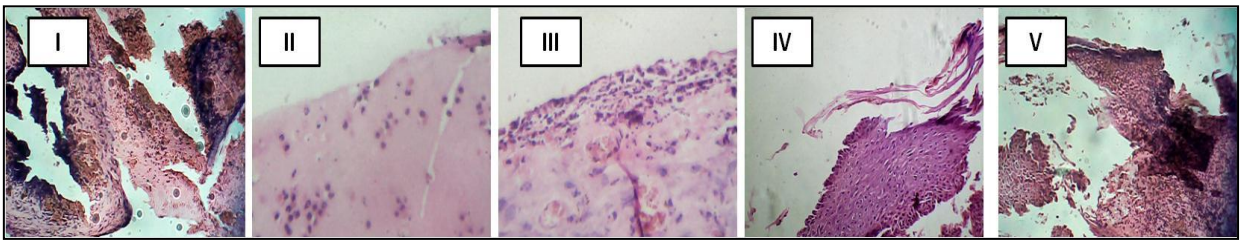

Figure 4. Histological sections of hematoxylin and eosin stained cutaneous wound site tissue of Group I-V on day 28 post-wounding

Group I (Control) shows abundant mononuclear inflammatory cells

Group II-V: granulation and remodeling, contraction with horizontally arranged fibrous connective tissue and lesser collagen fibres

CONCLUSION

Bio-epoxy resins produced from vegetable oils are the most important polymer in the categories of vegetable oil-based polymers. In the present study, bioepoxy resins were synthesized from epoxidized cottonseed oil (ECO) and evaluated for physicochemical properties and their potential as wound-healing dressing. The folding endurance, surface pH, swelling index, moisture absorption, water permeation, and mechanical properties were analyzed and bioepoxy resins films were found to exhibit the desired wound dressing properties. Wound healing activity was studied in vivo using an excision wound model on Wistar albino rats. All the animal groups treated with bioepoxy resins (A-D) showed significant improvement ($p<0.05$) in the percentage wound contraction rate and time of epithelization as compared to the control. Groups treated with bioepoxy resins showed significant granulation, remodeling, and contraction with horizontally arranged fibrous connective tissue, according to the histopathological analysis of samples of wound tissues. Thus, bioepoxy resin film dressings prepared from cottonseed oil can serve as an alternative for wound dressing films in the treatment of excision wounds.

CONFLICTS OF INTEREST

The author has declared no conflicts of interest.

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