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The effect of controlled electrical stimulation (ES) on some of biochemical characteristics and wounds healing in induced diabetic rabbits.

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Abstract. The current experiment was aimed to explore the effect of electrical stimulation (ES) on glucose concentrations, cholesterol levels and triglyceride levels in blood and its effect on wound healing in intact and induced diabetes rabbits. 40th male rabbit divided to five treatment, T1 intact animal as control (positive control), T2 intact animal treated with (ES), T3 diabetic rabbits treated with (ES), T4 diabetic rabbit treated with insulin and T5 diabetic rabbits with no treatment as a negative control. Three sub groups were designed as well, T2*, T3*, T4* each one of them is the same main group but with artificial wound on some of its animals. the results were shown a significant decreasing $p < 0.05$ in blood glucose concentration in T3 in comparison with T4 and T5. The cholesterol levels increased significantly $p < 0.05$ in T4 in compared with T3. The concentrations of triglycerides recorded significant $p < 0.05$ decreasing T3, T4 and T5 in compared with T1 and T2. In another hand the ES had a very obvious effect on wound healing in both T2* (intact animals) and T3* (diabetes animals) in comparison with T4* (insulin treated diabetes animals).

1. Introduction.

Electrical stimulation is conducting controlled electrical current applied on living tissues in different ways occurred by direct touching of electrodes on the tissues surface, using special probes to can get down to the depth of tissues for more effecting (get rid of electrical resistance), or by using electromagnetic waves currying on the demanded frequencies to permeate it as deep as to achieve the exact goal [1].

Tissues in living situations are already under control of electric pulse because of nervous system which connected with brain and any change in quality or quantity of pulses will affect directly the whole organ response [2, 3].

In general the electric current effects on all tissues and organs specially muscles, nerves, bones and blood which exhibit a direct response to electrical pulses causing a dramatical changes in their properties at short period, this changes were an indicator to a revolutionary progress in sciences especially in medical and agricultural fields [4].

It became a scientific reality that when exposed a damaged tissue (like wounds) to an electrical stimulation would accelerate wound healing by direct effect or by increasing the bio-performance of the whole body [5]. In the sixth decade of the last century researchers like Carley and Wainapel [6], Assimacopoulos [7] and Wolcott et al [8] found out the



positive effects of electricity on general health and the relation between electrical stimulation and treatment of many diseases, they found that butting a charged gold ply near skin of measles which due to blisters disappear.

About the effects of electrical stimulation on blood many researches had been carried on. Lee et al [9] referred to the decreasing in blood glucose concentration when body expose to electrical current in diabetic patients in addition of significant changes in other traits like triglycerides levels cholesterol profile and even hemoglobin concentration. More than one researches carried out to find the real mechanism behind this changes, one of them was the research of Maritim et al [10] who assumed that the positive effect of electrical current on the free radical that exist in blood stream and tissues which is the main cause of many of damage consequences which appear synchronizing with disease [11 , 12].

Because little or no research investigated electrical stimulation action on wound healing and blood glucose in diabetic animals so the current research attempt to investigate the changes would be occurred in some of biochemical parameters and wound healing a in diabetic and intact rabbits when 13 volt AC , 313 sine wave frequency electrical stimulation was exposed on skin by direct touching.

2. Materials and Methods

This study was concluded in the department of animal resourced/ university of karbala.

2.1. Animals:

Forty rabbit male at the age of 18- 24 months was used in experiment, their weights were about 1600-2000g, the rabbits were housed in 4x4 room dimensions at 22-25Co with lighting period reach two 14 hour a day. The feed and water were provided ad libitum. All animals were leaved for two week as an adaptation period before starting sampling.

Induce Diabetes Mellitus:

Inducing diabetes mellitus was as follows:

1. Obscured feeding for 14 hours.
2. The animals were injected with of Alloxane Monohydrate(150 mg/kg) intraperitoneal (IP).
3. Water with sugar 20% was offered the injected animals ad libitum for five days after treatment.
4. After five days of injection blood samples were tested to insure that diabetes was induced. The insurance was accepted when serum glucose is more than 200mg/ml [13].

2.2. Treatments:

The animals were divided to five treatments :

T1: intact animals for blood sampling only, positive control, (n=5).

T2: intact animals to be treated with ES, (n=10) five of them had an artificial wounding at the middle of their backs (cutting all the skin in circle shape with a area 1-1.5 cm². (T2*).

