



# Effect of Topical Treatment of *Rosmarinus Officinalis* Essential Oil on Wound Healing in Streptozotocin Induced Diabetic Rats

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## ABSTRACT

A common complication of diabetes is impaired wound healing. Systemic *Rosmarinus officinalis* oil improves healing in diabetics, which is dose dependent and may have side effects. There is very less information regarding topical *Rosmarinus officinalis* oil use. The objective of this study was to evaluate the effects of topical *Rosmarinus officinalis* oil on wound healing. Diabetes was induced in Wistar rats by using streptozotocin. The control group comprised age-matched animals not submitted to streptozotocin injection. Diabetic state was confirmed by glycosuria and hyperglycemia. Under tribromoethanol anaesthesia, four skin wounds (4mm diameter) were performed on shaved dorsal area (2 each side of median line). Topical *Rosmarinus officinalis* oil was applied daily only on both wounds on right side of median line. Animals were sacrificed on day 3 and 7 after surgery and tissue samples were prepared and observed under light microscopy. Histological, histometric and stereological methods were used for analysis. Topical *Rosmarinus officinalis* oil accelerated wound closure in diabetic and non-diabetic rats and the results were found to be more active than antibiotic treated controls. Topical *Rosmarinus officinalis* oil could be helpful in diabetics in order to improve the wound healing process avoiding possible adverse effects from systemic medication. All the values were statistically significant.

## INTRODUCTION

Diabetes Mellitus is a syndrome more than a disease and affects about 150 million people worldwide (Prakash et al. 1974). Studies have shown delayed wound healing in diabetics due to cell proliferation deficiency, infection, decreased cell surviving and reduced wound contraction (Nagy et al. 1961). Streptozotocin (intravascular) and injection of streptozotocin monohydrate produces insulin decreasing and hyperglycemia in a few days (Darby et al. 1997 & Ramamurthy et al. 1973). It is a natural cytotoxic chemical which is particularly toxic to pancreas and reduces insulin formation. Streptozotocin injection leads to the degeneration of the langerhans islets beta cells. Glucose level was increased in the blood of rat exposed to evaluate sublethal concentration of potassium dichromate (Job Gopinath 2001). The study of blood parameters has gained momentum in recent years in view of its importance to diagnose various abnormalities (Hawkins et al. 1954, Vahlquest 1950).

The aerial parts of the herb have a long tradition of use as an antispasmodic in renal colic and dysmenorrhoea, anti-rheumatic and anti-aging (Al-Sereiti et al. 1999, Abu-Rabia 2005, Al-Qura'n 2009) in relieving respiratory and digestive disorders, hypertension, kidney stones, sugar in blood (Everest & Ozturk 2005, Lev 2006), in stimulating circulation and nervous system and in treating skin diseases such

as hair loss, infections and healing of wounds (Abu-Rmaileh & Afifi 2000, Pieroni et al. 2004, Heinrich et al. 2006). Healing of wound is considered necessary for the removal of damaged tissues and/or invaded pathogens from the body as well as to restore the continuity and architecture of a cutaneous or visceral defect (Matsuda et al. 1998, Lerman et al. 2003). The present study aims to the initial phases of wound healing in the skin of normal and diabetic animals and to compare wound healing areas in diabetics and their controls after topical *Rosmarinus officinalis* oil use.

## MATERIALS AND METHODS

Wistar rats (*Rattus norvegicus*) were procured and maintained in the laboratory conditions. All the rats were placed in plastic boxes (40 × 32 × 17cm) under controlled light conditions (12 hours of light; 12 hours of darkness) and temperature (21-25°C). All the rats were separated into different groups. After 36 hours of food deprivation the diabetic group was injected with streptozotocin (40 mg/kg of body weight). 30 minutes after injection, food and water were offered ad libitum. The injected animals were tested 4 days after injection to verify the presence of gulcosuria. All the animals with gulcosuria were considered as diabetics. Subcutaneous injection of insulin (0.1U long duration insulin Humulin-Lilly), 100g body weight was followed every two days. The Group III animals were kept under insulin therapy

