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Wound Healing Activity of Hydroalcoholic Extracts of Lycopersicon Esculentum Seeds and Ficus Racemosa on Wistar Rats

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ABSTRACT: In the Present study Investigation on the preliminary phytochemicals screening of ethanolic extract showed the presence of Resin, Protin, Quinones, Anthraquinon, Carbohydrates, Alkaloids, Glycoside, Steroids, and Phenols. By ethanol extract using standardized procedure and also subjected to wound healing activity exhibited significant wound healing activity with respect to control biological effects of its extracts ointment, mainly the ones related to wound process. The present data clearly showed that the extract of dried Seeds of Lycoperscon esculentum seeds and Ficus racemosa has wound healing activity the highly significant response of some extracts .The result obtained from the excision wound model, Effect of EECG on Percentage Wound Contraction and Epithelialization Period. During the course of treatment the extract was found to show its preliminary effect from day 4 up to day 16.

Keywords: Hydroalcholic Extracts, Wistar rats, Ficus Racemosa, Wound Healing Activity

1. INTRODUCTION:

Herbal Medicinal Plant:-

The term medicinal plants include various types of plants used in herbalism and some of these plants have some medicinal activities. Medicinal plants are the “backbone” of traditional medicine, which suggests quite 3.3 billion people within the less developed countries consume medicinal plants on a daily basis. Medicinal plants are the rich resources of ingredients which will be utilized in the event and synthesis of medicine. Further that these

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plants play a crucial role within the development of human cultures round the whole world. India features a rich diversity of plant species in an in depth sort of ecosystems. Around 17.000 species of upper plants, of which approximately 8.000 species are considered medicinal which are employed by village tribal communities like the Ayurveda [1]

During the past decade, traditional systems of drugs became a topic of worldwide importance. Present evaluations suggest that, in many rising countries, an outsized section of the population depends on traditional practitioners and medicinal plants to satisfy the requirements of primary health care. Albeit modern medicines are available in these countries, herbal medicines (phytomedicines) have frequently maintained popularity for historical and cultural reasons. [2]

Raw materials of medicinal plants often used because the extraction of active ingredients that are utilized in the synthesis of various drugs. As within the case of laxatives, blood thinners, antibiotics, and anti-malarial medicines, all contain ingredients from plants. Moreover the active ingredients of Taxol, vincristine, and morphine isolated from periwinkle, yew, and opium respectively. [3]

As defined by WHO, health may be a state of complete physical, mental, and social wellbeing and not merely the absence of disease or infirmity. Medicinal plants can make a crucial influence on the WHO goal to make sure , by the year2000, thateachone peoples, worldwide[4]

The development and commercialization of medicinal plant-basedindustries within the rising countries are reliant upon the supply of amenities and knowledge regarding upstream and downstreambio processing, extraction, purification, and marketing of the economic potential of medicinal plants. Additionally, the absence of modernized socioeconomic and public healthcare systems emphasizes reliance onrural and lowerincome urban populations on the usage of traditional medicinal herbs and plants as corresponding aids to routine pharmaceutical market products. Modern evaluations suggest that over 9,000 plants have known medicinal applications in various cultures and countries, and this is often without having conducted comprehensive research amongst several indigenous and other communities [5]

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Plants in traditional medicines: - Four thousand years ago, the medical knowledge of the Indian subcontinent was termed as Ayurveda. Ayurveda remains an important system of medicine and drug therapy in India. Plant alkaloids are the primary active ingredients of Ayurvedic drugs. Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. As mentioned in the introduction only a certain percentage of plants are used in traditional medicines. It is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field. The therapeutic action of important medicinal plants and its parts used. The indigenous systems of medicine in India are reported in under supplementary material. The pharmacological properties of some Ayurvedic crude drugs support for their therapeutic claims. [6]

The World Environment has originated from French word "environ" mean surroundings includes each and everything outside the plant, which influences directly or indirectly the life of the plant. This is an integral part of the earth's ecosystem. Each component of the environment is called environmental factor. Plants grow best within certain ranges of various factors includes temperature, soil moisture, soil nutrients, light, air pollutants, humidity, soil structure and PSH. Although these factors affect all plants are frequently grown or kept in cultural particles (fertilization, irrigation, spraying with pesticides) that may affect their growth considerably.