T3: diabetic animals to be treated with ES, (n=10) five of them had an artificial wounding at the middle of their backs (cutting all the skin in circle shape with a area 1-1.5 cm². (T3*).

T4: diabetic animals to be treated with insulin, (n=10), five of them had an artificial wounding at the middle of their backs (cutting all the skin circle shape with a area 1-1.5 cm². (T4*).

T5: diabetic animal with no treatment as a negative control, five animals.

2.3. Electrical stimulation (ES):

Electrical stimulation device was designed and manufactured locally. This device has the ability to produce AC current with wide range of electrical waves in multi accordance's.

1. providing a range of voltage was 1mv to 30 volt.
2. providing a range of current was 1mA to 30 Amp.
3. providing a range of frequencies 0.001 HZ to 2megaHZ. (covered all kinds of frequencies except photo waves and microwaves).
4. providing three kinds of wave, sine, saw and digital.

The animals were exposed to ES each two days for one month with dose of 13V, 313HZ sine wave with adequate current, half hour per session. The positive and negative control were leaved without (ES), the insulin treatment animals were injected with 1.5 mg/ml, 1ml for each animal each two days subcutaneously. The blood samples were three ml drew from each animals after half hour from last treatment from heart directly.

The wounds were exposed at the beginning of the experiment as what was mentioned before. Each wound was measured before exposing to ES to see the differences would be occurred.



Picture 1. *Conducting wound artificially*

2.4. Blood samples:

Each blood sample put in normal tube (no anti-coagulation) and kept in 4Co for an hour before centrifuged at 4000 rpm for quarter hour to get serum. Serum had been collected and kept frozen in plastic tube at -20Co until be used.

Estimation Concentrations of Glucose, Cholesterol and triglycerides:

Glucose concentrations, Cholesterol and triglycerides were calculated in serum by using Spectrophotometer as what was described by Tietz, [14], with equipment's provided by Linear company.

2.5. Statistical analysis

The current data of all traits were be analyzed by complete random design (C.R.D). As well as, Dinkin's test used to find a significant variations between groups at probability 0.05[15].

3..Results and Discussion.

The current results showed incidence diabetes in T3,T4 and T5 group that treated by alloxan because the level of blood glucose was more than 200 mg/ml as well as by appearance of diabetic clinical signs as thirst, frequent urination, idle and skin dryness as compared with other non-treated groups. The results of electrical stimulation effects on some biochemical traits in rabbit were illustrated in table 1. the statistical analysis showed significant decline ($P<0.05$) in mean level of glucose in T3 that treated by electrical stimulation compared with T5 , as well as, T4 showed significant decline ($P<0.05$) in the mean value of serum glucose when compared with other group. These changes might be attributed to body response to electrical stimulation, the current results agreement with several finding of researcher who

Table 1. Impact of electrical stimulation on biochemical traits in induced diabetic rabbits.

Groups	Parameters (means± SE)		
	Glucose (mmol/L)	Cholesterol (mmol/L)	Triglycerides (mmol/L)
T1	1.56 ± 0.06 D	0.48± 0.01 AB	0.66 ± 0.01 A
T2	0.61± 0.10 E	0.50±0.005 AB	0.60 ± 0.05 A
T3	3.05 ± 0.02 B	0.54 ± 0.02 A	0.32 ± 0.04 C
T4	2.70 ± 0.11C	0.40 ± 0.05 B	0.50 ± 0.05 B
T5	3.62± 0.04 A	0.44 ±0.05 AB	0.49 ± 0.04 B

*The different letters in the one column are significant changes at <0.05.

Documented that the electrical stimulation caused significant lowering in glucose level during a period not exceeding 108 min after session in laboratory animals and human, as well as, these value might be returned to normal value when repeated the electrical stimulation courses [16]. The current data same as that finding by Price et al. [17] who concluded that the electrical impulse caused elevation the glycogenesis process in muscles and reduction of blood glucose rate to half due to increased insulin receptor's response leading to increase cell activity and energy production [18], furthermore, the percentage of glycogen quantity in muscles elevated twice time after 45 min of exposure to electrical stimulation [19], this aspect was very important in agricultural field because the muscle possessed medium to high percentage of glycogen was more perfect quality, stress resistance than other that had low glycogen percentage [20].

Beside, general health of animals that treated by electrical stimulation was be improved due to reduction in free radicle production by supplying electron from external source 'electrical impulse', this electron required for blocking free radicle

actions, this action reflected on general health, performance and production[9, 21].