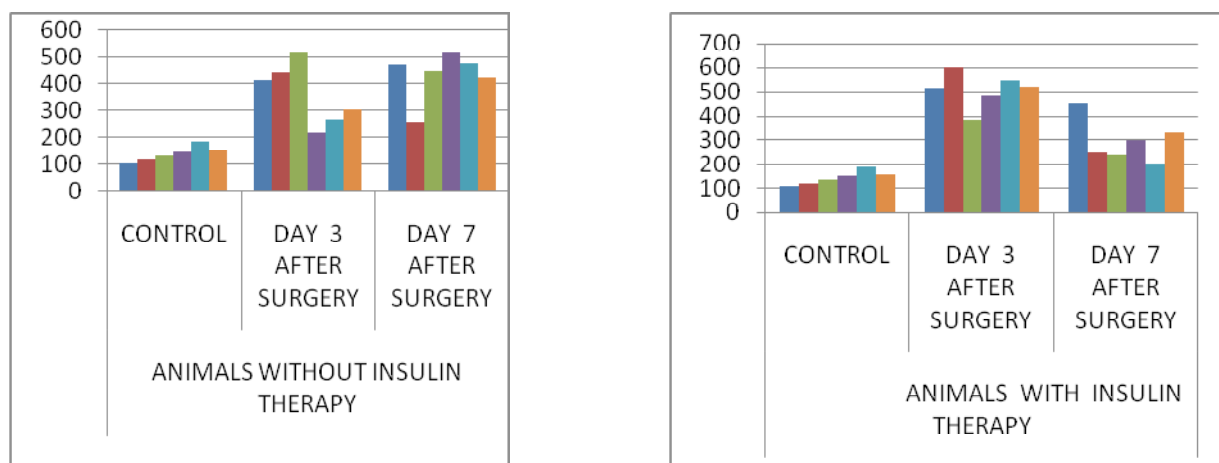


Fig. 1: Comparison of glycemia in animals without insulin and with insulin therapy.

Table 1: Blood glucose evaluation (mg/dL) in control and experimental animals.

Control	Day 3 After Surgery	Day 7 After Surgery
<b>Animals without insulin therapy</b>		
104.3±3.12	415.4±5.50	470±7.02
117.3±7.54	445.8±8.54	259.6±8.00
132.6±7.50	518.5±6.65	449.3±8.50
149.6±9.01	218.3±6.64	518.1±6.64
185.0±11.1	268±6.52	475.3±6.56
154.0±7.00	305.4±6.50	425.5±6.51
<b>Animals with insulin therapy</b>		
104.3±3.12	512.4±8.00	450±6.50
117.3±7.54	601.1±8.18	247.0±9.53
132.6±7.50	384.6±10.01	238.2±7.50
149.6±9.01	484.5±9.54	296.0±8.56
185.0±11.1	545.6±8.02	200.6±8.02
154.0±7.00	519.6±8.00	330.4±7.50

Values are mean ± SD of 100 individual observations. Values are significant at  $P < 0.001$ .

for 15 days before wound surgery. Blood glucose and biochemical factors were estimated by the methods given in Sigma Diagnostic kits (Sigma Analysis Catalogue) and histopathological study was carried out by Gutr (1959).

**Statistical analysis:** All the data were analysed and expressed as mean of six individual observations. Standard Error and Students 't' test, were performed as per the methods of Pillai & Sinha (1968).

## RESULTS AND DISCUSSION

Tables 1-3 and Figs. 1-4 indicate the results obtained in the present investigation. Hyperglycemia were observed in all diabetic animals. Mean blood glucose levels (368mg/dL on day 3 and 425 mg/dL on day 7 after surgery) were higher

compared to non-diabetic animals (156mg/dL on day 3 and 128mg/dL on day 7 after surgery).