2. MATERIAL AND METHODS

Experimental Animals:

Healthy Wister albino rats weighing between 150-250 gram were used in the present studies were procured from Bomb sniffer nachan dog trainer Indore (M.P.) India. The animals were fed with standard pellets diet and water. All the animals were acclimatized for acclimatized for a week before use and maintained at standard husbandry conditions, Room temperature27±3°C Relative humidity 65±10%, 12:12 hrs light/dark cycle and fed with standard diet and water during the study . All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by the

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committee for the purpose of Control and Supervision of Experiments on animals (CPCSEA).Animal experiment was performed in swami v

List of Instrument and chemicals:-Drugs:-

Providone Iodine Ointment USP 5% w/w (Cipla Ltd.), Mumbai -400013India

Chemicals:-

Ethanol (95% v/v) used in the present study were of general grade andwere procured from Sun-Chem. Chemicals Pvt. Ltd Satna, M.P.

Glassware:-

Soxhlet assembly, Round bottom flask, Beaker, Funnel, Glass rod, chain diss, Manufactured by (ASCO) Alka Scientific corporate manufacturer of scientific glass apparatus (Nagpur).

Evaluation of Wound

Statistic healing activity by using following models

I. Incision model
II. Excision model

Incision wound model

The incision wound model was studied. Under light ether anesthesia the animal was secured to operation table in its natural position. One paravertebral straight incision of 6 cm was made on either side of the vertebral column with the help of scalpel blade. Wounds were cleaned with 70% alcohol soaked with cotton swabs. They were kept in separate cages. The extract ointment 2% and 5% was applied for 10 days. The sutures.

were removed after 10 days, on tenth day the tensile strength was measure continuous constant water supply technique.

Excision wound model

Excision wounds were used for the study of rate of contraction of wound and epithelization. Animals were anaesthetized with slight vapour inhalation of di-ethyl ether and the right side of each rat was shaved. Excision wounds sized 300 mm 2 and 2 mm depth were made by cutting out layer of skin from the shaven area. The entire wound was left open. The treatment

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was done topically in all the cases. The extract 2%,5% ointment was applied +for 16 days. Wound areas were measured on days 1, 4, 8 and 16 for all groups, using a transparency sheet and a permanent marker.

Analysis :-

The data were expressed as mean ± standard error mean (SEM). The significance of differences among the group was assessed using one way analysis of variance (ANOVA) by prism software. The test was followed by Dunnett‟s, $p<0.05$ were considered as significant.

3.Experimental Work and Results:-

Collection of Plant:-

The plant of *Lycoperscon esculentum seeds and Ficus racemosa* were collected from the Indore region. (Madhya Pradesh.

Authentication of Plant:-

The plant was identified and authenticated Calotropis n. by Dr. S. K.Mahajan, Ex. Professor of Botany, Govt. P. G. College, Khargone, M.P.

Preparation of Extract:-

The Seed *Lycoperscon esculentum seeds and Ficus racemosa* were collected from local nimad region, Madhya Pradesh, India. This plant sample was authenticated by a botanist. Samples were washed in water and dry at room tempreture . After the sample were ground into cors powdered using a blender. 100gms. of powdered of *Lycoperscon esculentum seeds and Ficus racemosa* was dissolved in 500ml of solvent like ethanol and extracted using a Soxhlet apparatus. Distillation was carried out for individual samples separately with the solvents and the filtrate was collected and it was collected and it was concentrated by evaporating the solvent to get a final stock.

