# Study on effect of Vitamin C and E (normal and double dose) on skin wounds healing in experimental rats wistar albino

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#### Abstract

This present study was investigated the effect of certain nourishing factors such as vitamin C and E (normal and double dose) on skin wounds healing in Laboratory rats.

Total number of animals were (90) divided into (3) groups, with (30) rats in each. All animals were wounded on dorsal back within an area of  $(4\text{cm}^2)$ . Animals of fist group (GI) were treated after wounded intraperitoneally with 250mg/kg of vitamin C while control animals in this group treated with 0.01ml / gm of distilled water, where's the animals of second and third groups (GII,GIII)covered daily with normal dose of vitamin E(30mg/kg) and double dose (60mg/kg) respectively , while the wounds of control animals in these two groups covered with Vaseline only.Macroscopically changes accompanying wounds healing in both treated and control animals were daily recorded, also histological changes like average of inflammatory cells(Neutrophils and macrophages), Number of Fibroblasts, adipocytes density in addition to angiogenesis during healing was evaluated.Results clarified the effect of nourishing factors on collagen deposition , regeneration of skin layers and hair follicles appearance during periods (2,4,8,12,15,21) days post-wounding.

Wounds treated with vitamin C and normal dose of vitamin E showed early healing signs represented by sever hyperemia, redness, dryness of wounds edges and formation of scar tissue without scars and traces compared to control animals.

The study referred to an inhibition effect of double dose of vitamin E as wounds kept partially opened till the  $(15^{\text{th}})$  day with formation of scars and traces in the positions of wounds.

Data also shown an increase with significant difference (P<0.05)in rate of neutrophils and macrophages during (2<sup>nd</sup>) and (4<sup>th</sup>) days post-wounding in animals treated with vitamin C and double dose of vitamin E compared to animals treated with normal dose of vitamin E and control animals.

Proliferation of inflammatory cells gradually decreased in all wounds except that treated with double dose of vitamin E, which existence of neutrophils cells continuated in wounds.

The study elaborated as well as the effect of nourishing factors on regeneration of epidermis and dermis layers within the wounds of animals treated compared to control animals.

The histological sections shown complete regeneration of both treated wounds and control occurs on 21 days post-wounding.

Also the results of histological investigation stressed the role of these factors in the appearance of hair follicles, vitamin C and normal dose of vitamin E have positive role in the early appearance of hair vesicles during (4<sup>th</sup>) days post-wounding compare with wounds treated with double dose of vitamin E which hair follicles appeared till the (12<sup>th</sup>) day and control wounds on eight day. 1-Introduction

Healing of wounds whether caused by accidental injury or surgical intervention involves a complex series of interaction between different cells types and extra-cellular matrix (17).

The normal healing include hemostasis, inflammation, proliferation and mature phase or remodeling, although each phase is distinct but the healing processes continuous (16,23).

Successful wound healing requires adequate blood supply and nutrients to the site of damaged tissues, nutritional deficiencies can impede wound healing and several nutritional factors required for wound repair may improve healing time and wounds outcome (+1713).

Vitamin C is an essential cofactor for synthesis of collagen and procollagen which is necessary for conversion to collagen , in addition to collagen production, vitamin C enhances neutrophils function , increase angiogenesis and functions as antioxidant (14). Cutaneous wounds have been found to have lower vitamin C content than intact tissues, levels of vitamin C when compared with normal skin(22)

Vitamin E is popular among consumers for skin care and to prevent scar formation, it function as the major lipophilic antioxidant, preventing peroxidation of lipids and resulting in more stable cell membranes (11).

Another study identified the role of local application of vitamin E on wound healing with local inflammatory response (20).

Animal studies of vitamin E supplementation on surgical wounds show conflicting results, chicken treated with vitamin E had breaking strength less than half that of controls measured after days 7 and 45 from surgical repair (10).

Systemic vitamin E inhibit the inflammatory response and collagen synthesis, thereby possibly impeding the healing process so the daily dose must be not exceed 15mg/day orally (26,29).

The present study was therefore under taken to describe the effect of vitamin C and vitamin E (normal and double dose) on wounds healing .

#### 2-Material and methods:-

#### 2-1 Animals grouping

Total number of animals divided into 3 groups, with mean (30) rats in each as (18 treated rats and 12 control) on each period (2,4,8,12,15,21) days after wounds healing.

All animals were wounded on the dorsal back within an area of  $(4\text{cm}^2)$ . The animals of first group were treated with vitamin C (250 mg/kg) intra peritoneally, whereas the control animals injected with (0.01 ml/gm) of distilled water (14).

Animals in second and third groups were covered daily with normal and double dose of vitamin E (30 and 60mg/kg) while wounds of control animals in both groups were covered with Vaseline only (19).

2-2 Macroscopic examination

The macroscopically changes in both experimental and control animals concluded (wounds margin dryness, pus formation, Redness, Hyperemia and granulation tissue formation) were daily recorded.

Surface area of wounds and contraction percentage was also determined (1).

Wounds healing time in experimental and control animals was recorded as number of days needed for complete healing without any trace or scar (4).

2-3 Histological study

Skin specimen's from treated and control animals on each period (2,4,8,12,15.21) days post wounding were collected under anesthesia. The tissues processing for light microscopic examination according to (6).

All specimens were fixed with neutral formalin (10%), then preserved with phenol (4%), dehydrated with series of alcohol, clearing with chloroform, infiltrated with liquid paraffin and then embedding.