**Histopathological studies:** The epithelial neof ormation was evaluated taking the parameters: a) diabetics, without *Rosmarinus officinalis* oil treatment; b) topical *Rosmarinus officinalis* oil in controls; and c) topical *Rosmarinus officinalis* oil in diabetics. The results show that diabetes caused a delay in the epithelia neof ormation in the healing area, and the epithelial neof ormation in control and diabetic animals after the treatment of topical *Rosmarinus officinalis* oil.

**Evaluation of epithelial neof ormation:** The epithelial neof ormation was evaluated taking the parameters: a) diabetic (Table 1 and Fig. 1) animals insulin therapy comparison of glycemia in control animals without insulin and with insulin therapy (Table 2 and Fig. 2) Evaluation of relative percent on epithelium and pseudomembrane wounded areas in diabetic animals and their controls were evaluated wounded areas with (W/RO) and without (OUT/RO) *Rosmarinus officinalis* oil topical treatment on days 3 and 7 after surgery. The results show that diabetes caused a delay in the epithelial neof ormation in the healing area. The epithelial neof ormation in control diabetic animals after topical *Rosmarinus officinalis* oil (Table 3 and Fig. 3). Collagen evaluation in healing areas with *Rosmarinus officinalis* oil (W/RO) and without *Rosmarinus officinalis* oil (OUT/RO) on 3 days after surgery and 7 days after surgery respectively (Fig. 4). Diabetic animals wound healing in area on day 3 and day 7 after surgery observed the improved cicatrization and difference at right side of the wound.

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Table 2: Mean value of the percent of points on epithelium and pseudomembrane wounded areas in diabetic animals and their controls evaluated by wounded areas with (W/RO) and with out (OUT/RO) *Rosmarinus officinalis* oil topical treatment on 3<sup>th</sup> and 7<sup>th</sup> day after surgery.

Control		Diabetic W/cm		Diabetic Out/cm	
Epithelium	Pseudomebrane	Epithelium	Pseudomebrane	Epithelium	Pseudomebrane
<b>3th Day After Surgery</b>					
14.00±0.50	84.60±5.03	6.00±0.50	93.90±7.50	9.00±0.50	86.50±6.50
15.50±0.56	85.60±6.50	8.00±0.52	92.60±7.02	13.50±0.54	92.60±8.02
16.20±0.58	87.30±7.50	7.00±0.49	97.50±7.05	8.00±0.58	98.00±9.50
15.60±0.48	83.60±6.50	6.00±0.59	92.60±7.50	11.00±0.56	87.60±10.8
10.80±0.50	87.40±7.50	7.5.0±0.57	90.50±7.05	9.00±0.50	84.0±8.54
14.60±0.55	88.00±8.00	8.00±0.50	98.45±11.0	13.00±0.53	89.6±8.50
<b>7th Day After Surgery</b>					
69.0±7.50	33.6±4.50	47.5±8.00	52.70±8.50	47.0±9.40	45.40±8.00
93.6±7.53	3.50±5.63	44.0±7.50	56.30±8.41	89.6±8.50	11.00±0.56
64.0±8.02	36.3±6.20	38.0±6.00	68.00±9.00	96.0±9.53	5.00±0.53
95.5±8.56	1.00±0.50	56.2±9.01	44.20±8.52	88.6±9.00	1.00±0.54
93.0±7.50	0.5±0.50	30.5±7.54	65.80±7.50	90.0±9.01	0.76±0.25
86.8±8.23	13.0±0.52	46.6±6.50	55.60±8.02	85.20±9.00	10.0±0.50

Treatment 3<sup>th</sup> and 7<sup>th</sup> Day after surgery; W/RO = With *Rosmarinus officinalis* oil; OUT/RO = Without *Rosmarinus officinalis* oil = Statistically significant at P values < 0.05

