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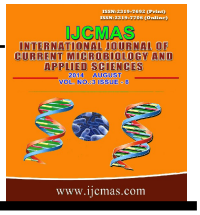

Original Research Article

Effects of topical *Cymbopogon martinii oil* essential oil in wound healing on diabetes induced rats

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Keywords

Topical *Cymbopogon martinii* oil Wistar rats, Oil-diabetes wound healing.

A B S T R A C T

A common complication of diabetes is impaired wound healing. Systemic Palmarosa oil improves healing in diabetics, which is dose dependent, and may have side effects. There is a very less information regarding topical Palmarosa oil use. The objective of this study was to evaluate the effects of topical Palmarosa 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 anesthesia, four skin wounds (4mm diameter), were performed on shaved dorsal area (2 each side of median line). Topical Palmarosa oil was applied daily only on both wounds on right side of median line. Animals were sacrificed on day 3 or 7 after surgery and tissue samples were prepared and observed under light microscopy. Histological, histometric and stereological methods were used for analysis. Topical Palmarosa oil accelerated wound closure in diabetic and non-diabetic rats and the results were found to be more active than antibiotic treated controls. Topical Palmarosa oil could be helpful in diabetics, in order to improve the wound healing process avoiding possible adverse effects from systemic medication. All the values are 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 naturally cytotoxic chemical that is particularly toxic to the pancreatic and insulin. Streptozotocin injection leads to the desgeneration of the langerhans islets beta cells. Glucose levels was increased in the blood of rat exposed to evaluate sublethal coccentration 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). Essential oils of plants show wound healing anti-microbial activity against a wide range of bacteria including antibiotic resistant species and fungal species (Carson et al., 1995; Carson and Riley, 1995).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 local Palmarosa oil use.

Materials and Methods

Wistar rats (Rattus norvegicus) were precured and maintained to the laboratory condition. All the rats were placed in plastic boxes (40 x 32 x 17cm) under controlled light conditions (12 hours of light; 12 hours of darkness) and temperature (21-25°C) in the Ecobiology lab of Voorhees College,Vellore. 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. The injected animals were controlled 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 for 15 days before wound surgery. A study on blood glucose was estimated by the method given in Sigma Diagnostic kits(Sigma Analysis Catalogue) biochemical factors and histopathological study were carried out as percentage collagen wound healing area of control animals.(Gutr 1959)

Statistical Analysis

All the data were analyzed and expressed as mean of six individual odservations. Standard Error and Students 't' test, were calculated as per the method of Pillai and Sinha (1968).

Results and Discussion

Tables1–3 and Plates1-2 indicate the results obtained in the present investigation. Hyperglycemia were observed in all diabetic animals. Mean blood glucose levels (360ml/dl on day 3 and 425mg/dl on day 7 after surgery) compared with non-diabetic animals (151mg/dl on day 3 and 139mg/dl on day 7 after surgery).

Histopathological Results

The epithelial neoformation was evaluated taking as parameters: a) diabetics, without Palmarosa oil treatment b) topical Palmarosa oil in controls and c) topical Palmarosa oil in diabetics. The results show that diabetes caused a delay in the epithelial neoformation in the healing area show the epithelial neoformation in control and diabetic animals after topical Palmarosa oil.

Evaluation of Epithelial Neoformation

The epithelial neoformation was evaluated taking as parameters: a) diabetics, Fig. (1). Animals insulin therapy comparison of glycemia in control the animals without insulin & with insulin therapy Fig. (2). Diabetics animals wound healing in area day 3th &7th after surgery observe the improved cicatrization and difference at right side of the wound Fig. (3). Evaluation of relative percent on epithelium and pseudomembrane wounded areas in diabetic animals and their controls were evaluated wounded areas with (w/CM) and with out (out/CM) *Cymbopogon martinii oil* Topical Treatment on days 3 and 7 after surgery.

