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In vivo study

Wound healing activity of *Tamarindus indica* Linn. seed and cork ash

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ABSTRACT

Introduction**: Chincha (Tamarindus indica* Linn.) is indicated for the treatment of wounds in Indian system of medicine, however there is no experimental data related to its efficacy in wound healing potential thus the present study was undertaken to evaluate the wound healing potential of the cork and seed ash of *Tamarindus indica* Linn. in Wistar albino rats. ***Methods: The excision wound was made on the dorsal side of the rats measuring an area of 2cm. [2]The animals were randomly divided into four different groups and treated with group specific drugs for 21 consecutive days. The number of days required for epithelialisation and percentage wound contraction was measured during the experimentation. The hydroxyproline estimation and histopathology was carried out in the healed skin. ***Results***: The test drug treated groups showed significant reduction in the period of epithelialization and percentage wound contraction compared to normal control. The cork ash of *Tamarindus indica* Linn. has shown considerable increase in the % wound contraction ($P<0.01$) on 9th, 12th,18th and 21st ($P<0.05$) post wounding day and seed *ash* has shown considerable increase in the % wound contraction on 18th($P<0.01$) & 21st($P<0.05$) day, as compared to control. The hydroxyproline content was increased in *T. Indica* cork treated group than *T. Indica* seed. The histopathalogical study revealed almost normal cytoarchitecture and complete re-epithelialization in test drug treated groups. ***Conclusion***: The results suggest that *T. indica* cork and seed *ash* has significant wound healing potential and comparatively *T. indica* cork ash has significant wound healing activity than *T. indica* seed.

KEYWORDS

Epithelialization, Excision wound, Histopathology, Hydroxy proline, percentage wound contraction.

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The universal role of plants in the treatment of disease is exemplified by their employment in all the major system of medicine irrespective of the underlying philosophical premise. The knowledge and other properties of plants is still transmitted from generation to generation so there is pressing need to preserve local knowledge before it is lost forever.[1] Several recent surveys have shown that using ethno pharmacology as a basis of selecting species for screening results in a significant increase in the "hit rate" for the discovery of novel active compounds compared with random collection of samples.[2] Ayurvedic texts claims that the plants available in vicinity are much beneficial in the management of diseases.[3] The usage of drugs which are commonly available, cost-effective and efficient should be encouraged rather than going for the expensive drugs. This will also reduce the burden over the routinely prescribed classical drugs thus preventing them from the verge of extinction and can be progressively used for longer time.

Tamarindus indica Linn. of Caesalpiniaceae is commonly identified and known as *Chincha* in Ayurveda system of medicine. Its fruit, tender leaves and flowers are used extensively in culinary preparations. It's a large wide spreading tree 12 to 18 m high. The trunk shows dark rough bark with deep cracks, and seeds are smooth, reddish brown, enveloped by tough leathery membrane. It has scientifically reported for several medicinal properties viz. anti-oxidant, anti-inflammatory, analgesic, anti-arthritic activity (for seed); anti-oxidant, anti-microbial activity (for fruit); antibacterial, hepatoprotective effect (for flowers); anti-microbial activity (for leaves); and hyperglycaemic, anti-microbial activity (for bark).[4-13] Various chemical constituents have reported from different parts of plants; tannins, saponins, glycoside, peroxidase and lipids have been isolated from the bark of *Tamarindus indica,* whereas the pipecolic acid , citric acid, 1-malic acid, lupanol, vitamin C, pectine, tannins, glycosides were isolated from leaves. The chemicals like furan derivatives, carbolic acid, citric acid, pectine and invert sugar were derived from fruit and campestrol, β- sitosterol, palmitic acid, linoleic acid, xylose, galactose and glucose were isolated from seed.[14]

Chincha is used in Ayurveda for the treatment of indigestion, as an appetizing agent, cardiac tonic, vermifuge, burning sensation and constipation in fever, seeds are useful in diarrhoea, dysentery, diabetes. Fruit juice is used for wound healing. The bark *kshara* (liquefied ash) cures colic and diminished digestive power.[15]

The cork and seed *masi* (ash) of *T. indica* has been successfully used by the folklore practitioners in the treatment of various types of wounds. Considering the traditional claim and reported activities, present experimental study was undertaken to evaluate the wound healing property of cork and seed ash of *T. indica* in Wistar albino rats using excision wound model.