Sections of  $(5\mu m)$  thickness were prepared and some stained with haematoxylin and eosin and others with Masson trichrome stain, mounted with D.P.X and examined with light microscopy to clarified histopathological changes accompany wounds healing phases.

2-4 Microscopic examination

Sections examined and the inflammatory response illustrated as the number of inflammatory cells (Neutrophils, macrophages) infiltrated on each period (2,4,8,12,15,21)days post-wounding, number of fibrocyte was counted at same periods, and regeneration of epidermis and dermis layer . 2-5 statistical methods

The results were analyzed statistically by used (spss) programs and the means compare according to the statistical difference (p<0.05).

#### 3-Results:-

#### **3-1 Macroscopic examination**

Results of experimental animals wounds treated with vitamin C and that treated with normal dose of vitamin E show clear effect on appearance of early healing signs represented by sever hyperemia, redness, dryness of wounds edges and formation of scar tissue without scars or traces compared with wounds of control group (plate 1-A-B-C-D).

Also wounds treated with V.C and normal dose of V.E show thin scab formed covered mid region of wounds on(4<sup>th</sup>)day post-wounding in comparison with control group(plate 2 A-B-C).

Recent study illustrate granulation tissue formed in all wounds except that treated with double dose of vitamin E which show wet wounds with sever redness (plate 2-A-D).

Results of macroscopically investigation shown epithelial layer formation (epithelization) starting at (8<sup>th</sup>) day post wounding in treated wounds with vitamin C and wounds treated with normal dose of vitamin E in comparison with wounds of control group and wounds treated with double dose of vitamin E(plate3A $\rightarrow$  D).

On (8<sup>th</sup> and 12<sup>th</sup>) days post wounding the contracted of wounds more obvious in wounds treated with V.C and normal dose of V.E compared with wounds treated with double dose of V.E and wounds in control group which appear more redness, irregular margins and wounds still partial opened (plate 3-4, A  $\rightarrow$ D).

Present study indicated that wounds treated with V.C and normal dose of V.E showed complete healing, regular margins and hair formation with scar tissue at (15<sup>th</sup> and 21) days post wounding in comparison to wounds treated with double dose of V.E and control group which still partial opened (plate 5,6 A  $\rightarrow$ D).

3-2Measurment of surface area, contraction rate and healing time.

Surface area of both treated and control wounds was measured on each period (2,4,8,12,15,21) days post wounding and contraction percentage rate was counted, wounds treated with V.C showed decrease in surface area with an increased in contraction rate reached to  $2.57\pm0.29$  cm<sup>2</sup> and  $35.75\pm10.39$  alternatively compared with other treated wounds and control group from  $(4^{th})$  day post wounding(Table-1).

Effect of V.C continue and very obvious on day ( $8^{th}$ ) with significant differences compared with control , also effect of normal dose of V.E beginning on this period , the surface area reached 1.7±0.36 cm<sup>2</sup> with contraction rate 57.5±9.29(Table-1).

Data on day  $(12^{th} \text{ and } 15^{th})$  showed decrease in surface area and an increase in contraction rate reached to  $99\pm1$  and  $95.25\pm7.2$  on day  $(12^{th})$  on wounds treated with normal dose of V.E and vitamin C respectively while the contraction rate reached to %100 in most treated wounds with significance differences (p<0.05) on day (15<sup>th</sup>) post wounding compared with control (Table-1).

Wounds treated with double dose of V.E still partial opened during period ( $12^{th}$  and  $15^{th}$ ) day with surface area ( $0.3\pm0.1$ )cm<sup>2</sup> and compared with control (Table-1).

Recent study showed total healing with equally contraction rate on all treated wounds and control on day (21) post wounding (Table-1).

Healing time was recorded (13day) on wounds treated with vitamin C and normal dose of vitamin E, also it was (16days) on wounds treated with double dose of vitamin E while control wounds needed (20days) for total healing (Table-1).

# Table (1) : illustrated surface area (Cm<sup>2</sup>) , percentage rate of contraction and healing time in treated wounds comparative to control wounds

Wound days	Nutrients factors	Surface area / Cm <sup>2</sup>	Contraction	Healing time
			rat	(Days)
Second day	Control animals	$3.6\pm0.35$	$10 \pm 0.6$	-
	Vitamin C	$3 \pm 0.17$	$25 \pm 4.3$	-
	Vitamin E(normal dose)	$3.3 \pm 0.4$	$17.5 \pm 2.8$	-
	Vitamin E(double dose)	$3.5 \pm 0.55$	$12.5 \pm 3.4$	-
Fourth day	Control animals	$3.4 \pm 0.35$	$15 \pm 0.6$	-
	Vitamin C	$2.57\pm0.29$	35.75 ± 10.39*	-
	Vitamin E(normal dose)	$3 \pm 0.38$	$25 \pm 9.46$	-
	Vitamin E(double dose)	$3.47\pm0.87$	$15 \pm 2.13$	-
Eighth day	Control animals	$2.5\pm0.58$	$37.5 \pm 4.4$	-
	Vitamin C	$0.97\pm0.45$	75.75 ± 11.5*	-
	Vitamin E(normal dose)	$1.7 \pm 0.36$	$57.5 \pm 9.29$	-
	Vitamin E(double dose)	$2.9\pm0.6$	$47.5 \pm 1.2$	-
Twelve day	Control animals	$1.9 \pm 0.52$	$52.5 \pm 12.7$	-
	Vitamin C	$0.19 \pm 0.28$	$95.25 \pm 7.2*$	-
	Vitamin E(normal dose)	$0.03 \pm 0.04$	99 ± 1*	-
	Vitamin E(double dose)	$0.67 \pm 0.21$	83.25 ± 5.13*	-
Fifteen day	Control animals	$1.3 \pm 0.12$	$67.5\pm2.89$	-
	Vitamin C	-	$100 \pm 0.00*$	day13
	Vitamin E(normal dose)	-	$100 \pm 0.00*$	day 13
	Vitamin E(double dose)	$0.3 \pm 0.1$	92.5 ± 2.53*	-
Twenty one	Control animals	-	$100 \pm 0.00$	20 day
	Vitamin C	-	$100 \pm 0.00$	13 day
	Vitamin E(normal dose)	-	$100 \pm 0.00$	13 day
	Vitamin E(double dose)	-	$100 \pm 0.00$	16 day