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

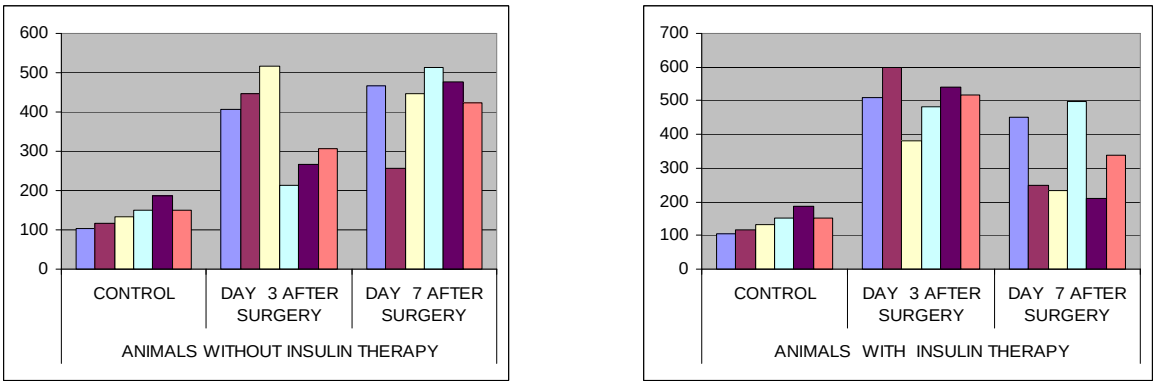

Fig.2 Healing area in diabetic animal, day 3 & 7 after surgery, observe the wound.

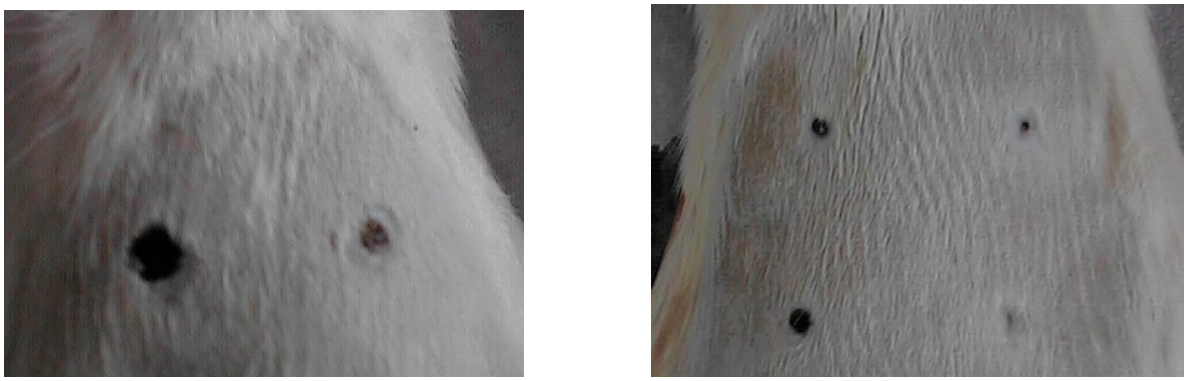

Fig.3 Evaluation of relative percent of points in neoformed epithelium surgical areas in 3th 7th days control animals with and without topical application of Palmarosa oil

