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Original Research Paper

PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL EVALUATION OF LEAVES OF *ZIZIPHUS MAURITIANA* FOR WOUND HEALING ACTIVITY IN ALBINO RATS

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ABSTRACT

Various medicinal plants are used in traditional system of medicine to treat various diseases and many of these plants have been evaluated for their different pharmacological activities *Ziziphus mauritiana* one of them. The present work deals with the study of ethanolic extract of leaves of *ziziphus mauritiana* for its wound healing potency. An excision wound was inflicted by cutting away a 500 mm^2 full thickness of skin from a predetermined area an impression was made on the dorsal thoracic region 1cm away from vertebral column and 5cm away from ear on the anaesthetized Rats. Wound contraction was measured as percentage contraction in each 2 days after wound formation from the healed wound. The wound healing was evaluated by using two different models viz. full thickness excision and dead space wound models. The reading were recorded on 1,3,5,7,9,11,13,15,17 and 19^{th} day after post wound day. The ZMLE and standard drug were applied daily to the wound area daily. The healing was assessed by physical parameters like percentage wound contraction, epithelisation and determination of hydroxyproline. The treatment with the ZMLE produced the anti-inflammatory activity against excision wound model and dead space wound model. The ZMLE showed anti-inflammatory activity against Excision wound when compared to the standard cipladine (povidine iodine 5%w/w). There were significant reduction in days required to healing and hydroxyproline and weight of wet and dry granuloma tissue were increased significantly by the treatment with ZMLE. Healing of excision wound were promoted significantly by treatment with ZMLE, ZMLE promoted the wound contraction and reduced the days required for epithelisation. ZMLE possesses significant anti-inflammatory and wound healing properties.

Keywords: *Ziziphus mauritiana,* Anti-inflammatory, Wound healing, Wound contraction, Epithelisation.

INTRODUCTION

Herbal medicines are used in treatment of various diseases in many developing countries. Herbal preparations are used for virtually all minor ailments. Over the past several decades, several scientific literature and articles are available on adverse drug effects of allopathic medicine so increasing awareness of scientific community to medicinal plants/herbs.[1] an increased interest in natural products by public and scientific community for the safe and less side effect of medicinal plants/herbs. *Ziziphus mauritiana* grows wild in forest and also on wastelands throughout India. In India it is commonly known as Ber and in English it is known as Indian Berry.[2] Ber consists of 45 genera and 550 species which are widely distributed

in tropical and subtropical climates in the world.[3] These trees grow up to 12m in height. It is found in arid region which can be grown successfully in saline soil under hot, arid environment.[4] Its fruits are palatable and delicious with a good amount of vitamin A, C and B complexes and minerals.[5] The leaves of *Ziziphus mauritiana* contain 5.6% digestible crude protein and 49.7% total digestible nutrients, making it a nutritive fodder for animals.[6] Leaves of ZM are traditionally used to cure kapha, biliousness, diarrhea, stomatitis, gum bleeding, syphilitic, ulcers and to reduce obesity.[7] The leaves are reported to show hypoglycemic activity in alloxan induced diabetes. It is also reported to possess significant weight reducing, hypophagic and hypolipidemic properties in sucrose induced obese rats.[8] The extract of leaves stimulates cell mediated immune system by increasing neutrophil phagocytic function.[9] In view of importance of medicinal plants/herbs the extract of leaves of *Ziziphus mauritiana* are applied on cuts and ulcers for wound healing activity.[10]

Figure 1: *Ziziphus mauritiana*. Leaves and flowers

Wound is a type of injury in which skin is torn, cut, or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin.[11] Most of us are likely to sustain different types of wounds throughout life as we participate in daily activities.[12] Many minor wounds result in damaged skin cells that lose their function and need time and simple treatment to heal.[13] Most common wounds are superficial.[14] The signs and symptoms depend on the wound site, depth and causative agent.[15] In general, wounds present with pain, redness, swelling, bleeding and loss or impairment of function to the wounded area.[16] Symptoms may include fever, malodorous pus drainage and heat, particularly in cases of infection.[17]

MATERIALS AND METHODS

Drugs

Dexamethasone (0.17mg/kg p.o,)
Povidine iodine USP (5% w/w topically)
ZMLE (200mg/kg b.w.,p.o.)

Chemicals:

All chemicals used were of analytical grade.

