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Effects of Topical Application of Green Apple Extract on Excisional Wound Healing in Female Rabbits

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Abstract
In the present study wound healing efficiency of green apple alcoholic extract of the whole fruit in the form of ointment , using one type wound model (full thickness incisional wound)was evaluated in twelve female rabbits. ethanol solvent was used to prepare the extract. chemical analysis of green apple alcoholic extract reveals the presence of alkaloid, saponin ,tannin in Flavonoid, Glycosides, polysaccharides , proteins, Triterpens and Carbohydrates. tow Circular wounds were inflicted on the animals which ,divided into three groups , four animals in each group. the ointment of green apple was applied topically with 5mg twice daily. Wound area was measured and the percentage of wound reduction (wound contraction rate) per a day was calculated. The effects were studied on excissional wound were hyperemia , exudation , were percent of wound contraction and length of wound macroscopically, and infiltration of inflammatory cell (macrophage ,neutrophils),granulation tissue formation microscopically. Treated wounds induced complete wound healing in shortest period (11 days) while control wounds show non complete healing till the end of experiment (14 days).
Keywords: rabbits, wound healing, green apple

1. Introduction

Wound healing is the physiological response to the tissue injury that results in the replacement of destroyed tissue by living tissue and thus restoration of tissue integrity. The mechanism of wound repair occurs by four basic processes such as inflammation ,wound contraction, epithelialization and granulation Tissue formation. Inflammation starts immediately after the disruption of tissue integrity (1). During this process, a set of complex biochemical events takes place in a closely orchestrated cascade to repair the damage. Errors in wound healing can lead to delayed healing or formation of hypertrophic scars (2). The goal for wound treatment is fast and scarless healing.

Apple is also very nutritious. As the saying goes – an apple a day, keeps the doctor away. Green apple is a favorite fruit of many. There are almost 7000 types of apple available in the world. The red apple is very popular as well as the green apple. Usually apples are sweet, but some are sour as well. The sour apples are used for cooking, refining it for the preparation of oil, as well as making wine. The green apple falls into the latter category. Green apple has a green colored radiant skin and is sweet and sour in taste. It is very juicy also and very tasty to eat.(2). Health Benefits of Green Apple:

The green apple not only tastes good but as several benefits. Let us have a look at the benefits. Some of the primary health benefits of green apple are: Contains a lot of fiber, which is good for digestion. The chances of colon cancer are less if one eats green apple .Relieves constipation. Beneficial in treating gout. Useful in controlling diarrhea. Reduces blood pressure .Blood sugar gets stabilized. Helps in treating rheumatism .Improves one's appetite. It contains carbohydrates and therefore provides much energy .It contains protein. Has plenty of vitamins, such as Vitamin C, Vitamin A, Riboflavin, Vitamin B6 and it is one of the most prominent green apple benefits .It also has plenty of minerals such as calcium, iron, magnesium, Manganese, Iron, Potassium, Copper, Zinc It helps in preventing liver problems. Lessens the chances of developing skin diseases. It consists of several anti-oxidants such as polyphenol as well as flavonoid that helps in reducing the risk of cancer by not allowing DNA damage to occur. It has less cholesterol and is therefore useful for those losing weight .As green apple contains a lot of Vitamin A,B as well C, it is very good for the nourishment of skin and also has a whitening effect on it .It purifies the blood as it contains a lot of minerals .One also less chances of suffering from liver problems .Apples have essential nutrients and green apple is no exception to it. Green apple has lots of benefits due to which it should be included in the daily diet (3&4).

Material and Methods

Preparation of extract

Air dried and coarsely powdered (70 gm) of green apple were placed in soxhlet extractor separately, using Diethyl ether about (300 ml) at 50 ^{o}C then successively with ethanol. The extracts were then concentrated to dryness under reduced pressure and controlled temperature, respectively and they were preserved in a refrigerator. (5).

Preparation of oil ointment

The ointment was prepared by using Vaseline base by titration method using spatula to admix the oil extract of green apple with gradual addition of Vaseline to obtain a homogenous ointment with ration of oil extract to Vaseline base was 1 : 3 respectively (6).