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Table No.1:- Extractive values of different extracts of Seeds of *Lycoperscon esculentum seeds and Ficus racemosa*

PLANT NAME	PARTS USED	METHOD	ETHANOL (95%)
Lycoperscon esculentum seeds	Seed	Soxhlet assembly	15.86
Ficus racemosa	Seed	Soxhlet assembly	15.86

Figure No. 1 - Soxhlet apparatus

Preparation of Formulation:-

Two types of ointment formulation 2% and 5%(w/w), were prepared from the extract where 2.5 gram ,2.5 gram and 5.0 gram and 5.0 gram of the extract of *Lycoperscon esculentum* seeds and *Ficus racemosa* respectively were incorporated into 100 g. of simple ointment base (BP), Respectively Povidone iodine ointment (5%w/w) was used as a standard drug for comparing the wound healing potential of the extract.

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100 g. Simple Ointment base (BP) contain:-

Wool fat 5g hard paraffin 5,cetostearyl alcohol 5g.,white soft paraffin 85g Types of preparation absorption ointment base procedure: Hard paraffin and cetostearyl alcohol on water -bath .wool fat and white soft paraffin are mixed and stirred until all the ingredients are melted and from homogeneous mixture, remove from the water bath and cool.

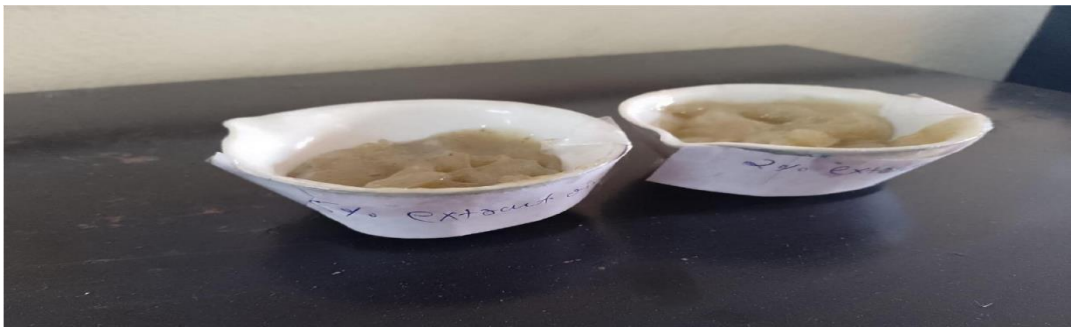

Figure No 2 - Extract ointment preparation

Preliminary Phytochemical Screening : Preliminary Phytochemical screening ethanol extracts of Seeds of Lycoperscon esculentum and Ficus racemosa

Test for Alkaloids

Mayer's test: A fraction of 1ml extract was treated with 1ml Mayer's test reagent and observed for the formation of cream coloured precipitate.

Wagner's test: A fraction of 1 ml extract was treated with few drops of Wagner's reagentand observed for the formation of reddish brown colour precipitate

Hager's test: A few ml of extract was treated with Hager's reagent and observed for the formation oprominent yellow precipitate

Test for Tannins

Acetic Acid Test: The extract was treated with acetic acid solutions and observed for the formation of red colour solution.

Dilute HNO3 Test: The extract was treated with dil. HNO3. The extract turns from reddish toyellow colour which indicates the presence of tannins.

Test for Phenols :

Ferric chloride test: The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour

Liebermann's test: The extract was heated with sodium nitrite, added H2SO4 solution diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue colour.

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Test for Flavonoids

NaOH test: A small amount of extract was treated with aqueous NaOH and HCl,observed for the formation of yellow orange colour.

H2SO4: A fraction of the extract was treated with concentrated H2SO4 and observed for the formation of orange colour.

Test for Terpenoids

Salkowski test: 2ml extract was treated with 1ml of chloroform and few drop con. H_2SO_4 were also added and formation of raddish brown precipitate indicated the positive result.

Test for aponins

Foam Test: The extract or dry powder was vigourosly shaken with water and not observed for the formation of persistent foam.

Test for Anthra quinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia and observed for the formation of pink or deep red colouration of aqueous layer.

Test of Protein

Ninhydrin test: The extract was treated with aqueous ninhydrin and observed for the presence of blue colour,indicating the presence of amino acid or purple colour indicating the presence of protein.