(\* refer to significant difference p < 0.05)

#### -3Microscopical examination

3-3-1 Effect of nutrients on neutrophils, macrophages and fibroblast cells infiltration.

Present study showed an increased with significant difference (p<0.05) on number of inflammatory cells(neutrophils) specially in wounds treated with vitamin C and double dose of vitamin E in comparison with control on day (2) post wounding , while macrophage recorded an increase in all treated wounds and control with significant difference (Table-2).

Also treated wounds with vitamin C showed increase with fibroblast cells with significant difference (p<0.05) compared with control wounds (Table-2).

Results indicated that decreased in neutrophils begin in all treated wounds from  $(4^{th})$  day except wounds treated with double dose of vitamin E while the mean reach to  $60-4\pm11.9$  cells. Also macrophages number continue increased in all wounds, and the fibroblast showed the same pattern through this period except the wounds treated with double dose of vitamin E (Table-2).

Our data referred that no neutrophils cells recorded on  $(8^{th})$  day in wounds treated with vitamin C and normal dose of vitamin E. also macrophage cells decreased in number in all treated wounds while the fibroblast cells increased with significant difference (p<0.05) in wounds treated with vitamin C and normal dose of vitamin E in comparison with their number in control wounds and wound treated with double dose of vitamin E(Table-2).

The neurtrophil disappear in all treated wounds and control during  $(12^{th})$  and  $(15^{th})$  days except that wounds which treated with double dose of vitamin E while their mean number reach  $35\pm0.5$  cell (Table-2).

The results also clarified the reduction in macrophage number starting from day  $(15^{\text{th}})$  and (21) post-wounding with significant difference (p<0.5) in treated wounds compared with control wounds (Table-2).

Inilitration	mean on treated	wounds comp	barative to con	trol wounds
Wound days	Nutrients factors	Neutrophils	Macrophages	Fibroblasts
Second day	Control animals	$70 \pm 36.6$	$10 \pm 0.7$	$2 \pm 0.5$
	Vitamin C	180 ± 2.1 *	40 ± 8.5 *	7 ± 2.1 *
	Vitamin E(normal	$90.5 \pm 3.9$	25.8 ± 4.6 *	6 ± 0.7 *
	dose)			
	Vitamin E(double dose)	100 ± 22.9 *	30 ± 2.3 *	3 ± 0.9
Fourth day	Control animals	$55.4 \pm 2.9$	$20.4 \pm 0.8$	15.3 ± 2.6
	Vitamin C	$25 \pm 2.8$	50.3 ± 1.8*	67.5 ± 3.9 *
	Vitamin E(normal dose)	$40.5\pm4.2$	31.2 ± 2.2 *	30 ± 0.9 *
	Vitamin E(double dose)	60.4 ± 11.9	36.7 ± 0.8 *	$20.3 \pm 2.8$
Eighth day	Control animals	$35 \pm 0.00$	$35 \pm 0.00$	$45\pm0.00$
	Vitamin C	$0.00 \pm 0.00*$	$10.5 \pm 0.7$	153.8 ± 4.3 *
	Vitamin E(normal dose)	$0.00 \pm 0.00*$	20 ± 4.9	120 ± 8.1*
	Vitamin E(double dose)	28.8 ± 1.5	21.7 ± 0.8	$40.67\pm0.9$
Twelve day	Control animals	$0.00 \pm 0.00$	$35 \pm 0.5$	$80 \pm 24.1$
	Vitamin C	$0.00 \pm 0.00$	$9.8 \pm 1.2$	107 ± 8.9 *
	Vitamin E(normal dose)	$0.00 \pm 0.00$	12.3 ± 1.1	175 ± 26 *
	Vitamin E(double dose)	$0.00 \pm 0.00$	20 ± 1.8	97 ± 3.3
Fifteen day	Control animals	$0.00 \pm 0.00$	$15 \pm 0.5$	$103.3 \pm 4.9$
	Vitamin C	$0.00 \pm 0.00$	7 ± 2.6	$48.8 \pm 2.9$
	Vitamin E(normal dose)	$0.00 \pm 0.00$	6 ± 1.6	77.5 ± 2.6
	Vitamin E(double dose)	$0.00 \pm 0.00$	$10.8 \pm 3.3$	151.7 ± 3.9 *
Twenty one	Control animals	$0.00 \pm 0.00$	8 ± 0.5	81.7 ± 2.5
	Vitamin C	$0.00 \pm 0.00$	$2 \pm 0.9$	42.1 ± 2.6
	Vitamin E(normal dose)	$0.00 \pm 0.00$	5 ± 0.01	69.5 ± 3
	Vitamin E(double dose)	$0.00 \pm 0.00$	7 ± 9.5	$58.3\pm6.5$

# Table (2) :illustrated difference in neutrophils, macrophage and fibroblasts infiltration mean on treated wounds comparative to control wounds

#### (\* refer to significant difference p < 0.05)

#### 3-3-2 Histological analysis

Microscopically examination of sections related to treated and control wounds illustrated scab formation which covering the surface area proliferation of inflammatory cells (neutrophils, monocytes, macrophages), also granulation tissue formed (consist of fine collagen fibers and small blood vessels) under the scab (plate 7- A  $\rightarrow$  D).