Preliminary chemical investigation

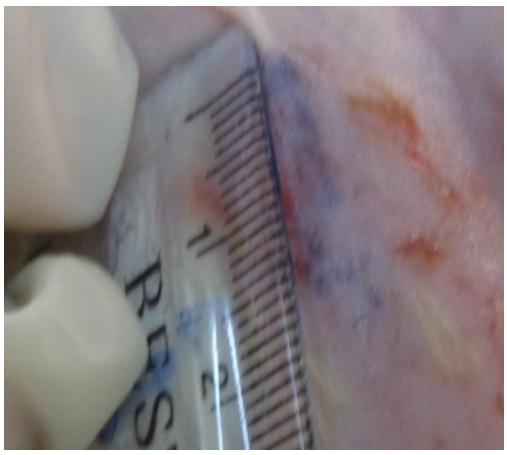
- ❖Carbohydrates test : By use molish reagent . (7).
- ❖Flavonoid test : By use Magnesium taringes and alcoholic Potassium hydroxide solution (8).
- ❖Saponin test : By use aqueous mercuric chloride (5%) , ($HgCl_3$ 5%) . (9).
- ❖Glycosides test : By use Benedict's reagent . (8) .
- ❖Polysaccharides test : By use Iodine reagent (10) .
- ❖Triterponoides test : By use sulfuric acid & chloroform (11).
- ❖Protein test : By use biuret Reagent . (12).
- ❖Aldehydes and Ketone's test : By use 2 , 4 Dinitro phenyl hydrazine reagent (13).

Animal and housing

eighteenth female rabbits , weighting 1.5 – 2 Kg . were distributed into three groups of six animals each . The rabbits were housed individual cages , maintained on 24 hour light , and given free access on *alfa alfa* and water , at room temperature .

Excision wound model

Excision wound models were used to evaluate the wound healing activity. Excision wound model was employed to have information about wound contraction and wound closure time on the three groups of animals. The back hairs of the animals were depilated by shaving. two wounds were created on the shoulder region of each standard made on both sides of shoulder regions , made by standard sharp blade the length of incision was 1.5 cm the right side was used as treated wound and the left was used as control. Fig(1). Wounds were left open and the medicine was applied topically twice a day (once in the morning and evening) on to each animal .(14). Then both treated and control wounds in each animal were treated with oil ointment and Vaseline base respectively with (0.5 g) of each , The treatment with ointment and Vaseline was continue for 14th day postoperative day ,finally wounds were covered with cotton vest to prevent detachment and self infliction Fig(2).



Figure(1)**:**show the length of the wounds were 1.5cm.

Figure(2):The cotton cloth vest to prevent attachments.

Measurement of wound area:

The progressive changes in wound area were monitored every fourth day. The size of the wound was also measured using a scale daily and the wound area was calculated. Wound contraction was calculated as percentage of the reduction in wound
area (15):

$$\textbf{Percentage of wound contraction}=\frac{(\text{Initial wound area} - \text{Specific day wound area})}{\text{Initial wound area}} \times 100$$

Macroscopically

Both wounds (treated and control) were examined daily to observe the pathological changes which take place in

wounds , regarding to the severity of hyperemia (redness degree) exudation (seous , seroupurulant and purulent) , (16).

Wound biopsy

The animal at each intervals (3rd , 7th , and 14th) day post were euthanized under general anaesthesia with Intramuscular of a combination of Ketamin HCl (50 mg /Kg) and xylzin hydrochloride (10 mg/Kg) body weight (17) . The samples taken for histopathological examinations were prepared using classical processes, and then the paraffin-embedded blocks were cut to a thickness of 5µ and stained with hematoxylin and eosin stain. The tissues were scored histopathologically in terms of re-epithelization, granulation development, collagen accumulation, inflammatory cell infiltration and angiogenesis .granulation tissue development; progress of epithelization and presence of infection were evaluated macroscopically. Progress of wound contraction and epithelization were accepted as criteria for wound healing.

Results

Table 1. The chemical constituents of green apple alcoholic extract

Chemical Constituents	Results
Glycosides	+
Flavonoides	+
Proteins	+
Polysaccharides	+
Saponins	+
Triterpens	+
Carbohydrates	+
Aldehydes and Ketones	+

The preliminary chemical analysis of green apple alcoholic extract showed the presence of all chemical constituents that recorded in the above table.