MATERIALS AND METHODS

Plant materials

The seed and cork of *T. indica* were collected from Udupi and authenticated in the department of Pharmacognosy, SDM Research centre Udupi. The cork and seeds were dried in sun light and were made into fine powder for further usage.

Preparation of the *Masi* (ash)

The cork and seed of *T. indica* was taken and pounded to fine powder separately. These fine powders were taken in separate open pan and roasted with frequent stirring until turns into black soot like powder. This black soot like powder was, again taken in clean *Khalva Yantra* (the instrument used for triturating) and triturated further to obtain fine homogenous powder as mentioned in classics. The each powder was later separately preserved in suitable airtight containers.

Drugs and chemicals

Chincha (Tamarindus indica Linn.) – Cork and Seed *Masi* (Ash) was prepared locally and Povidone iodine procured from A. B. Enterprises Mumbai.

Experimental animals

Wistar albino rats of weighing 200 ± 25g body weight of either sex were procured from animal house of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. The experimental protocol was approved by the institutional animal ethical committee (CPCSEA/10/IAEC/15-16/12103/2015). Rats were fed with normal rat diet and water *ad libitum* throughout the study.They were acclimatized in the laboratory condition for two weeks prior to the experimentation. The experimental rats were maintained at natural 12:12h light and dark cycle, temperature of 25 ± 2^0C and relative humidity of approximately 50%.

Study design

The healthy rats were inflicted with excision wounds as described by Morton and Malone, under light ether anaesthesia.[16] One excision wound was made by cutting away a 2 cm^2 full thickness of skin from the depilated area, the wound was left undressed to open environment. The animals were randomly divided into four different groups of 6 rats each. Group 1 animals were left untreated and served as control. Group 2- served as reference standard and treated with povidone iodine ointment topically. Group 3 and 4 were treated topically with the paste of *T. indica* cork and seed ash respectively. The group specific drugs were topically applied once a day, starting from the day of the operation, till complete epithelization. The change in the wound shapes, the wound margins were traced on a trace paper from the day of wounding and continued till the complete healing of the wound. The wound area was calculated using millimetre scale graph paper. The percentage wound contraction was calculated on the 3rd, 6th, 9th, 12th, 15th, 18th and 21st post wounding days using the following formula.

$$\% \text{ wound contraction} = \frac{\text{Initial wound size- specific day wound size}}{\text{Initial wound size}} \times 100$$

These wounds were also observed for epithelialization by noting the day of escher fall. On the 21st day the animals were sacrificed and a piece of skin was collected from the wound area for hydroxyproline and histopathalogical examination.

Estimation of hydroxyproline concentration

Wound bed sample weighing 20 mg was taken and the colorimetric assay was performed with known standards of hydroxyproline at 560 nm using double beam spectrophotometer. The hydroxyproline contents of individual samples were expressed as μg hydroxyproline/mg muscle wet weight. The amount of hydroxyproline in the final colorimetric reaction represents a proportion (1.5 ml/2.5 ml) of the total hydroxyproline in the final toluene extract. Multiply the result by (2.5/1.5) to obtain the total amount of hydroxyproline present in the final extract. Divide the result by the amount of muscle (wet weight) contained in the initial sample (0.5 mg) to obtain the hydroxyproline content (μg hydroxyproline/mg muscle).[17]

Histopathology of skin

On 21st day animals were sacrificed by deep ether anaesthesia. The full thickness skin from wound bed was dissected and then transferred to 10% formalin. The tissue was embedded in paraffin. The section was cut into 5 μm thickness and stained with haematoxylin and eosin stain. Sections were qualitatively assessed under the trinocular light microscope. Stained slides were observed for assessing epithelialization, angiogenesis, collagen formation and for presence of leucocytes, these observation is compared with control group.[18]

Statistical Analysis

The data was expressed in Mean ± SEM and statistically analyzed by employing one way ANOVA followed by Dunnet's multiple t- test as post hoc test. Graph pad prism Inst 3 was used for this purpose and $p<0.05$ and $p<0.01$ considered as statistically significant.