Recent study indicate to an increase with adipocytes density on the surface layers of wounds treated with vitamin C and normal dose of vitamin E compared to control wounds (plate  $7A \rightarrow D$ ).

Histological examination of tissue sections clarified the appearance of epithelial layer on  $(4^{th})$  day in all wounds treated with vitamins compared to control (plate  $8-A \rightarrow D$ ), in spite of the wounds treated with double dose of vitamin E show blood vessels congestion and dense proliferation of endothelial cells (plate 8-D-dd).

Early appearance of hair follicles was observed in wounds treated with vitamin C on (4<sup>th</sup>) day, these follicles formed in epidermal layer then extend deeply on dermis layer while wounds treated with double dose of vitamin E and control wounds didn't show any appearance of hair follicles at this period (plate 8,B,D,dd).light microscopy examination showed that epidermis layers were differentiate in all treated wounds with an increase in dermal papillae depth except wound treated with double dose of vitamin E and control wounds which show flattened epidermis and the dermal ridges less depth(plate 9 A  $\rightarrow$  D).

At (21) day post wounding all treated wounds and control show normal skin layers with hair follicles and sebaceous glands, also dense collagen fibers were deposited while fibroblast,  $A \rightarrow D$  cells and blood vessels decreased (plate 12 A  $\rightarrow D$ ).

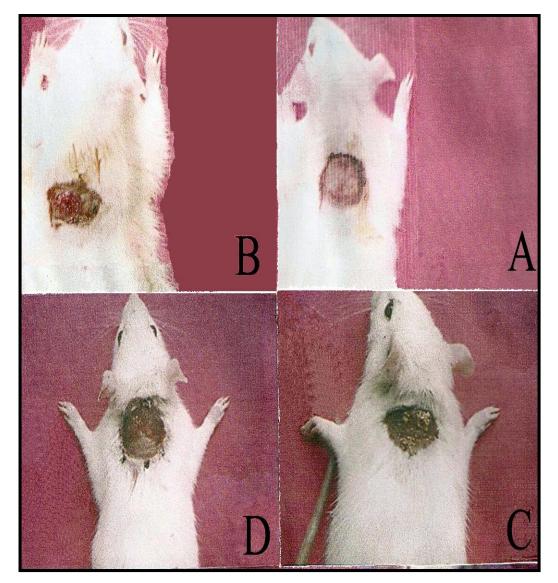


Plate (1) :Showed morphological differences in treated animals wounds ( B- vitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animals wounds A after (2) days post- wounding

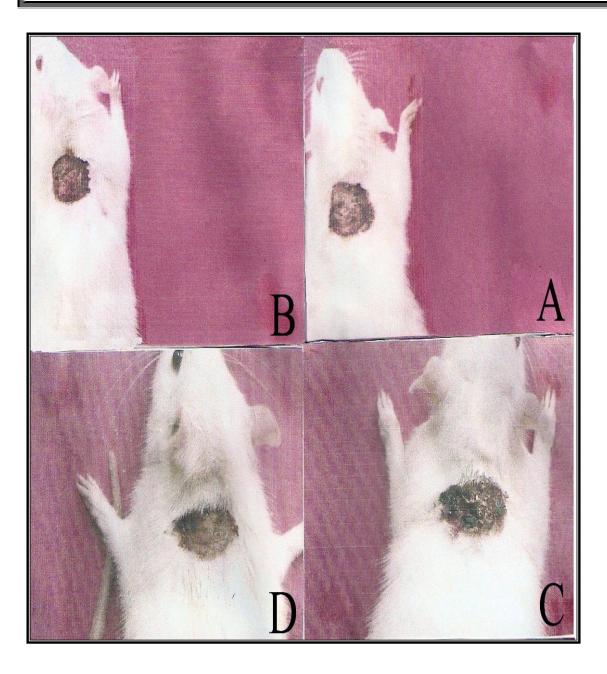


Plate (2) :Showed morphological differences in treated animals wounds (B-vitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animals wounds A after (4) days post-wounding

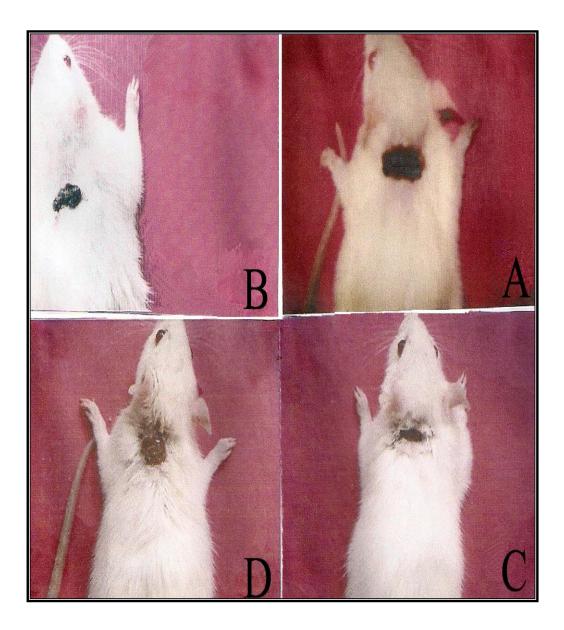


Plate (3) :Showed morphological differences in treated animals wounds ( B- vitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animals wounds A after (8) days post- wounding