Macroscopic evaluation

Table 2 . The effect of green apple alcoholic extract on macroscopic wound healing categories

Days	Groups	Hyperemia	Exudation
3rd day	Treated	-	-
	Control	+ +	+ +
7th day	Treated	-	-
	Control	+++	+++
14th day	Treated	-	-
	Control	+	+

*** Note : (-) Absent „ (+) Mild „ (++) Moderate „ (+ + +) Severe**

Topical applications of green apple alcoholic extract for the treated wound showed absence of hyperemia and exudation through the period of experiment ,figures: (1, 2 , 3) while the severity of these categories (Hyperemia and exudation) ranged between moderate at third day to sever at 7th day and reduce to be mild at 14th day postoperative day .As explained in Table (2) .

The decreasing in the length of the wounds

Table 3. The effect of green apple alcoholic extract on decrease the wound length

Groups	0 Day	3rd Day	7th Day	14th Day
Treated	1.5 cm	1.1 cm	0.8 cm	0 at 11th
Control	1.5 cm	1.4 cm	1 cm	0.3 cm

The above table explains the decreasing in the length of the wounds in both (treated and control wound throughout the period of the experiment.

Table 4. The effect of green apple alcoholic extract on wound area and wound contraction of wounds

intervals		Wound area	Wound contraction
3rd day	treated	0.95	46
	control	1.54	12
7th day	treated	0.50	71
	control	0.78	55
14th day	treated	0	100 at 11th day
	control	0.07	96

Figure1. The length of treated wound was 1.1cm at 3rd postoperative day.

Figure2. The length of control wound was 1.4cm at 3rd postoperative day.

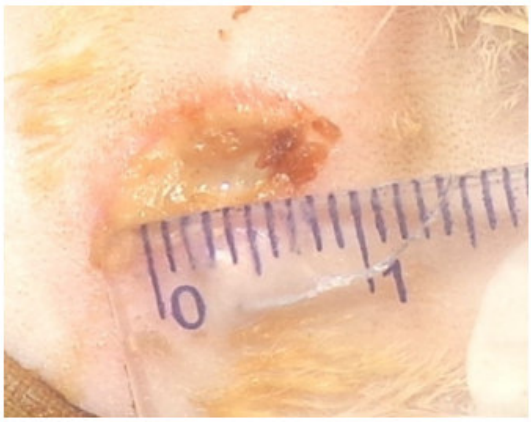


Figure3.The length of treated wound was 0.8 cm at 7th postoperative day.

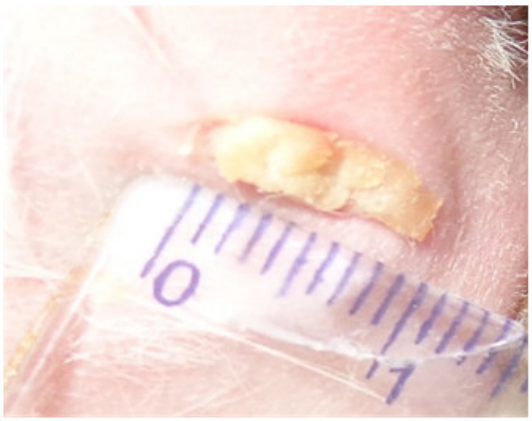


Figure4. The length of control wound was 1 cm at 7th postoperative day.

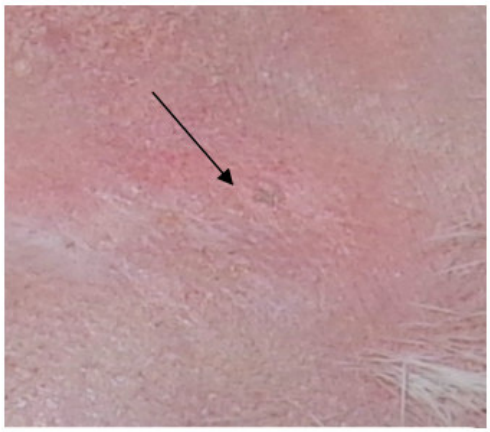


Figure5.Treated wound showed complete healing at 11th postoperative day(arrow).