- Anesthetic ether
- Alcohol (70%)
- ZM extract (Ointment)
- Surgical blade

Instruments

- GC-MS (Shimadzu QP2000)
- Incubator
- Autoclave

- Muffle Furnace
- Rotatory evaporator

Animals

Albino Wister rats were allocated within the protocol-specified weight range (i.e of 180 to 200 gm). After randomizing, the rats were assigned into this study**.** The present study is been approved by Instiutional Animal Ethics Committee (IAEC) and given approval no. **MGCPS/IAEC/2014/02**. Animal was maintained under standard condition in animal house approved by the Committee for Purpose of Control and Supervision on Experiments on Animal (CPCSEA).

Collection, Identification and Authentication of Plant

This includes Plant parts of *Ziziphus mauritiana* collected from Jobner local area of Jaipur District. Authentification done by Department of Botany University of Rajasthan and registration number given is as **RUBL211421**.

Extraction of Plant Material

The leaves of *Ziziphus Mauritiana* were dried under shade and powdered. The dried leaves powder 30.0 g was successively extracted by using ethanol as solvent. The last trace of the solvent was removed under reduced pressure by rotary evaporator. The dried crude ethanolic extract was used for the study.

GC-MS analysis

Analytical GC-MS was carried out on a Shimadzu QP2000 instrument at 70 eV and 250˚C.

GC-MS data Identification

Data were evaluated and matched with the inbuilt NIST library. All the peaks were identified with their comparative area and area % MS library identified the compound on the basis of mass.

Pharmacological Screening on Animal Models

Models for evaluating wound healing activity are-

- Excision wound healing model
- Dead space wound healing model

Excision Wound Model

Drug

Povidine iodine USP 5% w/w
ZMLE ointment 5 % w/w

Study Design

All the animals were divided into three groups of five animals of approximately same weight range (180-200 g) and same and same age (12-13 weeks) in each group.
Group I - Control Untreated group
Group II - Standard Povidine iodine treated
Group III -Test group-I treated with 5 % ZMLE ointment

Dosing Schedule

For assessment of excision wound healing activity Standard formulation Povidine iodine ointment (5 % w/w) and test drug ointment was formulated in ointment by using simple ointment BP as base. 5% (w/w) ointment was applied where 2.5 g of the ethanol extract was incorporated in 50 g of simple ointment base BP. 0.5 g of the extract ointment and Povidine iodine ointment was applied once daily to treat different groups of animals on wounded area from initial day to complete healing.

Investigate Parameters after create Excision Wound Model

- Wound Scars on every two days.

- Epithelisation time.
- % Wound Contraction rate.

Dead Space Wound Model

Drug

Dexamethasone (0.17 mg/kg) p.o.
ZMLE (200 mg/kg) p.o.

Study Design

The animals were divided into three groups of five rats each-
Group I Control untreated group
Group II Standard treated with dexamethasone (0.17 mg/kg p.o.)
Group III Test group- II treated with ZMLE (200 mg/kg b.w. p.o.)

Dosing Schedule

For the assessment of wound healing activity by dead space wound model, freshly prepared ZMLE extract 200 mg/kg b.w. and standard drug dexamethasone 0.17 mg/kg p.o was given orally from 0 to 10^{th} post wounding day.

Determination of Hydroxyproline (HP)

The amount of hydroxyproline in tissue was estimated according to the method of Neuman RE et al (1949).[18]

Weight of Wet Granulation Tissue

The tissue was collected on 10^{th} post wound day and weight of wet granulation tissue was calculated.

Weight of Dry Granulation Tissue

After the wet granulation weight tissue was dry and the weight of dry granulation tissue was calculated.

Determination of Hydroxyproline content

The pieces of granulation tissue was collected and dried at 60ºC for 24 hrs to get constant weight and weighed. Tissue was then used for determination of Hydroxyproline content.