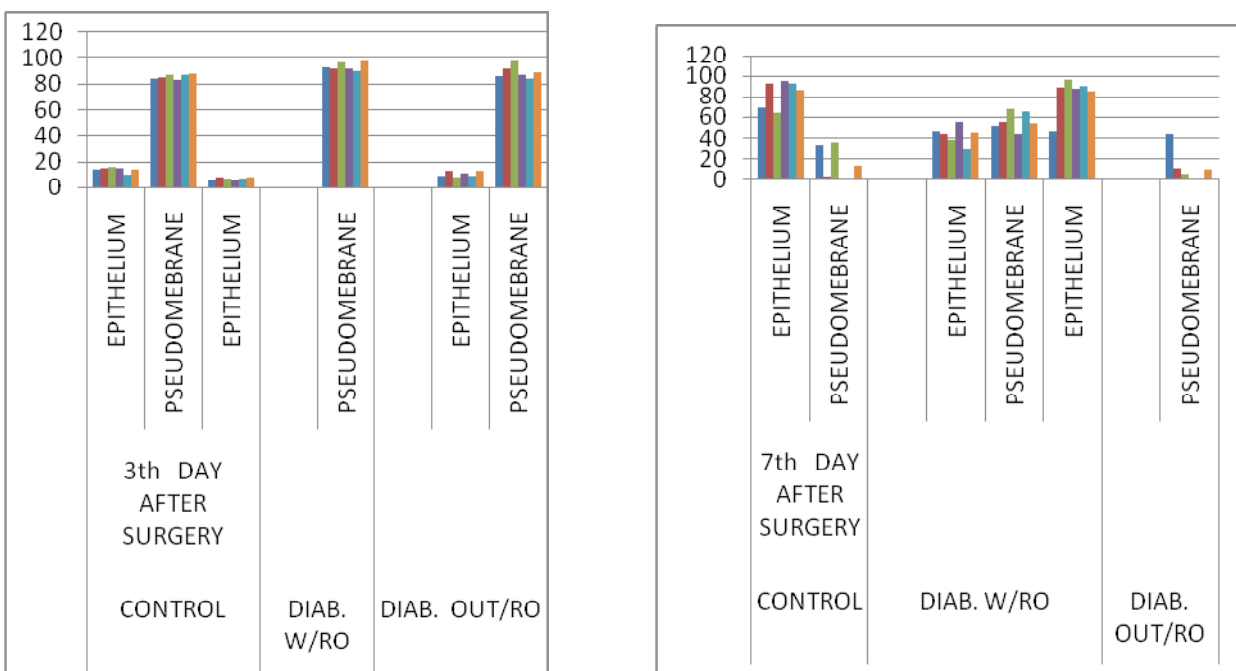


Fig. 2: Evaluation of relative percent of points in neoformed epithelium surgical areas in 3<sup>th</sup> and 7<sup>th</sup> day control animals with and without topical application of rosmary oil.

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Table 3: Collagen evaluation in healing areas with *Rosmarinus officinalis* oil (W/RO) and without *Rosmarinus officinalis* oil (OUT/RO) on day 3 and 7 after surgery.