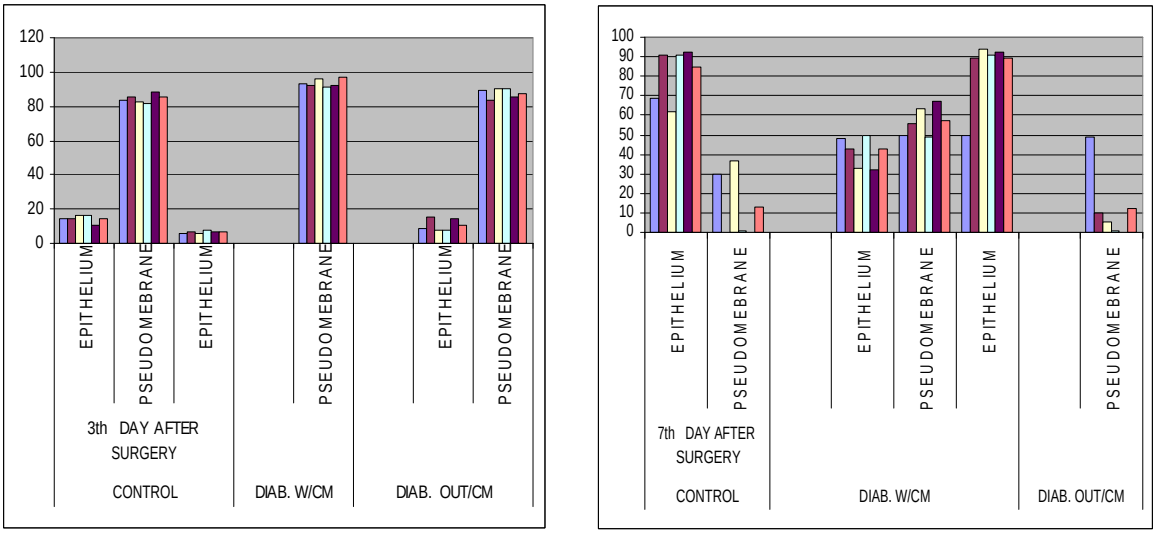

Fig.4 Collagen Evaluation in Healing Areas Diabetic with Insulin therapy (W/CM) &(Out/CM) on Day 3 & 7After surgery

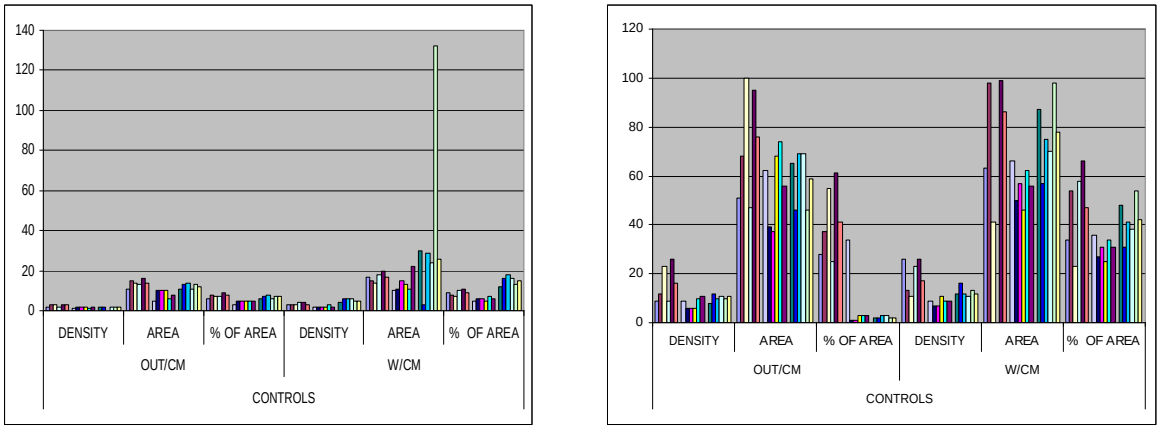

Table.1 Blood Glucose Evaluation (mg/dl)

ANIMALS WITHOUT INSULIN THERAPY		
CONTROL	**DAY 3 AFTER SURGERY**	**DAY 7 AFTER SURGERY**
104.66 ± 6.02	408.00 ± 0.00	467.33 ± 8.04
117.0 ± 8.00	447.66± 8.56	258.66 ± 8.11
133.33 ± 12.34	518.38 ± 8.03	447.60 ± 8.02
150.00 ± 9.00	212.0 ± 8.14	512.00 ± 7.00
187.00 ± 8.00	266.00 ± 9.53	476.00 ± 9.00
151.00 ± 7.00	307.66 ± 8.02	425.33 ± 8.02

ANIMALS WITH INSULIN THERAPY		
CONTROL	**DAY 3 AFTER SURGERY**	**DAY 7 AFTER SURGERY**
104.66 ± 6.02	508.00 ± 9.00	451.33 ± 7.51
117.0 ± 8.00	600.66 ± 8.02	248.00 ± 9.00
133.33 ± 12.34	382.66 ± 8.50	233.33 ± 12.34
150.00 ± 9.00	484.00 ± 9.00	499.66 ± 9.01
187.00 ± 8.00	542.60 ± 8.02	209.00 ± 8.00
151.00 ± 7.00	517.00 ± 8.00	338.00 ±19.52