RESULT AND DISCUSSION

A significant wound healing was observed in standard and test drug treated groups of animals, when compared to the control group. There was a significant reduction in wound size from day 9 onward in ZMLE treated animals and also on subsequent days. Rapid wound closure rate was observed in ZMLE treated groups when compared to the control treated group. In past many studies have been done using natural products for the treatment of wounds, but these were mainly aimed to control infections. Although the present study did not explore the exact mechanism of prohealing by ZMLE, it could be attributed to both anti-inflammatory and antiseptic properties, revealed in the literature review. In dead space wound model ZMLE were used as test compound and dexamethasone as a standard drug. There was significant increase in test group hydroxyproline when compared to the control group. ZMLE were administered from 1^{th} to 10^{th} post wound day there were significant increase in dry tissue weight and hydroxyproline concentration found in test and standard group when compared to the control group. This showed significant increase in tissue formation in dead space wound model. This may due to active principles present in to ZMLE like-alkaloids, flavanoids, phytolterpenoids, dibutyl pthalate (esters), diallyl disulphide, benzenepropanoic acid, present into the ethanolic extract of *Ziziphus mauritiana* extract.

Table 1: The Wound Contraction and Epithelisation in Excision Wound Model

Group	Mean Wound Area mm^2										Mean Period Epithelisation (Day)
	Day1	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Day13**	**Day15**	**Day17**	**Day19**	
Control	432±4.30 (0.0±0.0)	417.4±9.31 (16.52±1.86)	387±7.14 (22.6±1.42)	317±8.66 (36.6±1.73)	268.4±5.31 (46.32±1.06)	184.6±4.87 (63.08±0.97)	111.2±7.01 (77.76±1.40)	85.4±4.82 (82.92±0.96)	43±3.80 (91.6±0.76)	18.4±2.07 (96.32±0.41)	22.6±1.14
Povidine Iodine 5% W/W	416.8±11.27 (0.0±0.0)	379.8±6.09*** (24.04±2.25)***	311.4±5.72*** (37.72±1.21)***	257±12.08*** (48.6±1.14)***	193.4±8.53*** (61.32±2.41)***	143.2±4.65*** (71.6±1.70)***	68.8±8.87*** (86.24±0.67)***	41.6±5.89*** (91.68±1.77)***	12.8±1.30*** (97.44±1.17)***		21.2±1.30
Ziziphus Extract	427±7.14 (0.0±0.0)	409.2±6.05^{ns} (18.12±1.21)^{ns}	390.8±3.34^{ns} (21.84±0.66)^{ns}	282±8.03*** (43.6±1.60)***	199.8±5.67*** (60.04±1.13)***	164.4±6.54*** (67.12±1.30)**	97.8±3.76* (80.44±0.75)**	64.6±7.63*** (87.08±1.52)**	32.4±3.36*** (93.52±0.67)*	13.2±1.48 (97.36±0.29)	20.4±1.14

Values are expressed as ±SEM and compared the different group with ANOVA. N=5
*p<0.05, **p<0.01, ***p<0.001, ns Non significant when compared to the control group