3 <sup>rd</sup> day after surgery			Controls		
Density	Out/cm Area	% of Area	Density	W/cm Area	% Of Area
2.00±0.50	11.0±0.50	6.00±0.50	3.03±0.41	16.0±0.41	9.03±0.55
3.00±0.60	15.0±0.51	7.20±0.45	4.00±0.53	15.0±0.54	7.06±0.40
3.30±0.30	16.0±0.54	8.00±0.56	2.90±0.57	14.0±0.61	8.03±0.55
2.00±0.51	13.0±0.45	7.00±0.61	4.00±0.60	17.0±0.63	10.03±0.45
3.00±0.52	16.0±0.6	9.10±0.50	5.10±0.50	20.9±0.67	11.0±0.57
3.10±0.54	14.1±0.56	8.06±0.50	3.00±0.54	16.0±0.56	9.10±0.54
<b>Diabetic Without Insulin Therapy</b>					
1.00±0.5	5.03±0.6	3.00±0.51	1.90±0.51	10.03±0.40	5.60±0.50
2.00±0.51	10.0±0.57	5.00±0.53	3.00±0.41	10.9±0.41	6.00±0.56
2.00±0.53	10.0±0.62	4.10±0.56	1.00±0.52	14.0±0.50	6.96±0.54
2.13±0.55	9.10±0.53	5.10±0.44	2.00±0.42	14.9±0.45	5.06±0.58
1.03±0.52	6.10±0.58	6.10±0.60	3.00±0.53	10.9±0.60	7.06±0.52
2.03±0.50	8.13±0.50	5.10±0.45	2.00±0.61	22.0±0.90	6.06±0.50
<b>Diabetic With Insulin Therapy</b>					
1.96±0.50	11.0±0.5	5.00±0.52	4.00±0.52	29.0±1.00	10.96±0.45
2.06±0.57	13.1±0.58	7.00±0.41	5.90±0.58	3.03±0.53	14.9±0.40
0.50±0.51	12.10±0.45	8.00±0.51	6.00±0.56	28.0±1.00	17.0±0.40
2.06±0.53	13.1±0.40	6.00±0.59	5.00±0.44	25.00±1.00	16.0±0.59
2.00±0.5	14.1±0.52	7.00±0.43	5.20±0.60	13.0±1.50	12.0±0.60
2.10±0.5	15.0±0.59	8.00±0.43	6.00±0.53	24.0±1.50	14.0±0.55
7 <sup>th</sup> day after surgery			Controls		
9.00±0.5	50.0±6.00	27.0±3.00	25.0±0.52	61.6±6.50	35.0±5.56
8.03±0.6	65.6±9.10	38.6±5.50	12.0±0.56	96.3±8.02	52.3±5.03
22.0±1.00	96.6±10.1	24.3±2.57	10.0±0.54	41.0±5.70	21.6±5.03
9.00±0.50	46.6±9.00	26.7±2.08	21.3±1.15	0.51±0.50	58.3±8.56
27.0±1.00	95.0±11.0	59.3±5.03	23.0±2.64	97.6±7.52	46.3±8.50
15.0±0.51	73.6±6.02	41.0±6.0	20.6±8.32	87.6±8.02	35.6±6.02
<b>Diabetic Without Insulin Therapy</b>					
9.00±0.52	62.7±5.60	34.0±4.00	13.8±8.80	65.3±9.00	26.0±6.00
7.00±0.62	40.6±5.03	1.00±0.51	7.76±1.60	52.3±5.50	30.0±5.00
4.00±0.56	38.0±8.00	2.00±0.50	7.73±0.20	57.6±7.50	23.6±4.50
6.00±0.72	68.3±8.02	1.96±0.45	10.03±1.26	74.0±7.00	33.3±5.50
11.0±0.59	75.0±7.00	3.03±0.45	10.03±1.04	60.0±5.00	30.6±7.02
12.0±0.59	56.6±7.50	3.10±0.44	9.36±1.09	57.6±8.02	47.3±7.50
<b>Diabetic With Insulin Therapy</b>					
8.00±0.57	65.3±6.50	2.96±0.50	11.0±1.30	86.6±7.02	31.3±7.50
12.00±0.50	48.0±8.00	1.96±0.51	14.0±1.32	57.0±7.54	38.3±2.08
8.00±0.60	69.0±5.50	3.03±1.52	13.7±1.57	74.0±6.00	39.3±9.50
12.00±0.52	68.0±7.54	1.96±0.53	11.0±2.23	72.3±6.11	55.0±7.00
10.0±0.50	47.6±8.02	3.03±0.45	12.0±1.28	94.0±9.00	34.0±6.00
12.0±0.56	58.0±8.00	2.06±0.50	12.0±0.50	76.6±8.50	42.0±6.55