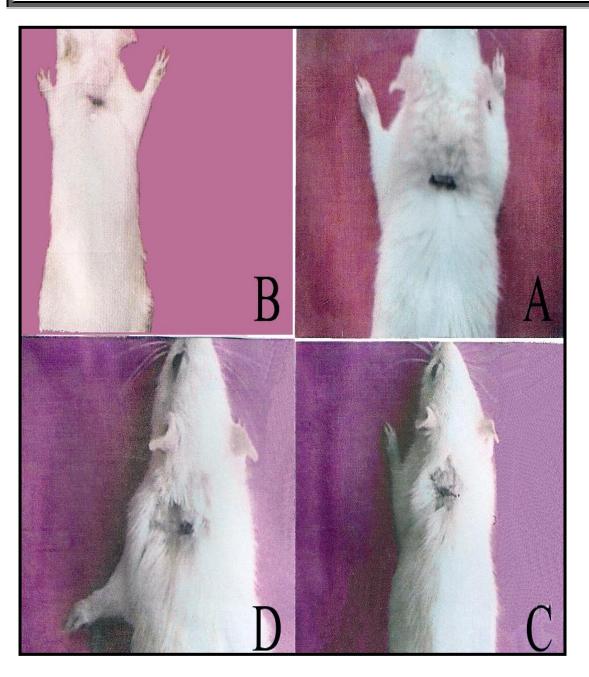


Plate (4) :Showed morphological differences in treated animals wounds (Bvitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animals wounds A after (12) days post- wounding

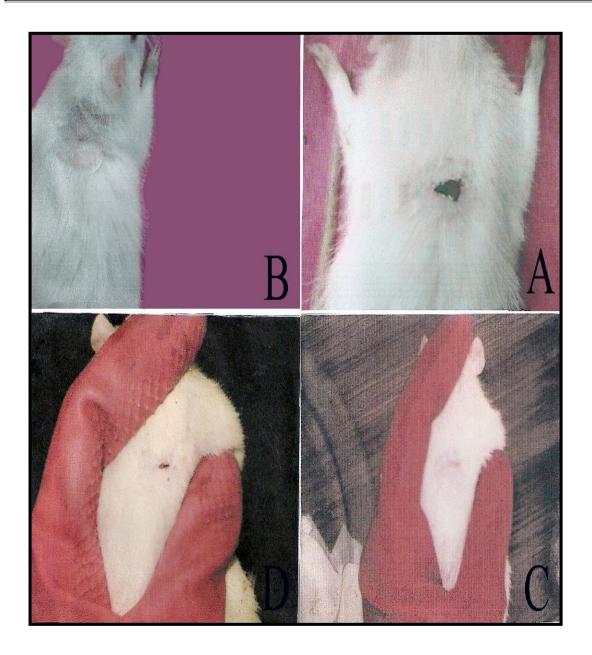


Plate (5) :Showed morphological difference in treated animals wounds ( B- vitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animals wounds A after (15) days post- wounding