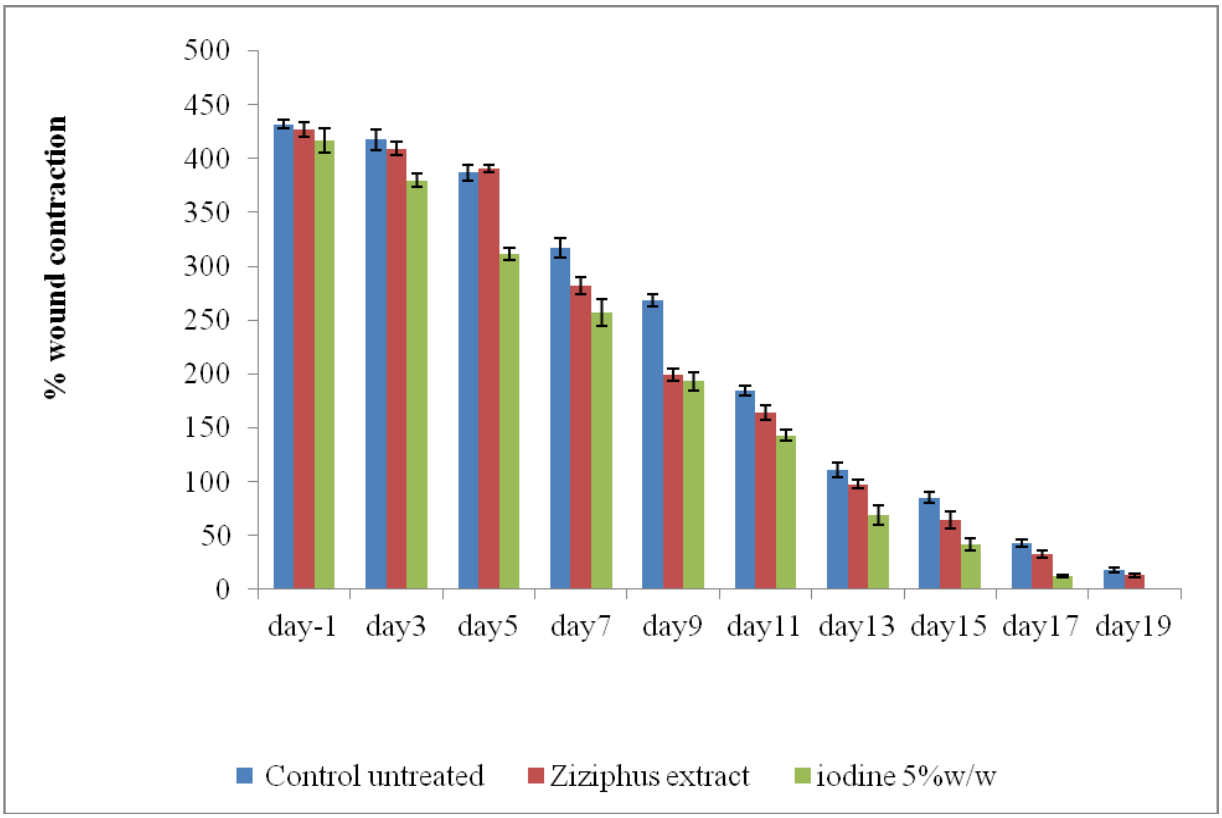

Figure 2: The % wound contraction of Excision wound model

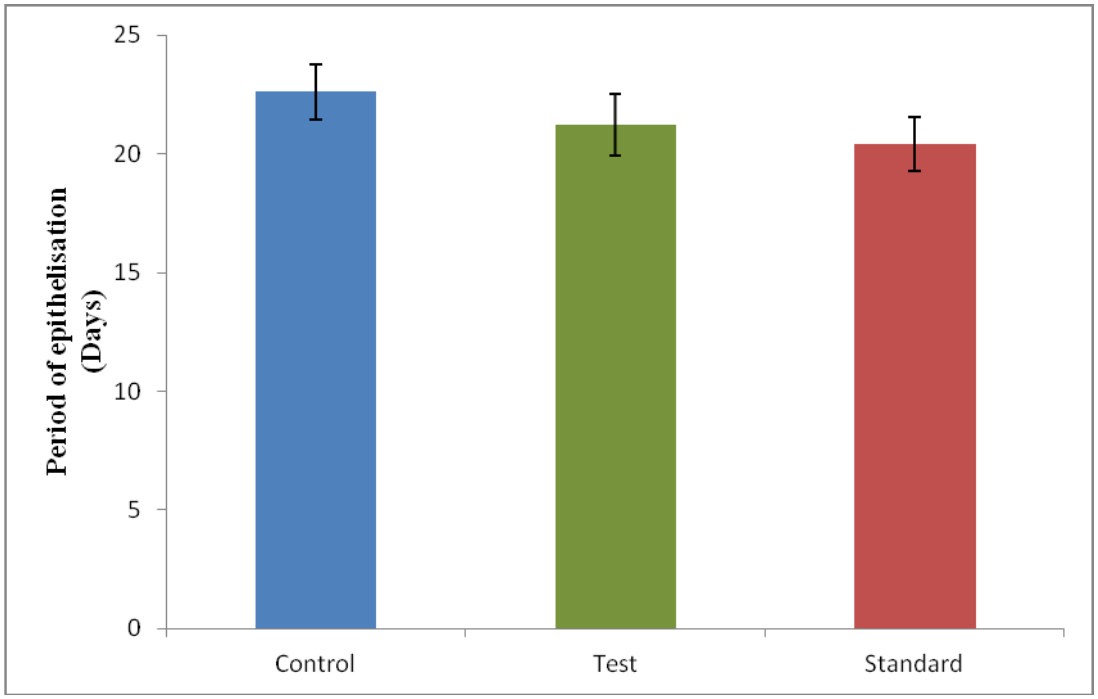

Figure 3: The period of Epithelisation in Excision model

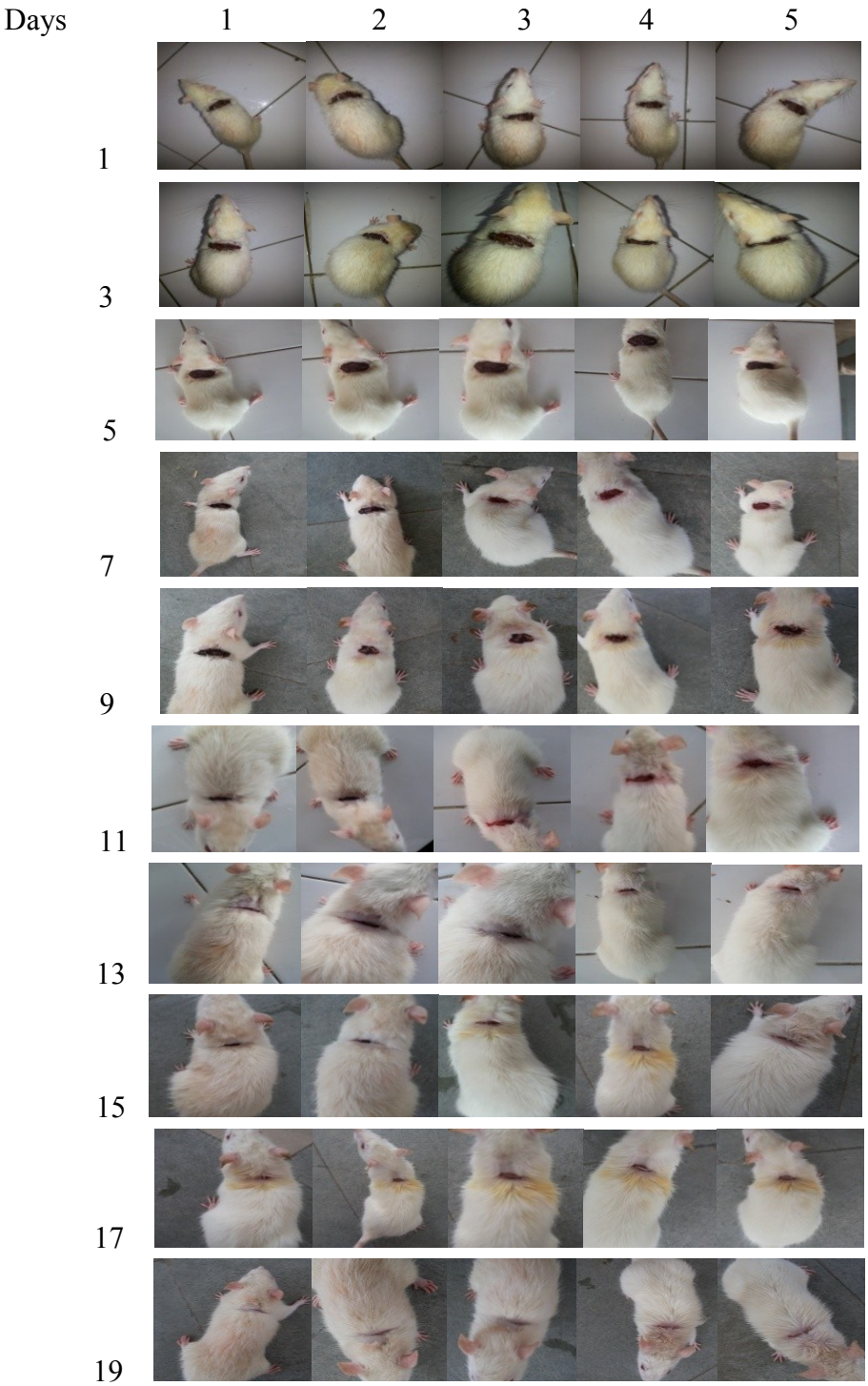

Figure 4: Excision wound control group animals

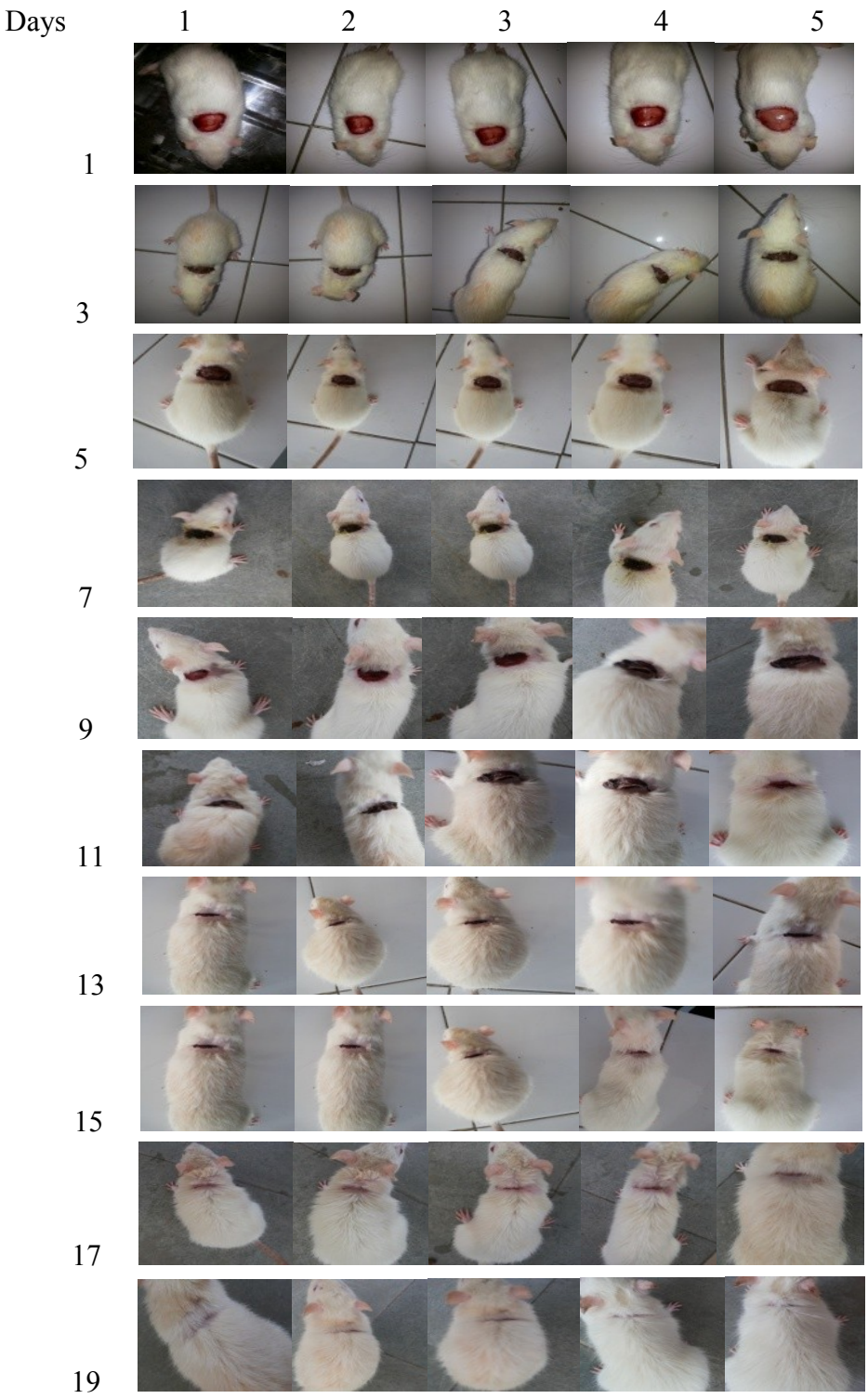

Figure 5: Excision wound test group animals

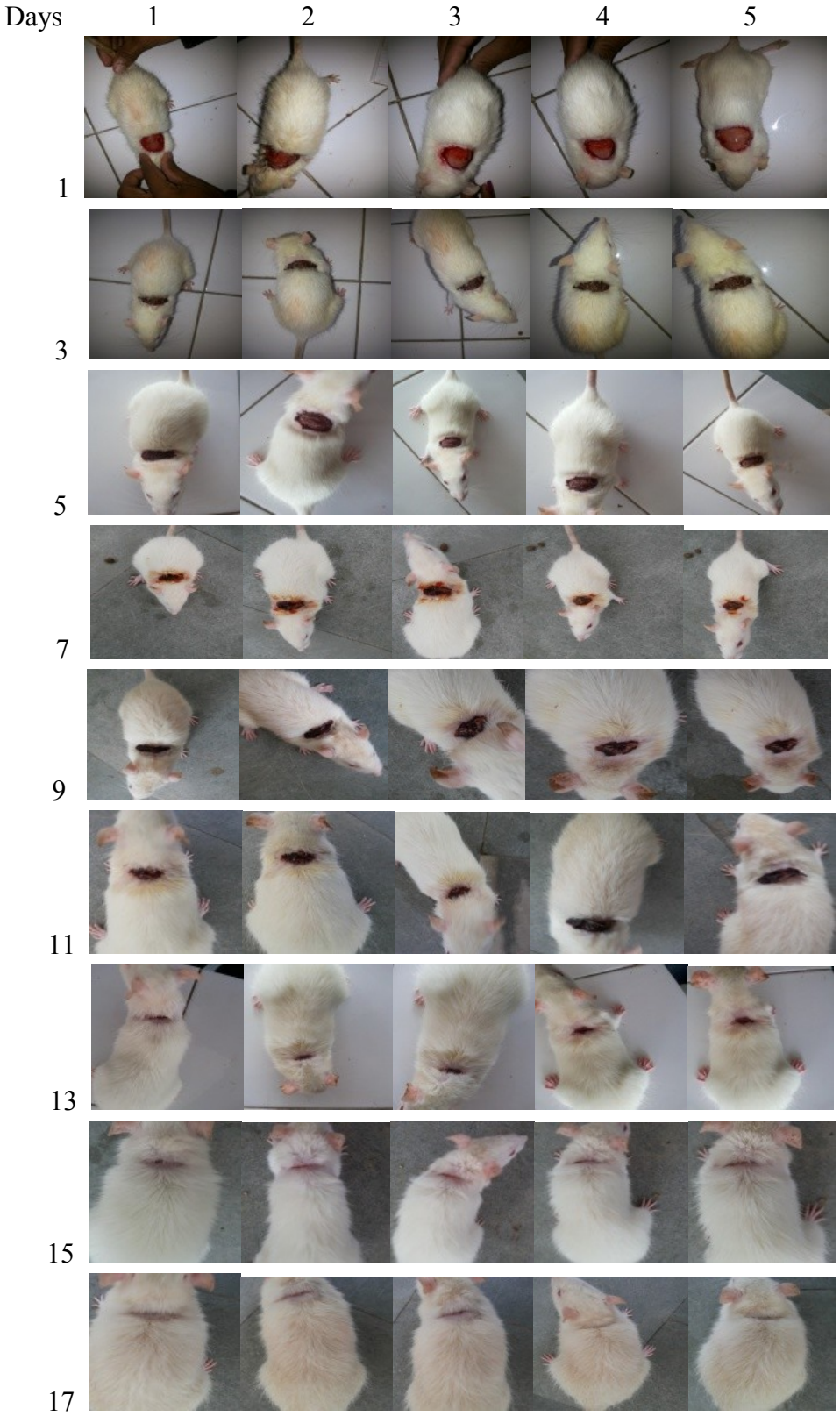

Figure 6: Excision wound standard group animals