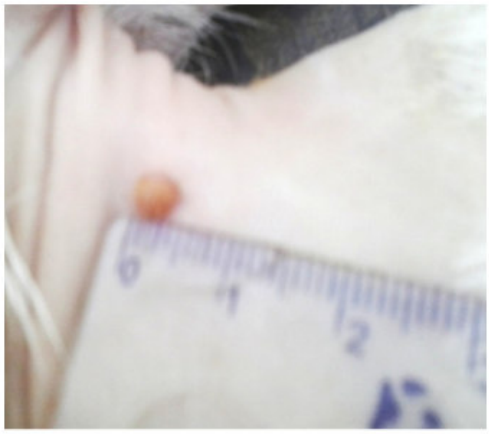


Figure6.control wounds showed non complete healing, ,the length was 0.3 at 14th postoperative

Microscopic Evaluation

Table 4. The effect of green apple alcoholic extract on microscopic categories

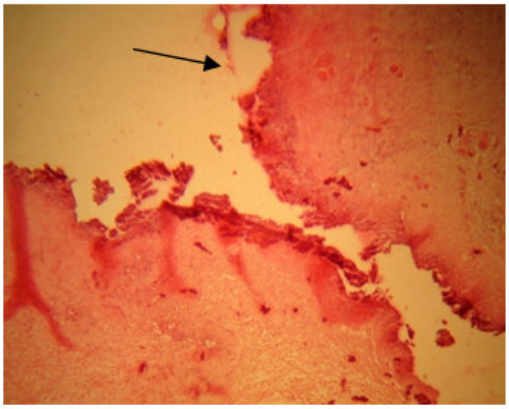
Days	Groups	Neutrophils	Macrophages	Granulation tissues	Re-epithelialization
3rd day	treated	+	+	+	-
	control	+ +	-	-	+
7th day	treated	+ +	+ + +	+ + +	+ + +
	control	+ + +	+ +	+ +	+ +
14th day	treated	-	+ + +	+ + +	(11th day)+ + +
	control	+	+ +	+ + +	+ + +

Note : (-) Absent , (+) Mild , (++) Moderate , (+ + +) Severe

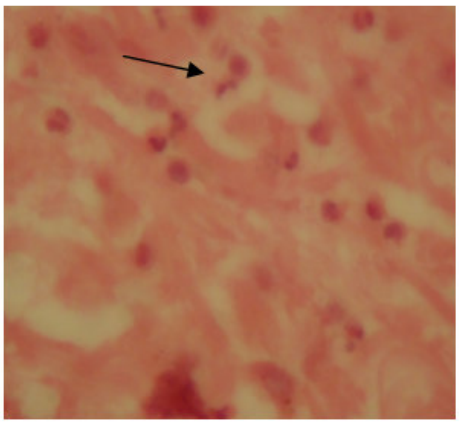
The result of the effect of green apple alcoholic extract on the histological elements of wound healing was showed in table (4) and gross figures . At 3rd day the infiltration of neutrophils was less in treated wound figure(2, 4) and still lesser in treated wound than control on 7th day figure(6,8) . The infiltration became mild on 14th day in control and disappeared completely in treated wound figure(10,12) .

The infiltration of macrophage was higher in treated wound than in control at 3rd and 7th and decreased in their infiltration at 14th day figure(2,4,6,8,10,12) .The granulation tissue appear early at 3rd postoperative day and become obvious on 11th day . Throughout period of experiment , the progression of new epithelium to cover the wound area in the treated wound more than control wounds figures: (1,3,5,7,9).

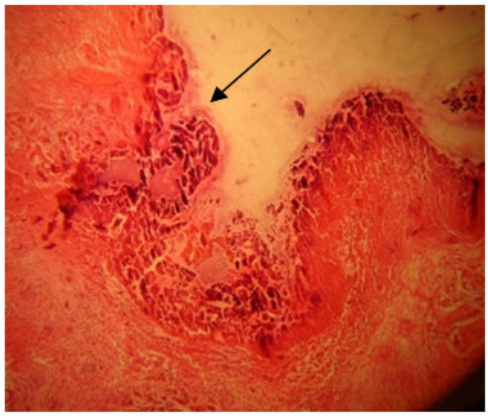
Histopathological figures



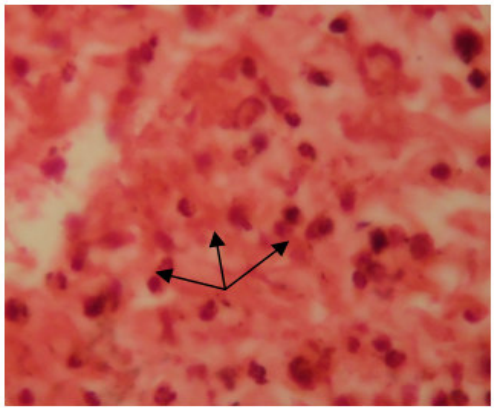
Figure(1): treated(3rd day)mild re-pitheliazation . Hand E stain. 10x.