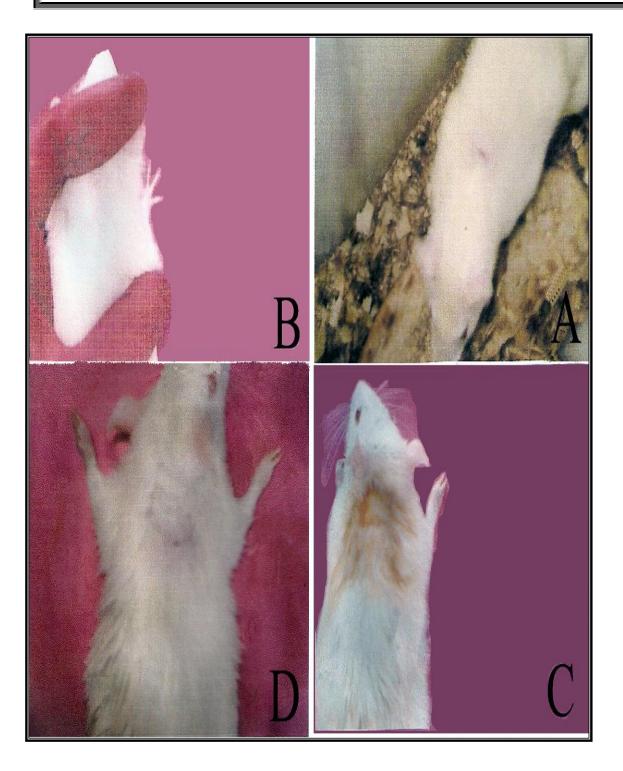
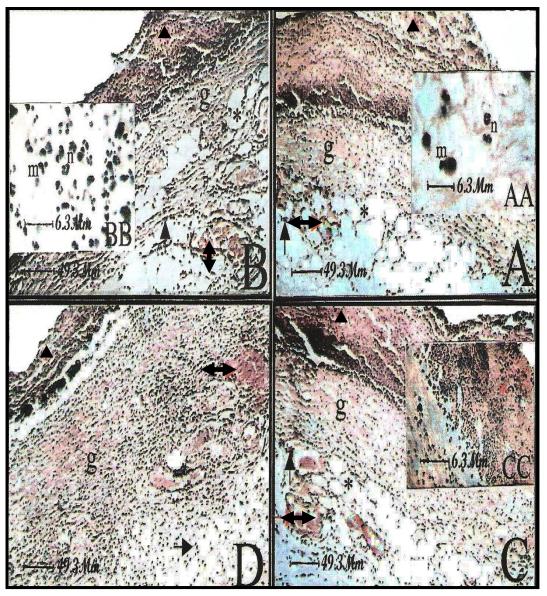
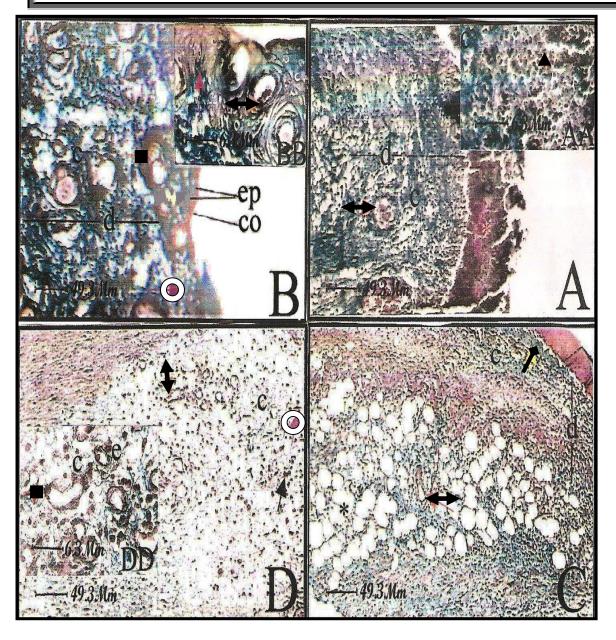


Plate (6) :Showed morphological differences in treated animals wounds (B- vitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animals wounds A after (21) days post- wounding.



Plate(7): Sections illustrate histological changes in treated animals wounds (B- vitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animal after (8) days post wounding 10x (A→D) 100 X (AA,BB,CC). H & E stain cortex , \*adipose tissue , g- granulation tissue, n- neutrophil cells, m- macrophage blood vessels , → inflammatory cells, .



#### Plate (8): Sections illustrate histological changes in treated animals wounds ( B-

**vitamins C, C-** normal dose of vitamins E, D- double dose of vitamin E) comparative with control animal after (4) days post wounding  $10x (A \rightarrow D) 100 X (AA \rightarrow DD)$  cortex, \*adipose tissue,, Co collagen fiber, ep. Epidermal layer, C- corneum layer, d- dermal layer, e- endothelial layer, m- macrophage blood vessels, hair follields epidermis appearance  $\rightarrow$  inflammatory cells

fibroblast. O Masson trichrome stain.

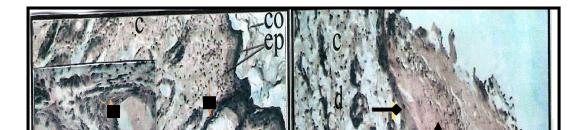


Plate (9): Sections illustrate histological changes in treated animals wounds (B-vitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animal after (8) days post wounding  $10x (A \rightarrow D) 100 X (AA,BB,CC)$ . H & E stain  $\blacktriangle$  cortex , \*adipose tissue , , C- collagen fiber , ep. Epidermal layer , Co corneum layer , d- dermal layer , e- endothelial layer , m- macrophage blood vessels ,  $\blacksquare$  hair follicles – dermal papillae  $\blacksquare$  pidermis appearance  $\rightarrow$  inflammatory celkes  $\bigcirc$  fibroblast, k-keratinocytes ,  $\blacksquare$  ebaceous glands .

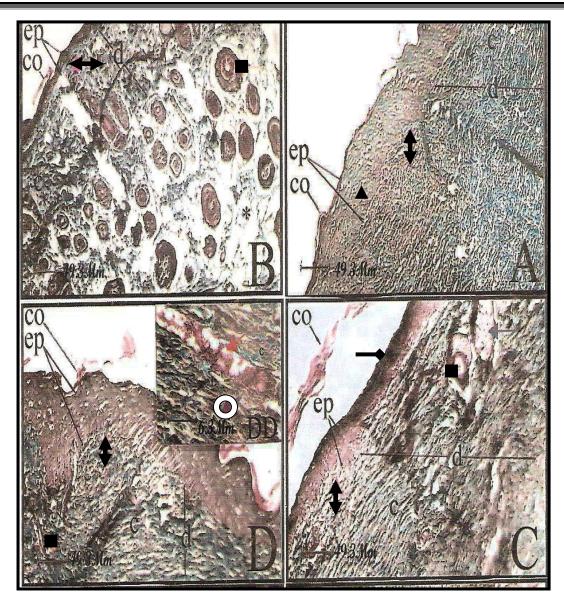
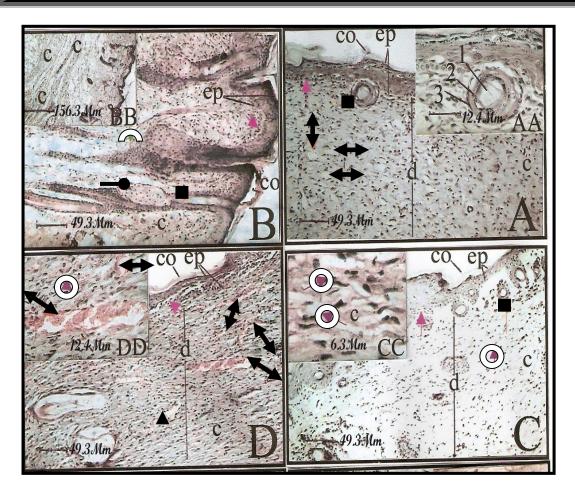