RESULT AND DISCUSSION

Effect on wound contraction

The percentage of wound contraction was significantly increased in *Tamarindus indica* cork and seed *masi* group when compared to control group. The test drug *T. indica* cork has shown considerable increase in the % wound contraction at 3rd, 6th, 9th, 12th, 15th 18th and 21st day measurement and found to be statistically significant in comparison to normal control group. While the Test drug *T. indica* seed ash has shown significant increase in the % wound contraction on 18th & 21st day measurement in comparison to normal control group (Figure 1). There was increase in percentage wound contraction in standard group when compared to the control group, the observed increase was found to be statistically significant in 6th, 9th, 18th day measurement (Table 1).

Table 1. Effect of *Tamarindus indica* cork and seed on wound contraction at different intervals

Group	Percentage wound contraction measured at different time interval [in no. of days]						
	3rd	6th	9th	12th	15th	18th	21st
Control	13.48 ± 5.48	43.52 ± 5.74	66.660 ± 5.31	87.10 ± 3.80	92.37 ± 2.56	95.82 ± 1.33	98.31 ± 0.76
Standard	23.66 ± 4.96	61.82 ± 1.84**	86.07 ± 2.22**	93.41 ± 1.87	96.27 ± 1.91	98.60 ± 0.22*	99.37 ± 0.16
Test1 (Cork)	33.39 ± 3.79*	66.51± 3.65**	90.62 ± 1.18**	97.00 ± 0.56*	99.34 ± 0.26*	99.90 ± 0.04**	100 ± 00*
Test2 (Seed)	12.33 ± 2.62	35.89 ± 2.90	74.45 ± 4.63	88.54 ± 1.40	96.96 ± 0.47	99.17 ± 0.31**	99.87 ± 0.09*

Data expressed in Mean ± SEM, *P<0.05, **P<0.01 in comparison to control group

Effect on day of escher fall

The test drug *T. indica* cork ash (TICA) has shown early escher falling compared control group and found to be statistically significant (P<0.05), whereas the *T. indica* seed ash (TISA) considerable decrease in the number of days taken for escher fall however it is found to be no significant different among control group (Table 2).

Table 2. Effect of *Tamarindus indica* cork and seed on number of days taken for eschar fall

Group	Day of Eschar fall	% Change
Control	9.83 ± 0.79	
Standard	8.83 ± 0.30	10.172 ↓
Test1(*T.indica* Cork)	7.66 ± 0.42*	22.075 ↓
Test2(*T. indica* Seed)	9.33 ± 0.21	5.086 ↓

Data: MEAN ± SEM,*P<0.05 in comparison to Control group

Effect of*Tamarindusindica* cork and seed ash on Hydroxyproline concentration

The hydroxyproline content was relatively higher in TICA treated group compared to other groups, however the difference was statistically non-significant (Table 3).

Table 3. Effect of *Tamarindus indica* cork and seed on hydroxyproline concentration