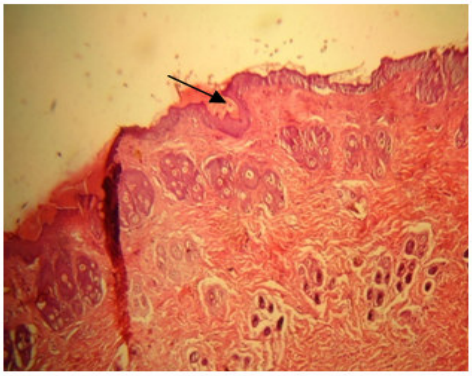
Figure(2): treated(3rd day) infiltration of inflammatory cells was mild(arrow) . Hand E stain. 40x.



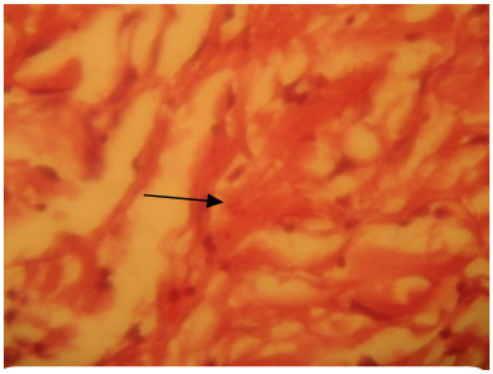
Figure(3): no re-epitheliazation arrow. H and E stain. 10x.



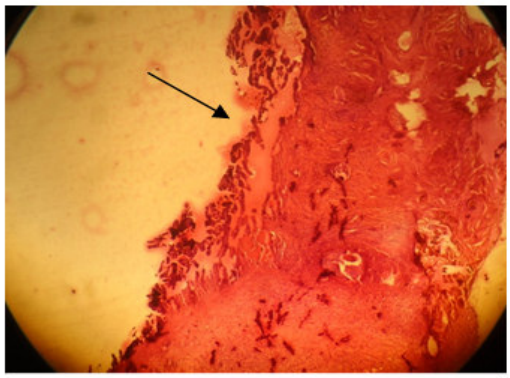
Figure(4): control (3rd day) infiltration of inflammatory cells was sever(arrows). Hand E stain. 40x.



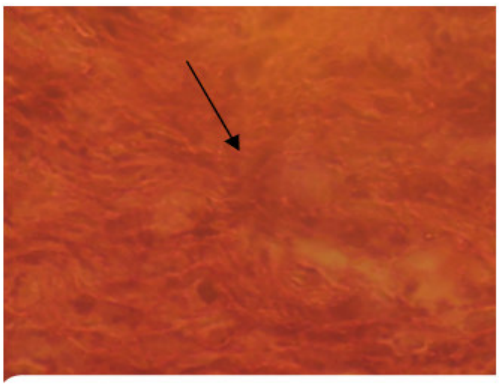
Figure(5): treated wound moderate re-epithelialization, at7th day postoperative. H&E stain.10x



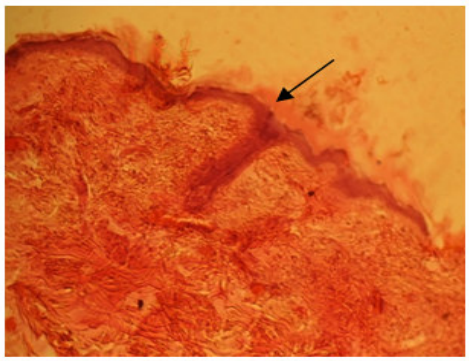
Figure(6): treated wound Granulation tissue was clear. at7th day postoperative.10x H&E.



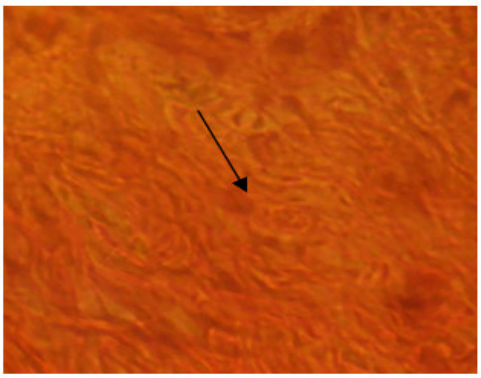
Figure(7): control wound mild re-epithelialization till 14th day , arrow. postoperative. H&E stain.10x