Plate (10): Sections illustrate histological changes in treated animals wounds (B- vitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animal after (12) days post wounding  $10x (A \rightarrow D) 100 X (AA,BB,CC)$ . cortex, \*adipose tissue,, C- collagen fiber, ep. Epidermal layer, Co corneum layer, d- dermal layer, eendothelial layer, m- macrophage

 $\leftarrow$  blood vessels, mair follicles – dermal papillae  $\leftarrow$  pickermis appearance  $\rightarrow$  inflammatory cells  $\triangle$  fibroblast, k-keratinocytes- Masson trichrome stain.



#### Plate (11): Sections illustrate histological changes in treated

animals wounds (B- vitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animal after (15) days post wounding  $10x (A \rightarrow D) 100 X (AA,BB,CC)$ .H & E stain

▲ cortex , \*adipose tissue , , C- collagen fiber , ep. Epidermal layer , C- corneum layer , d- dermal layer , e- endothelial layer , m- macrophage blood vessels ,

 $\blacksquare hair follicles - dermal papillae epidermis appearance <math>\rightarrow i$  in the matory cells fibroblast, k-keratinocytes- sebaceous glands

Mass (O)richrome.

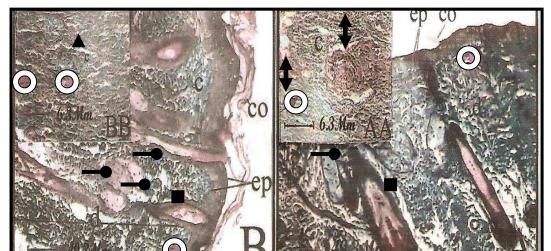


Plate (12): Sections illustrate histological changes in treated animals wounds (B- vitamins C, C- normal dose of vitamins E, D- double dose of vitamin E) comparative with control animal after (21) days post wounding 10x (A→D) 100 X (AA,BB,CC). cortex , \*adipose tissue , , C- collagen fiber , ep. Epidermal layer , Co -corneum layer , d- dermal layer , bod vessels , hair follicles – domal papillae epidermis appearance fibroblast, k-keratince es- sebaceous glands . Masson trichrome stain.

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#### 4- Discussion

Wounds healing process needed physiological environment include repair and damage tissue regeneration, so the nutrition status play an important role through healing phases (3,30).

This study focuses primarily on the effect of nutrients factors on full thickness wounds healing, the treated wounds show signs of healing from early stages concluded sever hyperemia, dryness of margins and this may be related to decrease with tissues fluids transduction which it is sign to an inflammation.

Many studies confirmed that signs of inflammation appear as redness , hyperemia and fluids transduction (28,16,7).

In the present study the scab formation with irregular contraction over surface layer in wounds treated with vitamin C and normal dose of vitamin E maybe caused by myofibroblast cells which increased in periphery regions of wounds, and this agreed with other observations confirmed that fibroblast differentiate to myofibroblast play an important role in wounds contraction irregularly (7).

The study also indicated that wounds treated with vitamin C show sever redness at early days post wounding , this may be regarded to formation of new blood vessels (angiogenesis) to supply oxygen and nutrients to damage area , this result agreed with studies identified an important role of vascularization to damage area for regeneration and healing (9,24,15,25).

Scab formation more obvious in the wounds treated with vitamin E compared with other treated wounds and this may be caused by cell mitotic activity and infiltration of an inflammatory cells, also these wounds more moisture than other wounds which show dryness, these results similar to other studies which proved an importance of vitamin E as an essential factor in skin care and prevent scar formation (11, 2).

The study referred to an inhibition effect of double dose of vitamin E on healing wounds as the wounds kept partially open till (15<sup>th</sup>) day post wounding with presence of scars or traces in the position of wounds and this may be related to blood vessels congestion, thin scab covered surface area and less deposition of collagen fibers so the wounds region became fragile and bleeding easily. Other studies showed that double dose of vitamin E causes damage to cross linkage of collagen fibers and blood vessels which formed and then the healing area appear congested (26, 32).

Our data showed variation in cellular infiltration at all healing stages depend on healing phase and first cells found at early inflammation stage, then macrophages which continue at proliferation phase and at last the fibroblast which being the dominance cells at maturation and remodeling stage, so this may be suggested to tissue damage, debris of dead tissue, inflammatory factors and formation of clot at early stage, all these may attracked an inflammatory cells to wound area these results in similarity with many studies reffered to importance role of leukocytes to invade wounds region and phagocyte the foreign materials (28, 14).

Also vitamin C and E support the immune system and increase an immune cells infiltration (26,8). Recent study showed significant increase from  $(2^{nd})$  day in angiogenesis, collagen fibers deposition and contraction rate in wounds treated with vitamin C and normal dose of vitamin E and this may be regarded to the role of vitamin C as antioxidant factor and its role in collagen fibers synthesis, this agreed with the role of vitamin E and C in cell membranes stability and tertiary helical structure of collagen fibers (21,12).