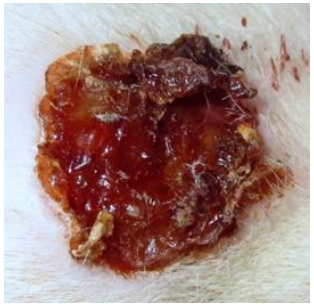
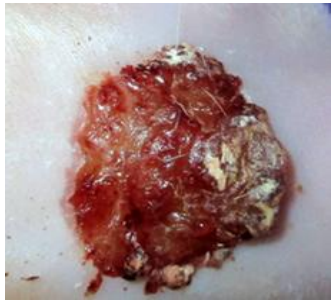
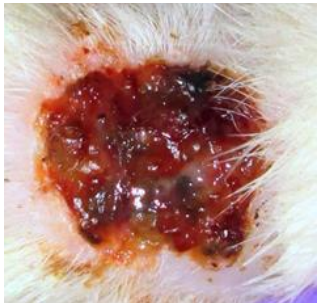
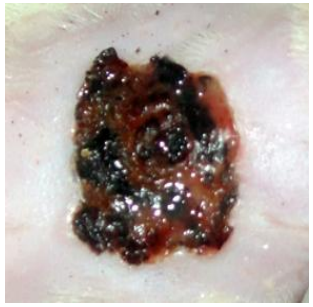
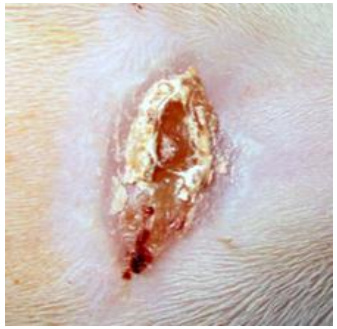
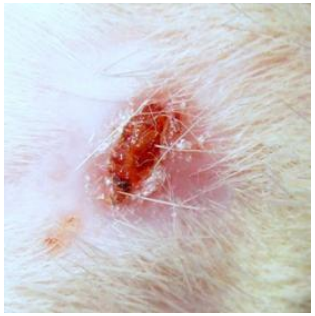
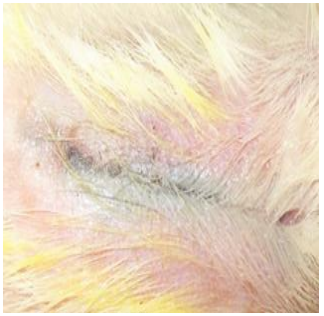
Group	Hydroxyproline estimation (µg/mg)	% change
Control	6752.59 ±183.22	
Standard	3769. 62 ± 395.04**	44.1751 ↓
Test1(*T.indica* Cork)	7958.51 ± 663.60	17.8586 ↑
Test2(*T. indica* Seed)	5609.26 ± 360.47	16.9317 ↓