Test for Quinones

A small amount of extract was treated with concentrated HCl an observed forthe formation of yellow colour precipitate

Table No. 2- Result of Preliminary phytochemical screening of ethanol extracts of seeds of *Lycoperscon esculentum and Ficus racemosa*

Sr. No.	PhytochemicalConstituents	Ethanol (95%)
1.	TEST FOR ALKALOIDS	
	Mayer's test	++
	Wagner's test	++
	Hager's test	++
2.	TEST FOR TANNINS	
	Acetic Acid Test	+

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	Dilute HNO3 Test	+
3.	**TEST FOR FLAVONOIDS**	
	NaOH test	+
	H2SO4 test	+
4.	**TEST FOR PHENOLS**	
	Ferric chloride test	+
	Liebermann's test	+
	TEST FOR TERPENOIDS	
	Salkowski test	+
5.	**TEST FOR SAPONINS**	
	FoamTest	-
6.	**TEST FOR ANTHRAQUINONES**	
	Borntrager's test	+
7.	**TEST FOR PROTEINS**	
	Ninhydrin test	++
8.	**TEST FOR QUINONES**	
	++	
Present ++ Moderately Present Absent - +		

Determination of wound healing evaluation parameters:- Measurement of Wound Contraction and Epithelialization Period. In the excision wound model, wound area was measured by tracing the wound with the help of transparent sheet using millimeter based graph paper on days 0, 4, 8, 12, and 16 for all groups. Wound contraction was measured every 4th dayuntil complete wound healing and represented as percentage of healingwound area . Percentage of wound contraction was calculated taking the initial size of the wound as 100% using the following formula:

% wound contraction

= (Initial wound area − Specific day wound area)Intial wound area × 100.

Epithelialization period was calculated as the number of days required for falling off the dead tissue remnants of the wound without any residual raw wound

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Measurement of Tensile Strength. The tensile strength:-

The tensile strength of a healing skin wound indicates the degree of wound healing. It represents how much the healed tissue resists to breaking under tension and may identify the quality of healing tissue. On the 10th day, all the animals were anesthetized by light diethyl ether.the sutures were removed, and the healed tissue was excised from all animals. Tensile strength of excised tissue was measured with the help of tensiometer. Primary skin irritation test: The skin irritation test was done by the method described with some minor modifications. The hairs from the rat dorsal part was removed by hair removing cream (Veet). A 2-cm2 dorsal area was shaved and cleaned with surgical spirit. The animal did not show any toxic effect when 2%,5% w/w extract ointment was applied to a shaved area of rats.Hence the prepared extract ointment was considered safe for topical application.

Wound Healing Studies:-

Excision model andIncision model

1. Excision Model

Test compound:- The Ethanolic extract of Seeds of Lycoperscon esculentum and Ficus racemosa and standard drug providon iodine solution 5 % w/w were used.

Chemicals and Reagent:- simple ointment base ointment (BP)

Experimental Animal :-

Rat(150-250gm) used in the present study. The animals were fed with pellet diet and water add libitum. All the animals were acclimatized for a week before use.

Procedure:- Excision Wound Model:-

Excision wound were used for the study of rate of contration of wound and epithelization. five groups of animals each containis rats were shaved on the dorsum portion using depilatory cream (veet) and anesthetized using light di- ethylether. An impression was made on shaved dorsal region and area of the wound to be created was marked. A full thickness excision wound with a circular area of 314 mm2 was created along the marking using toothed forceps, a surgical blade, and pointed scissors. Rats were left undressed to the open environment.

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The simple ointment base, formulated extract ointment, and standard drug were applied once daily from the day of the operation until the complete healing. In Journal of Pharmaceutics this model, wound contraction and epithelialization period were evaluated. Wound contraction was measured as percent contraction every 4th day after wound formation.