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

Table.2 Mean Value of the Percent of Points on Epithelium and Pseudomembrane Wounded Areas in Diabetic Animals and Their Controls were Evaluated Wounded Areas with (w/CM) and with out (out/CM) *Cymbopogon martinii oil* Topical Treatment 3th and 7th Day after surgery

CONTROL		DIAB. W/CM		DIAB. OUT/CM	
3th DAY AFTER SURGERY					
EPITHELIUM	PSEUDOMEBRANE	EPITHELIUM	PSEUDOMEBRANE	EPITHELIUM	PSEUDOMEBRANE
14.56±0.45	84.82±9.00	6.40±0.50	93.37±9.99	9.95±0.29	89.01±8.99
14.80±0.49	85.86±9.13	7.11±0.52	92.53±10.00	15.92±0.50	84.07±9.10
16.32±0.57	83.26±9.50	6.31±0.51	96.77±5.96	8.96±0.47	90.50±10.23
16.70±0.50	82.17±9.47	8.20±0.54	91.10±9.21	8.70±0.50	90.57±10.16
11.48±0.52	88.18±9.51	7.92±0.49	92.05±8.96	14.45±0.51	85.30±8.94
14.70±0.58	85.29±9.92	7.25±0.46	97.70±8.99	11.76±0.45	87.52±10.44

CONTROL		DIAB. W/CM		DIAB. OUT/CM	
7th DAY AFTER SURGERY					
EPITHELIUM	PSEUDOMEBRANE	EPITHELIUM	PSEUDOMEBRANE	EPITHELIUM	PSEUDOMEBRANE
69.95±5.98	30.00±4.00	48.49±2.35	50.97±4.21	50.49±4.21	49.35±4.94
91.92±7.90	0.76±1.25	43.23±5.10	56.12±5.68	89.77±8.29	10.27±0.57
62.05±8.99	37.33±6.00	33.81±6.63	63.94±5.31	94.78±9.27	5.00±0.50
91.19±8.55	1.33±1.22	50.65±3.94	49.27±4.98	91.95±9.02	1.01±0.98
92.7±7.42	0.41±0.71	32.89±5.53	67.86±5.68	92.42±8.78	0.41±0.71
85.48±9.52	13.41±0.57	43.45±5.26	57.65±5.03	89.64±8.94	12.96±0.55

Treatment 3th and 7th Day after surgery W/CM = With *cymbopogon martinii oil* OUT/CM = Without *cymbopogon martinii oil* = Statistically significant. P values< 0.05

Table.3 Collagen Evaluation in Healing Areas with *Cymbopogon martinii oil* (W/CM) and without *Cymbopogon martinii oil* (OUT/CM) on Day 3 After Surgery

CONTROLS					
OUT/CM			W/CM		
DENSITY	AREA	% OF AREA	DENSITY	AREA	% OF AREA
2.50±0.50	11.46±0.55	6.44±0.50	3.71±0.51	17.23±0.45	9.85±0.26
3.39±0.520	15.36±0.70	8.73±0.412	3.27±0.49	15.0±0.43	8.30±0.523
3.09±0.510	14.26±0.45	7.94±0.410	3.02±0.50	14.1±0.65	7.780±0.2234
2.99±0.65	13.7±0.520	7.61±0.524	4.04±0.54	18.7±0.46	10.25±0.58
3.61±0.540	16.80±0.50	9.31±0.620	4.46±0.56	20.8±0.51	11.4±0.53
3.11±0.523	14.4±0.514	8.03±0.50	3.77±5.00	17.20±0.53	9.41±053
DIABETIC WITH OUT INSULIN THERAPY					
1.24±0.48	5.30±0.50	3.28±0.54	2.23±0.50	10.30±0.50	5.72±0.50
2.24±0.50	10.20±0.56	5.72±0.54	2.44±0.53	11.0±0.53	6.08±0.44
2.26±0.532	10.33±1.04	5.76±0.84	2.43±0.56	15.01±0.53	6.21±0.52
2.24±0.50	10.16±0.54	5.84±0.51	2.53±1.45	13.4±0.59	5.61±0.54
1.97±0.59	6.63±5.05	5.04±0.56	3.01±0.52	11.2±0.53	7.53±0.43
2.02±0.54	8.96±0.452	5.18±0.50	2.43±0.53	22.4±0.50	6.24±0.26
DIABETIC WITH INSULIN THERAPY					
2.52±0.50	11.20±0.50	6.50±0.52	4.84±0.51	30.4±0.50	12.57± 0.52
2.94±0.521	13.4±0.521	7.54±0.56	6.68±0.56	3.20±0.57	16.90±0.54
0.9±0.541	14.40±0.560	8.02±0.57	6.84±0.54	29.33±0.511	18.53±0.66
2.13±0.351	11.50±0.541	6.01±0.32	6.46±0.50	24.96±0.45	16.40±0.59
2.62±0.54	13.60±0.521	7.60±0.45	5.25±0.45	13.32±0.532	13.40±0.53
2.88±0.56	12.96±0.410	7.87±0.58	5.98±0.50	26.96±0.42	15.41±0.67