Data: MEAN ± SEM **P<0.01 in comparison to Control

Histological observations

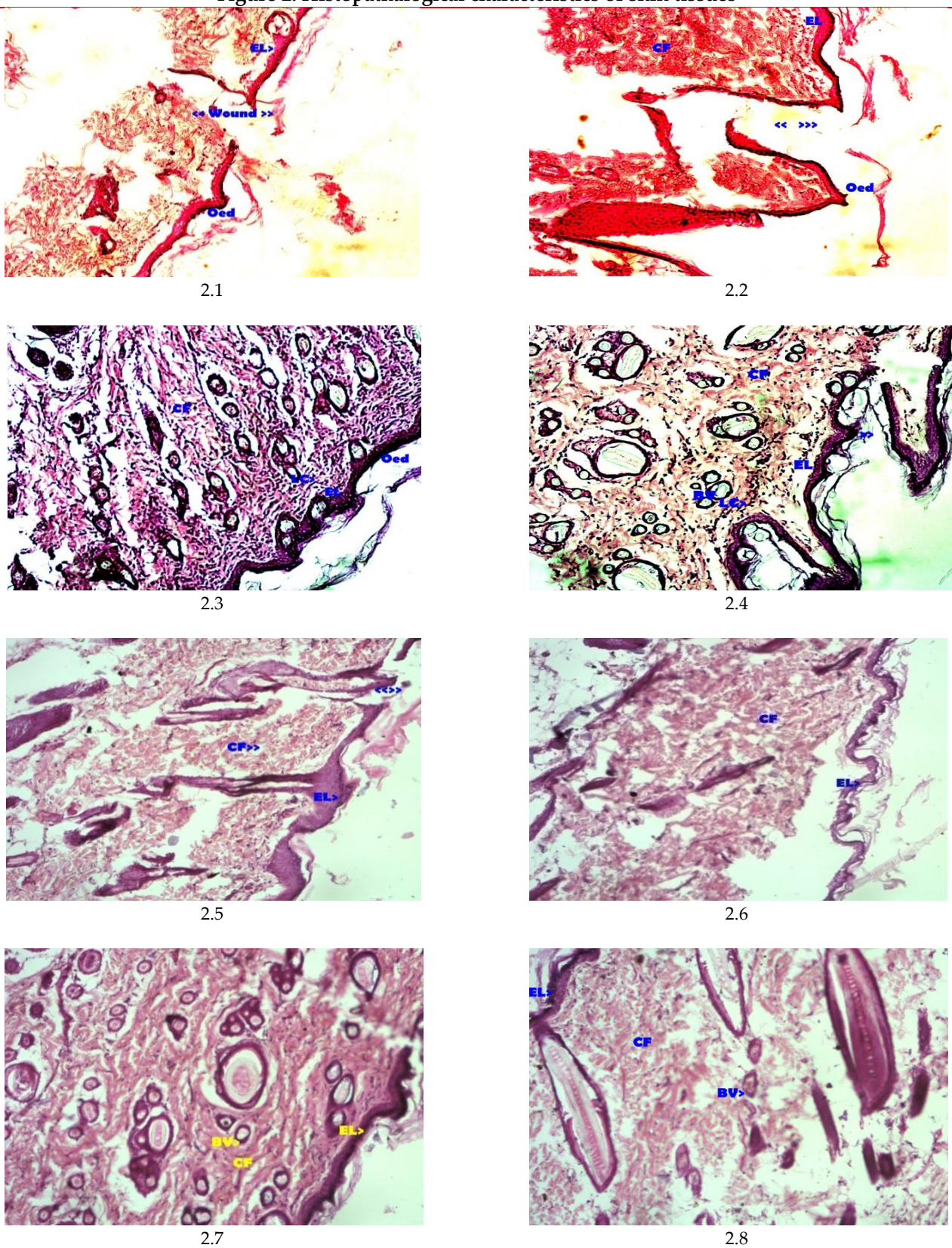
The photomicrograph ofwound sections from control group rats has shown reduced collagen formation, moderate and incomplete re-epithelialization, comparatively less vascularization, presence of leucocytes was sparse. In standard group,normal collagen formation, enhanced epithelialization, blood vessels were prominent and the leucocytes were also noted. In test drug treated groups (*Tamarindus indica* cork and seed ash) complete re-epithelialization, normal collagen formation and few leucocytes were observed. Between the two test groups no remarkable difference was observed (Figure 2).

Figure 1. Different stages of wound healing measured at different time interval

Days of observation	CONTROL	STANDARD	TICA	TISA
7th Day	 1.1	 1.4	 1.7	 1.10
14th Day	 1.2	 1.5	 1.8	 1.11
21st Day	 1.3	 1.6	 1.9	 1.12

Control group (1.1-1.3), Standard group (1.4-1.6), *T. indica* cork [TICA] treated group (1.7-1.9), *T. indica* seed [TISA] treated group (1.10-1.12)

Figure 2. Histopathalogical characteristics of skin tissues



Control group (2.1 and 2.2), Standard group (2.3 and 2.4), *T. indica* cork treated group (2.5 and 2.6), *T. indica* seed treated group (2.7 and 2.8).

Epithelial layer (EL), oedema (Oed), collagen fibres (CF), Leucocytes (LC), Blood vessels (BV)

Wound healing is a complex and dynamic process of restoring cellular structure and tissue layers in damaged tissue as closely as possible to its original state. The excision wound healing has three important phases such as inflammatory phase, which is

characterised by haemostasis and inflammation, Proliferative phase characterised by angiogenesis, collagen deposition and remodelling Phase characterised by wound undergoes contraction and granulation tissue formation.[19]

In Ayurveda and different traditional system of medicine hundreds of herbs were mentioned for its medicinal use. In present study we have taken one such reference regarding wound healing property of two different parts i.e. cork and seed of *T. indica* in experimentally induced wounds in rats. The results of the present study substantiate the use of *T. indica* cork and seed masi in folklore medicine for the treatment of wounds.