Table No.3: Excision wound model induced in rats

Treatment	% of wound contraction				
	4th day	8th day	12th day	16th day	Epithelialization period (days)
Group I (untreated)	4.39 ± 0.82	18.62±0.69	42.62±2.56	68.87 ±0.91	19.16 ± 0.7
Group II (ointmentbase treated)	4.21 ± 0.19	19.48 ±0.93	42.76± 1.36	71.2 ± 0.93	19.66 ± 0.66
Group III (Standard)	14.55 ±87	38.39 ±0.46	67.38 ±.01	94.3 ±0.43	17.33 ± 0.4
Group IV (2 % w/wointment)	13.83 ± 1.0	31.11 ±0.83	56.46 ±.79	80.21 ±0.27	18.33 ± 0.22
Group V (5 % w/wointment)	14.33 ±.94	34.82 ±0.67	59.64 ±.18	93.58 ±0.76	17.66 ±0.7

All values are represented as mean ± SEM, = 6 animals in each group. Data were analyzed by one-way ANOVA, followed by prism software. Multiple Comparisons Test. a: significant difference as compared to untreated group (group I); b: significant difference as compared to ointment base treated group (group II); c: significant difference as compared to standard group (group III), and **P < 0.01, ***P < 0.001.

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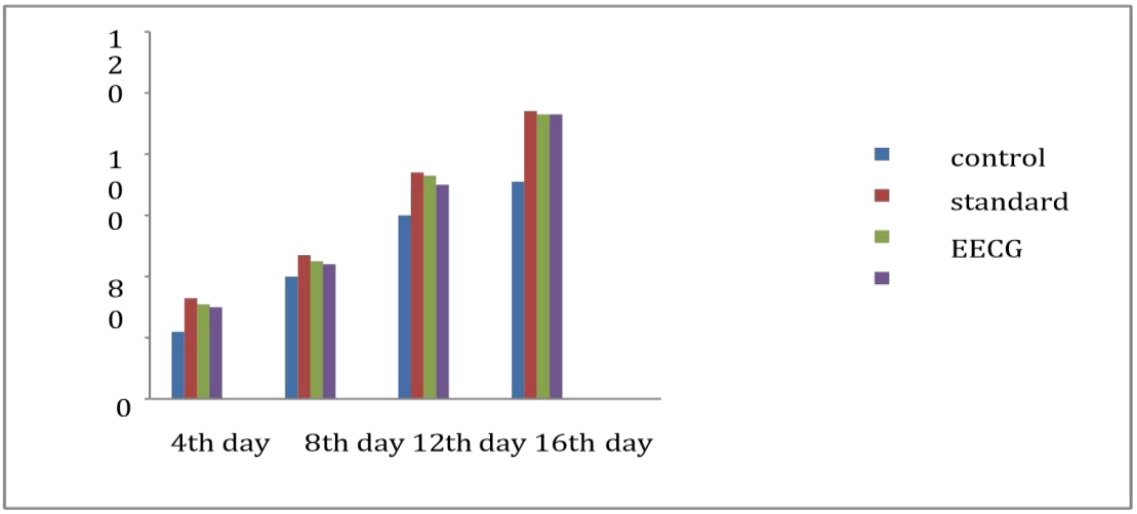

Graph No. 3:- Excision Wound Model Induced

DIFFERENTIATION OF DOSES

Group (I) = (untreated)
Group (II) = (ointment base treated
Group (III) = (standard) Providon iodine ointment
Group (IV) = (2%w/w ointment)
Group (V) = (5% w/w ointment)

I .INCISION WOUND MODEL:-

Test compound:- The Ethanolic extract of Seeds of Lycoperscon esculentum and Ficus racemosa.

Drug :- Providon iodine ointment

Experimental Animal :- Wister albino Rats (150-200gm) used in the present study. The animals were fed with pellet diet and water add libitum. All the animals were acclimatized for a week before use.

Procedure:- In incision wound model, all the animals of each group were anaesthetized under light ether anesthesia. Two full thickness par vertebral long incisions were made through the skin at the distance of about 1 cm from midline on the each side of the depilated back of rat .After the incision was made the both edges of skin kept together and stitched

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with black silk surgical thread (no.000) and a curved needle (no.11) was used for stitching . The sutures were removed on the 7th day. wound breaking strength was measured.