Dead Space Wound Model

The concentration of hydroxyproline after 10th post wound day of group 1, group2 and group 3 were found to be 1.62±0.27,3.21±0.14 and 4.61±0.55.

Table 2: Effect of Ziziphus mauritiana extract on healing in Dead space wound model

Treatment	Wet tissue weight (mg)	Dry tissue weight (mg)	Concentration of Hydroxyproline (mg)
Control untreated	84.4±3.57	43.4±3.97	1.62±0.27
Test ZM Extract 200 mg/kg p.o.	113.4±2.60***	51.8±2.16**	3.21±0.14***
Standard (dexamethasone 0.17mg/kg p.o.)	145±2.23***	54.4±2.88***	4.61±0.55***

Values are expressed as±SEM,Compared the different group by ANOVA,N=5
*p<0.05, ***p<0.001,ns Non significant when compared to control group

CONCLUSION

All these results revealed that the topical ZMLE can be used to assist healing of cuts, scrapes and even skin ulcers which may be due to synergistic action of active principles present in ethanolic extract of *Ziziphus mauritiana.* ZMLE given orally at a dose of 200 mg/kg significantly suppressed the response of inflammation of dead space wound model. So, anti-inflammatory activity was observed with ZMLE in albino rats. The results of above study also provide a rationale for the use of ethanolic extract of *Ziziphus mauritiana* in the traditional system of medicine as antiinflammatory and to promote wound healing.

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