Collagen Evaluation in Healing Areas. Without *Cymbopogon martinii oil* (OUT/CM) and with *Cymbopogon martinii oil* (W/CM) on Day 7 After Surgery

CONTROLS					
OUT/CM			W/CM		
DENSITY	AREA	% OF AREA	DENSITY	AREA	% OF AREA
9.70±0.74	51.27±4.95	28.0±2.36	26.19±2.61	63.24±6.47	34.80±3.40
12.67±0.59	68.79±5.49	37.17±3.85	13.25±1.16	98.81±9.45	54.22±5.41
23.10±1.82	100.71±6.52	55.99±4.65	11.98±0.89	41.23±3.05	23.70±2.29
9.01±0.50	47.05±4.04	25.74±2.49	23.47±2.30	0.98±8.51	58.17±4.95
26.39±1.23	95.01±7.86	61.96±5.97	26.34±2.88	99.52±9.00	66.13±6.00
16.14±1.10	76.30±6.99	41.98±3.96	17.34±1.74	86.33±8.00	47.28±4.35
DIABETIC WITH OUT INSULIN THERAPY					
9.73±0.50	62.06±6.15	34.13±3.07	9.78±6.71	66.03±6.00	36.32±3.00
6.22±0.58	39.9±3.57	1.99±1.15	7.63±0.67	50.24±4.96	27.90±2.70
6.99±0.50	37.91±2.60	1.94±1.12	7.92±0.50	57.10±5.01	31.40±3.10
6.86±0.51	68.13±5.90	3.27±1.89	11.83±0.52	46.20±3.90	25.71±2.59
10.62±0.53	74.13±7.10	3.82±2.21	9.65±0.40	62.03±6.00	34.22±3.42
11.30±0.54	56.13±7.10	3.10±1.88	9.51±0.35	56.67±4.95	31.11±3.29
DIABETIC WITH INSULIN THERAPY					
8.95±0.51	65.03±5.95	2.73±1.57	12.8±0.51	87.90±7.99	48.67±4.40
12.09±0.50	46.0±4.00	2.80±1.61	16.33±1.09	57.29±3.52	31.92±2.99
10.75±0.64	69.36±6.00	3.81±2.19	12.55±0.52	75.30±6.85	41.70±2.94
11.43±0.56	69.37±6.02	3.64±2.10	11.19±0.30	70.44±7.00	38.71±3.80
10.90±0.54	46.47±3.90	2.50±1.44	13.39±0.54	98.24±9.04	54.21±5.01
11.23±0.57	59.12±4.99	2.45±1.41	12.11±0.52	78.20±7.00	42.64±4.02

Treatment of Collagen Evaluation in Healing Areas 3th and 7th Day after surgery W/CM = With *Cymbopogon martinii oil* OUT/CM = Without *Cymbopogon martinii oil* = Statistically significant. P values< 0.05

The results show that diabetes caused a delay in the epithelial neoformation in the healing area. the epithelial neoformation in control diabetic animals after topical Palmarosa oil, Fig. (4). Collagen evaluation in healing areas diabetic with insulin therapy (W/CM) on Day 7After surgery 3 and 7 after surgery respectively.

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