ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Issue 3, 2013

ISSN - 0974-2441

Research Article

WOUND HEALING ACTIVITY OF TOPICAL *MENTHA PIPERITA* AND *CYMBOPOGAN CITRATUS* ESSENTIAL OIL ON STREPTOZOTOCIN INDUCED RATS

UMASANKAR.K, BALWIN NAMBIKKAIRAJ*, MANLEY BACKYAVATHY.D

PG and Research Department of Zoology, Voorhees College, Vellore. Email. K.umasankar.1986@gmail.com

Received: 25 April 2013, Revised and Accepted: 24 May 2013

ABSTRACT

A common complication of diabetes is impaired wound healing. Systemic Mentha piperita and Cymbopogan citratus essential oil improves healing in diabetics, which is dose dependent, and may have side effects. There is a very less information regarding topical Mentha piperita and Cymbopogan citratus use. The objective of this study was to evaluate the effects of topical Mentha piperita and Cymbopogan citratus 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, Diabetic induced infected wound treatment with topical Mentha piperita ointment treatment and their another essential ointment in Cymbopogan citratus wound contraction studies a circular piece 08 mm^2 in area 20^{th} days compared wound healing study on the wound contraction studies a circular piece 08mm^2 in area 18^{th} days highly effective in Mentha piperita ointment. Then non diabetic wound healing control on the wound contraction studies a circular piece 07 mm^2 in area12^{th} days, complete wound healing activity and diabetic wound control compared with diabetic infected wound treatment Mentha piperita ointment with highly effective wound healing activity histological, histometric and stereological methods were used for the analysis. Topical Mentha piperita and Cymbopogan citratus accelerated wound closure in diabetic and non-diabetic rats and the results were found to be more active than antibiotic treated controls. Topical Mentha piperita and Cymbopogan citratus 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.

Keywords: Topical *Mentha piperita* oil, *Cymbopogan citratus* oil, Wistar rats, Hematological and Histopathological factors, diabetes wound healing.

INTRODUCTION

Diabetes Mellitus is a syndrome more than a disease and affects about 150 million people worldwide [9] . Studies have shown delayed wound healing in diabetics due to cell proliferation deficiency, infection, decreased cell surviving, and reduced wound contraction [7]. Streptozotocin (intraperitoneal) and injection of streptozotocin monohydrate produces insulin decreasing and hyperglycemia in a few days [3,1,10,4]. 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 [12].

Essential oils and various extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [13]. It is Particularly, the antimicrobial and antivirus activities of plant oils and extracts have formed the basis of applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [6].

The present study aims to the initial phases of wound healing in the skin of normal wound healing control and diabetic induced infected wound healing control and diabetic induced infected wound and diabetes induced infected wound treatment with essential oil to compare wound healing areas in diabetics and their different essential oil controls after local *Mentha piperita and Cymbopogan citratus* [2].

MATERIALS AND METHODS

Toxicity Evaluation (LD_{50})

The LD_{50} for The Wistar rats were procured and acclimatized to laboratory condition. They were maintained on commercial diet supplied by "Hindustan Lever Limited" Bombay, marketed under the trade name "Gold Mohur Feeds" water provided ad libitum. Fourity eight (24) adult healthy male wistar rats with body mass of approximately 200–225 g were used. Streptozotocin-induced (intraperitoneal) and injection (60 mg/kg,) dissolved in 0.01 M citrate buffer, pH 4.5, immediately before use. Three days later blood glucose levels were determined in diabetic animals were further divided into 4 groups of 6 rats each group. The rats were divided into 4 groups

Group I : Normal rat

Group II : Diabetic Induced wound healing control

Group III: Diabetic Induced wound healing + infected microorganism

Group IV: Diabetic Induced wound healing + infected microorganism + Treatment

Analysis carried out

Hematological parameters such as Haemoglobin ,Total WBC count, Differential Leucocyte Count, Erythrocyte Sedimentation Rate, Total RBC count, Platelets, Packed Cell Volume, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, Colour Index ratio and histopathological study All the analysis are carried out by the method of Sigma Diagnostic kits (Sigma Chemical Company Catalogue, 1997) and [2].

Statistical Analysis

All the data were analyzed as per the method of Pillai and Sinha (1968)[8].

RESULTS AND DISCUSSION

Tables1-4, Fig 1-4 and Plates 1-6 indicate the results obtained in the present investigation. Hyperglycemia were observed in all diabetic animals. Normal wound healing and diabetic induced wound healing activity was compared, Hematological parameter and histopathological studies were carried out.

Diabetic induced wound control animal and diabetic induced wound artificial infected microorganism such as *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabillus, Candida albicans,* and

Aspergillus fumigates, It also infected control animals then diabetic induced wound artificial infected microorganism treatment with topical *Mentha piperita and Cymbopogan citratus* oil wound healing compared in *Mentha piperita* oil in 18th days wound healing activity and *Cymbopogan citratus* oil in 20th days wound healing activity, Compared wound healing activity highly effective in 18 days topical *Mentha piperita* ointment [5,11] .Normal rats wound creation in wound healing activity in 12th days compared wound healing activity diabetic induced wound control and diabetic induced artificial infected microorganism wound control then diabetic induced artificial infected microorganism treatment in topical *Mentha piperita* and *Cymbopogan citratus* ointment comparison in study.

Table 1:The Wound Area (Mm²) Normal wound control compared Diabetic Induced Wound Control.

Experimental Rats	2nd	4th	6th	8th	10th	12th	14th	16th	18th	20th
Normal Wound Control	217±4.40	145±4.41	70±2.00	35±1.00	17±0.54	08±0.50	-	-	-	-
Diabetic Induced Wound Control	225±5.00	232±4.00	236±4.59	244±2.14	248±2.51	256±3.14	252±2.00	246±4.00	237±2.52	228±2.51

Values are mean ± SD of 6 individual observations.Values are significant at P < 0.001.