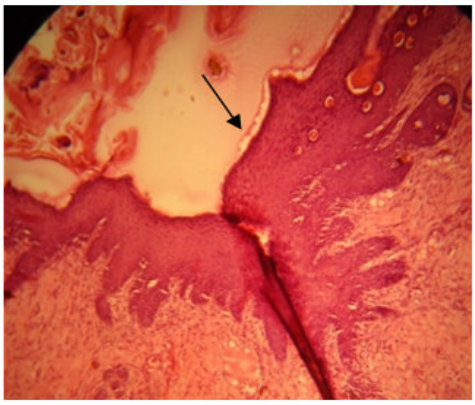
Figure(8): control wound mild Granulation tissue.at7th day postoperative. H&E. stain.10x



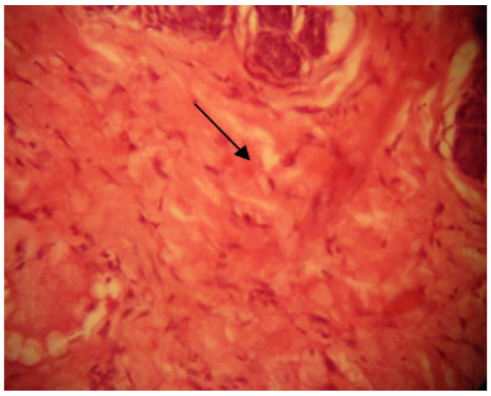
Figure(9): treated wounds showed complete re-epithelialization at 11th postoperative .10 x . H&E stain



Figure(10):): treated wounds Collagen accumulation and mature granulation tissue at 11th postoperative. arrow10x.H&E stain



Figure(12): control wounds showed immature granulation tissue and presence of inflammatory at 11th postoperative. arrow10x.H&E stain



Figure(11): control wounds showed non-complete re-epithelialization till 14th .H&E

Discussion:

In this study , topical applications of alcoholic extract showed clearance of treated wound from inflammatory elements such as hyperemia and exudates as compared with control wounds, according to chemical alcoholic extract of green apple contains important chemical constituents such as saponins which acts as an antiseptic agent (18) , and this explains the clearance of treated wounds from exudates than control wound .

Wound healing is divided into three phases: inflammatory response, proliferation stage, and scar formation (19). Inflammation is an important factor affecting the outcome of wound healing (20,21). We found that treatment with alcoholic ointment of green apple reduced inflammatory cell infiltration in the wound also induced rapid closure of the wound and increased the formation of granulation tissue at 7 days post-wounding. Furthermore, re-epithelialization was accelerated following treatment with this ointment.

Wound contraction is considered an important factor in the evaluation of healing process in large open wounds (22).In our study, the typical application of alcoholic extract on full thickness excissional wounds results in more accelerated contraction of treated wounds with 100% contraction rate at 11th day post-wounding, while in control wounds the contraction rate was 78% at the end of experiment. polysaccharides in Aloe which increase collagen activity and promote wound contraction, while (22) found in his study that polysaccharides activate macrophages and stimulate the fibroblast proliferation with subsequent proliferation of myofibroblast at the periphery at the periphery of wound, and the contraction of these cells play important role in wound contraction (22, 23and 24).

From the previous facts we discussed the result of our study, that the excesional wounds were treated with alcoholic extract of green apple accelerated the contraction rate for the treated wounds as compared with control wounds. (24).Found in their study, The wound healing process depends on the local circulation as well as formation and deposition of collagen (25).

In this work the treated wound showed more accelerated healing with complete re-epithelialization at 11th postoperative day , while control showed incomplete healing till the end of the experiment. Green apple alcoholic extract has important ingredients such as polysaccharides , triterpens , flavonoides , glycosides , which

are responsible on wound healing(18 , 25) , found in their study that all these ingredients increase collagen activity and promote healing process .

(18) showed that alkaloids ingredient that present in *Nilumbo nucifera* (Lotus) dilate blood vessels and make more oxygen available to improve collagen formation for wound healing . During the wound healing process , epithelial cells proliferate and migrate from the edges of the wound and eventually cover the wound with new epithelium (24). In this study , the treated wounds were showed decreasing in the length of wound which become chiefly at 11^{th} day postoperative day , this may regard to the presence of alkaloid in alcoholic extract that improve blood supply and improve the healing process (18,16) .

As a result the green apple is affective topically in improvement of the healing process and decrease the length of treated wounds as compared with control wounds.

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