Treatment of collagen evaluation in healing areas 3<sup>th</sup> and 7<sup>th</sup> day after surgery; W/RO = With *Rosmarinus officinalis* oil; OUT/RO = Without *Rosmarinus officinalis* oil = Statistically significant P values < 0.05

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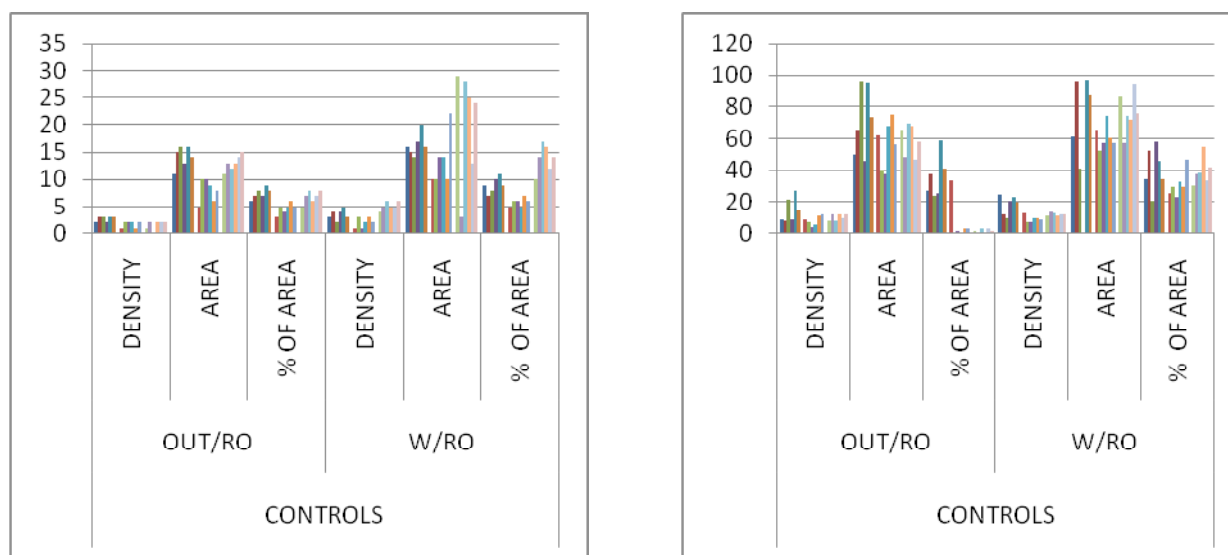


Fig. 3: Collagen evaluation in healing areas of diabetic with insulin therapy (W/RO) and diabetic without insulin therapy (OUT/RO) on day 3 and day 7 after surgery.

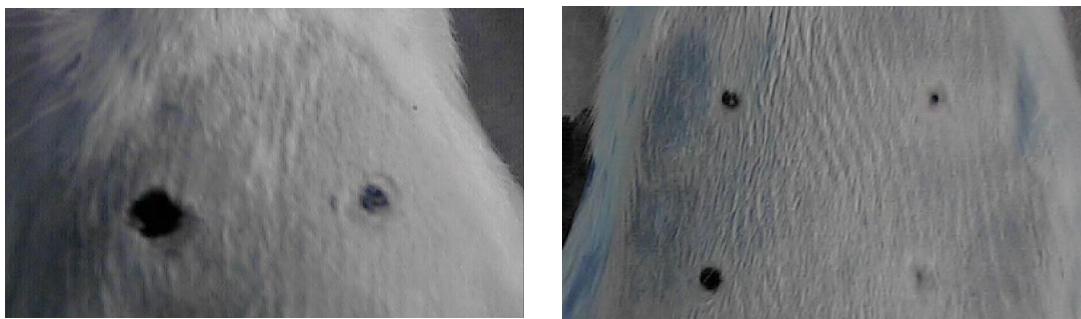


Fig. 4: Healing area in diabetic animal, day 3 and day 7 after surgery, observe the wound.

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