Table No 4:- Incision wound model in rat

S. NO.	TREATMENT	BREAKING STRENGTH(g)
1.	Control	168.35± 3.53
2.	Ointment base	172.95 ± 1.24
3.	Providon ointment	210.76 ± 6.65
4.	2% Ointment	191.35 ± 6.43
5.	5% Ointment	201.83 ± 4.98

All values are represented as mean ± SEM, = 6 animals in each group. Data were analyzed by one-way ANOVA, followed by prism software Multiple Comparisons Test. a: significant difference as compared to untreated group (group I); b: significant difference as compared to ointment base treated group (group II); c: significant difference as compared to standard group (group III), and $* < 0.05$, $* * < 0.01$, And $* * * < 0$**.001.**

GRAPH NO. 2 :- INCISION WOUND MODEL INDUCED IN RAT

DIFFERENTIATION OF DOSES

Group (I)

Untreated Group (II) Ointment base

Group (III) Standard Drug (Providon IodineOintment)

Group (IV) Ethanolic Extract 2%Ointment
Group (V) Ethanolic Extract 5%Ointment

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Jayant Kanungo *et al*, International Journal of Pharmaceutical Sciences and Medicine (IJPSM), Vol.9 Issue. 10, October- 2024, pg. 1-16

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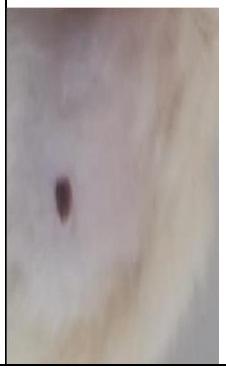
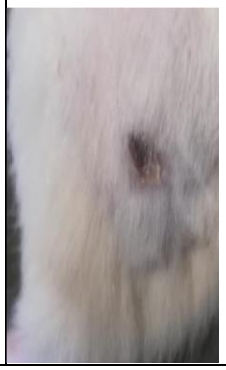
Day	Group I	Group II	Group III	Group IV	Group V
Day 4					
Day 8					
Day 12					

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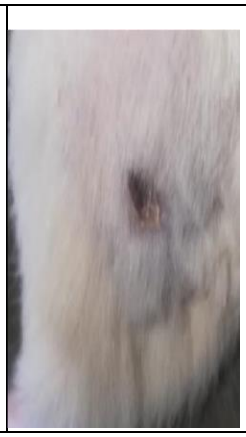
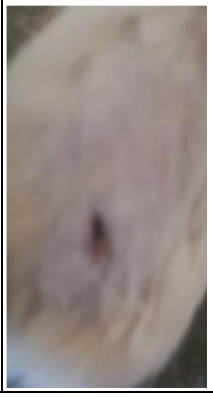
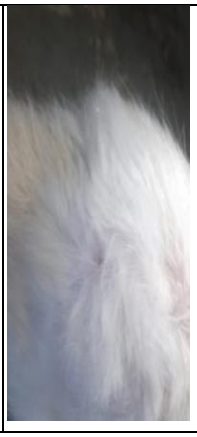
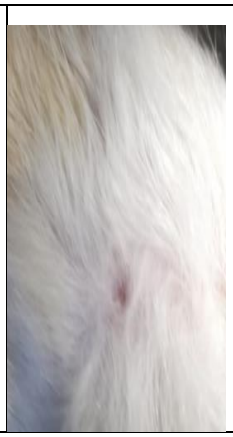
Day 16					

Figure 4: Photographs of wound repair at different time interval in excision wound model in rats

4. CONCLUSION: Investigation on the preliminary phytochemicals screening of ethanolic extract showed the presence of Resin, Protin, Quinones, Anthraquinon, Carbohydrates, Alkaloids, Glycoside, Steroids, and Phenols. By ethanol extract using standardized procedure and also subjected to wound healing activity exhibited significant wound healing activity with respect to controlbiological effects of its extracts ointment, mainly the ones related to wound process. The present data clearly showed that the extract of dried Seeds of Lycoperscon esculentumseeds and Ficus racemosa has wound healing activity the highly significant response of some extracts .The result obtained from the excision wound model, Effect of EECG on Percentage Wound Contraction and Epithelialization Period. During the course of treatment the extract was found to show its preliminary effect from day 4 up to day 16

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