Results showed that differentiation of skin layers start from (4<sup>th</sup>) day specially on wounds treated with vitamin C and an increase with dermal papillae deep which considered because of mitotic activity of keratinocytes and their migration upstart, this result agreed with studies clarified that

keratinocytes migration result either by chemical material or dissolve of desmosomes and hemidesmosomes (31,27).

Keratinocytes play an essential role in healing and they start migration from wounds margins , the basal keratinocyte (5,24)

In this study early appearance of hair follicles in wounds treated with vitamin C and normal dose of vitamin E, this result in agreement with other studies identified the role of vitamins in skin care and hair growth by activation either the blood cycle in scalp or immune function and protein (keratin) synthesis specially hair growth needed nutrients , blood vessels oxygen and vitamins (18,32).

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دراسة تأثير فيتامين C وفيتامين E (الجرعة الاعتيادية والمضاعفة) على التئام الجروح في الحراية

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الخلاصية:-

تم خلال الدراسة الحالية دراسة تأثير بعض العوامل المغذية مثل فيتامينC وفيتامينE(بجر عتين الاعتيادية والمضاعفة) على مراحل عملية التئام الجروح الجلدية في الجرذان المختبرية .

قسم العدد الكلي للحيوانات المستعملة(90)جرذ إلى (3) مجاميع وبمعدل (30)جرذ لكل مجموعة أحدثت الجروح في الجهة الظهرية بمساحة سطحية مقدار ها(4cm<sup>2</sup>)، عوملت حيوانات المجموعة الأولى بعد إحداث الجروح فيها مع فيتامين C تحت البريتون بجر عة (250mg/kg) بينما عوملت حيوانات السيطرة بجرعة (0.01ml/gm) من الماء المقطر في حين غطيت حيوانات المجموعتين الثانية والثالثة خارجيا وبصورة يومية بجرعة اعتيادية ومضاعفة من فيتامين E بجرعة (30mg/kg) و(60mg/kg)على التوالي بينما جروح حيوانات السيطرة في كلا المجموعتين فقد تم تغطيتها بالفازلين فقط.

لوُحَظْتُ التغيرات العيانيةُ المرافقة لعمَّلية التئام الجروح في الحيوانات المعاملة وحيوانات السـيطرة يوميا ، كذلك درسـت التغيرات النسيجية المرافقة لعملية الالتئام مثل معدل ارتشاح الخلايا الالتهابية ( العدلة والبلعمية)خلال الطور الالتهابي ، وإعداد الخلايا المولدة للألياف وكثافة الخلايا الدهنية فضلا عن تكوين الأوعية الدموية الجديدة خلال مراحل الالتئام .

أوضحت النتائج أيضا دور العوامل المغذية في ترسيب الكولاجين وإعادة تجديد طبقات الجلد وظهور حويصلات الشعر خلال المدد الزمنية (21,15,12,8,4,2)يوم بعد إحداث الجروح .

أظهرت الجروح المعاملة بكل من فيتامين Cوالجرعة الاعتيادية من فيتامين Eتأثيرا واضحا في ظهور علامات الشفاء المبكر متمثلة بالتبيغ الشديد والاحمرار المفرط وجفاف حافات الجروح وتكوين النسيج البديل دون ترك آثار وندب مقارنة مع حيوانات السيطرة .

أشارت الدراسة أيضا إلى التأثير المثبط للجرعة المضاعفة من فيتامين E إذ بقيت الجروح مفتوحة جزئيا حتى اليوم الخامس عشر بعد إحداث الجروح مع وجود ندب أو آثار في مواقع الجروح.

جاءت نتائج الفحص ألمجهري لتوضح زيادة وبفارق عالى المعنوية (p<0.01)في معدل ارتشاح الخلايا العدلة والبلعمية خلال اليومين الثاني والرابع بعد إحداث الجروح في الحيوانات المعاملة بكل من فيتامين c والجرعة المضاعفة من فيتامين E مقارنة مع جروح حيوانات السيطرة والجروح المعاملة بالجرعة الاعتيادية من فيتامين E ثم بدأ فيما بعد نقصان تدريجي في معدل ارتشاح الخلايا الالتهابية في جميع جروح الحيوانات المعاملة ماعدا تلك المعاملة بالجرعة المضاعفة من فيتامين E حيث استمر

كما أكدت الدراسة تُأثير العوامل المغذية على إعادة تجديد طبقات البشرة والأدمة في جروح الحيوانات المعاملة مقارنة مع جروح السيطرة وأوضحت نتائج الفحص المجهري إعادة تجديد تام في جميع طبقات الجلد خلال اليوم الحادي والعشرون بعد إحداث الجروح .

أوضـحت نتائج الفحص النسـيجي أيضـا دور هذه العوامل في الظهور المبكر لجريبات الشـعر وكان لفيتامين C والجرعة الاعتيادية من فيتامين E التأثير الايجابي في الحث المبكر على ظهور جريبات الشـعر في مناطق الجروح خلال اليوم الرابع مقارنة مع الجروح المعاملة بالجرعة المضاعفة من فيتامين E حيث لم تظهر هذه الجريبات حتى اليوم الثاني عشر وفي جروح حيوانات السيطرة التي بدأ ظهور الجريبات فيها في اليوم الثامن بعد إحداث الجروح .