On other hand, the present results registered significant elevation ($P<0.05$) in cholesterol concentration in T3 group as compared to T4 groups, intergroup comparison there were no significant between T3 and T2, T1 and T5, this regarded a positive aspect when this elevation reflected on increasing a high density lipoproteins 'HDL' percentages and decline low density lipoprotein 'LDH' in blood at rate 23 and 20% ,respectively, leading to minor increment in cholesterol concentration [22] the current results agreement with that finding by Sharma et al., [21] who registered minor elevation in cholesterol concentration might be attributed to short duration exposure to electrical stimulation and / or low density of electric current.

Also, the statistical analysis showed a significant decline ($P<0.05$) in triglyceride concentration 'TG' in T3 in compared to T4, T5, T2 and T1, the present changes might be attributed to high level of glucose in blood causing to delay repose of TG for that changes. The current data dis-agreement with Sharma et al[21] who concluded that an electrical stimulation could cause non-significant reduction in TG concentration in human.about the effect of ES on wounds healing the results have been briefed in table 2.

Table 2.The effect of ES on wound healing in intact and diabetes rabbits.

Treat.	The changes in wound area cm ² with proceeding sessions						
	Session number (two days between each two session)						
	1	2	3	4	5	6	7
T2*	1.5 ±0.01 A	1.0 ±0.1 B	0.8 ±0.02 B	0.4±0.01 C	0.1± 0.03 D	00	00
T3*	1.5±0.02 A	1.2±0.01 A	1.0 ±0.03 B	0.8± 0.02 B	0.5±0.04 C	0.25±0.01 D	00
T4*	1.5± 0.03 A	1.5±0.02 A	1.4 ±0.02 A	1.4±0.002 A	1.3±0.02 A	1.0 ±0.1 B	1.0± 0.01 B

*The different letters in the one column are significant changes at <0.05.

It could be seen obviously that the time of wounds healing in ESed animals had a significant shortening. The area of wound in T2* (no diabetes animal treated with ES) decreased about 0.5-0.3cm² per each session (fast style healing), while T3* (diabetes animals treated with ES) decreased about 0.3-0.2 cm² in each session (slowly style healing). The T4* animals (diabetes animals treatment with insulin) showed no differences in the first five session, the differences began to appear (in a very slowly style) in the session number six with no significant again with session number seven. The current results improved the effect of ES on wound healing, the complete healing was achieved after five session only (ten day) in intact animals. The diabetes animals healed up from wound just after one days from intact ones. While diabetes animals treated with insulin (no ES) never healed up even after completed seven session.



Picture 2. measuring wound area.

The current results agreement with that obtained by [23] who showed a semi- completed healing of ulcer in patients exposed to ES for 20 min during 12 weeks.

Adunsky [24] showed a significant differences between ES and non ES patients in wound area. The current results agreement with [25,26] whom found a reduction in wound area about 31% for ES in comparison with 4% in non ES cases. All that changes might be attributed to the plasma membrane of each cell in body worked as electrical unite and had a DC exchange-like activity on ions exchange electrochemical physiology, so, injuring the epithelial layer disrupts the natural electrical current occurring in the body, therefore creating an electrical field [26]. This electrical field help the epithelial cells by guiding them to the wound area. Applying external electrical current on the injury place enhancing the epithelial cells movement. In addition to epithelial cells the fibroblasts migrates faster to the injury location when ES apply, but the direction of them is quite related with the type of electrodes [27].

In vitro several studies investigated the effect of ES on wounds healing had been shown that the ES with DC current imitates the natural electrical current that occurred in injury skin, the ES decreasing the time of replication of the epithelial and fibroblasts to the half [28]. In another study there it would be found that the ES was a direct factor to stimulate mitogen-activated protein (MAP) kinase activation which have central role in in initiating cells responses and lead to proliferation, also ES is a factor that increases the migration of neutrophils and macrophages to the injury location [29].



Picture 3: image show a semi-healed wound.

4. Conclusions:

It can be concluded that the concentration of glucose will be decline when electrical current would be exposed to body. This decreasing reflexes positively to general animals health, because of transferring all this glucose and interring muscles as glycogen which enhancing health profile and meat producing quality in future. The cholesterol would be in the normal concentration with a light enhancing in it. Triglycerides change as well and stay in healthy levels.

Much more studies have to be proceeded to study the relationship between electrical stimulation and body tissue especially that related with animal health and fattening by using a different frequencies and electric profiles. Study maybe connected in medicine field as well because of its importance in human health also.

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