We consider plants are the rich sources of therapeutically diverse molecules with potential therapeutic value. The phyto-constituents such as tannins, alkaloids, coumarins and flavonoids has a strong antioxidant, anti-inflammatory and anti-microbial activities.[17] In excision wound initially there will be production of reactive oxygen species (ROS) as a part of tissue injury. These ROS were responsible for initiating the inflammatory response followed by interference in the wound healing process.[18] In majority cases there will be infection due to microbial attack. Hence the primary aim of therapy for wound healing is to prevent further tissue injury due to oxidative stress or either prevention of inflammatory process or prevention of microbial infection.[20] *T. indica* seed has showed the presence of flavonoids which is established to possess potent anti-oxidant activity, free radical scavenging effect along with anti-inflammatory and antimicrobial activity thus the collective anti-inflammatory, anti-oxidant and anti-microbial property of *T. indica* cork and seed could be the responsible factor for its wound healing property. Comparatively *T. indica* cork masi has shown highly significant wound healing activity than *T. indica* seed masi. Hence from these findings we can conclude that the *T. indica* cork masi can be a potential therapeutic agent and used against wound caused by tissue injury. This supports its traditional usage of *Chincha* (*T. indica*) in wound healing as mentioned in Ayurvedic text and folklore claim.

CONCLUSION

From the present study, we can conclude that *Tamarindus indica* cork and seed masi exhibited potent wound healing action against excision wound model. Comparatively *T. indica* cork masi has shown highly significant wound healing activity than *T. indica* seed masi. However, still further research is warranted to delineate the mechanism of present findings and to evaluate the major phytoconstituents responsible for the wound healing property exhibited by cork and seed of *T. indica.*

CONFLICT OF INTEREST

Nil

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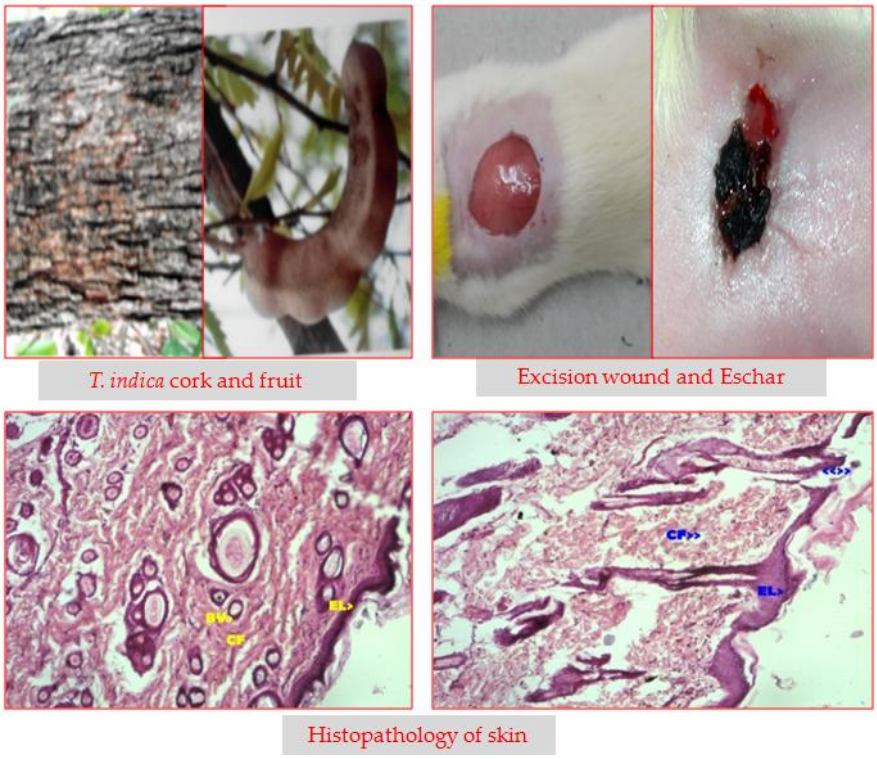
GRAPHICAL ABSTRACT



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