Table 2: The Wound Area (Mm²) Streptozotocin Induced Infected Wound Microorganism Treatment With Topical Peppermint Ointment.

Experimental Rats	Days	*P.aeruginosa* ATCC 31480	*S.aureus* ATCC 25923	*Prot.mirabillus* ATCC 49565	*C.albicans* ATCC 10231	*A .fumigatus* ATCC 46445
Diabetic Induced Infected wound	1st	225±2.16	224±2.15	220±2.14	235±2.51	230±2.14
	3rd	230±2.14	228±2.19	228±2.17	244±2.13	236±2.52
	6th	236±2.52	235±1.90	236±2.54	250±2.00	242±2.18
	9th	242±2.18	246±2.10	242±2.17	254±2.54	248±2.26
	12th	247±2.24	250±2.00	248±2.10	258±2.18	254±2.12
	15th	254±2.12	254±2.15	255±2.12	262±2.40	258±2.18
	18th	248±2.26	252±2.21	253±2.20	257±2.32	256±2.37
Diabetic Induced	1st	230±2.32	228±2.20	225±2.16	230±2.12	225±2.15
Infected Wound Treatment with Peppermint	3rd	215±2.14	213±2.15	218±2.08	217±2.18	214±2.00
Ointment	6th	183±2.10	186±2.14	183±2.10	185±2.15	189±2.17
	9th	124±2.00	128±2.10	120±2.00	122±2.18	128±2.10
	12th	70.0±1.41	78.0±1.52	60.0±1.30	74.0±1.45	73.0±1.38
	15th	35.0±1.21	38.0±1.18	28.0±1.20	33.0±1.00	34.0±1.14
	18th	09.0±0.51	10.0±0.50	08.0±0.58	09.0±0.50	10.0±0.54

Values are mean ± SD of 6 individual observations, Values are significant at P < 0.001.

Table 3: The Wound Area (Mm²) Streptozotocin Induced Infected Micro Organism Wound Healing Treatment With Topical Lemongrass Ointment.

Experimental Rats	Days	*P.aeruginosa* ATCC 31480	*S.aureus* ATCC 25923	*Prot.mirabillus* ATCC 49565	*C.albicans* ATCC 10231	*A .fumigatus* ATCC 46445
	2nd	230±2.28	225±2.00	230±2.17	225±2.00	224±2.14
	4th	235±2.34	223±1.90	236±2.54	230±2.10	228±2.16
	6th	241±2.20	240±2.24	241±2.15	236±2.25	235±2.50
Diabetic Induced Infected wound	8th	246±2.25	245±2.28	248±2.38	244±2.28	244±2.40
	10th	251±2.30	253±2.30	254±2.50	254±2.17	249±2.42
	12th	256±2.26	258±2.54	258±2.56	259±2.32	254±2.48
	14th	260±2.46	263±2.63	264±2.58	265±2.58	258±2.28
	16th	265±2.55	267±2.58	268±2.50	269±2.53	260±2.35
	18th	262±2.51	264±2.56	265±2.52	266±2.54	258±2.30
	20th	257±2.46	258±2.40	259±2.47	260±2.50	254±2.58
Diabetic Induced	2nd	235±2.24	228±2.30	235±2.36	226±2.12	228±2.17
Infected Wound Treatment with Lemongrass	4th	225±2.04	224±2.00	228±2.10	223±2.16	223±2.08
Ointment	6th	200±1.85	205±1.80	208±1.90	207±1.86	206±1.80
	8th	186±1.74	186±1.76	189±1.78	192±1.94	192±1.90
	10th	163±1.54	167±1.58	164±1.56	174±1.50	175±1.51
	12th	126±1.46	128±1.48	128±1.45	135±1.52	143±1.54
	14th	86.0±1.34	83.0±1.32	82.0±1.30	78.0±1.28	70.0±1.20
	16th	43.0±1.26	40.0±1.20	42.0±1.23	37.0±	35.0±1.17
	18th	18.0±1.12	20.0±1.00	18.0±1.14	17.0±1.10	17.0±1.08
	20th	09.0±0.58	10.0±0.50	08.0±0.54	09.0±0.50	08.0±0.52

Values are mean ± SD of 6 individual observations.Values are significant at P < 0.001.

Table 4: Diabetic Induced Rats and Normal Control Rats Compared In ematological parameter

TEST	NON INDUCED IN CONTROL	STZ INDUCED WITH OUT INSULIN HERAPY IN RATS
Hb gms%	14±0.50	18±0.54
TC cells/cu.mm	8500±450	11500±500
DLC Neutrophils %	62±2.00	78±2.12
Eosinophils %	3.0±0.50	7.0±0.53
Basophils %	00	3.0±0.50
Lymphocytes%	30±2.00	48±3.00

Monocytes %	5.0±0.52	9.0±0.54
ESR 20 min	2.0±0.50	5.0±0.54
40 min	5.0±0.54	12±0.58
60 min	9.0±0.58	22±1.00
TRBC Million cells/cub.mm	5.9±0.12	6.8±1.00
Platelets Lakh cells/cu.mm	2.6±0.10	3.5±0.14
PCV %	52±2.00	58±2.40
MCV FI	88±1.20	85±1.40
MCH Pg	23±1.00	26±1.20
MCHC %	26±1.14	31±1.00
CI	0.9±0.30	1.0±0.54

Values are mean ± SD of 6 individual observations. Values are significant at P < 0.001.

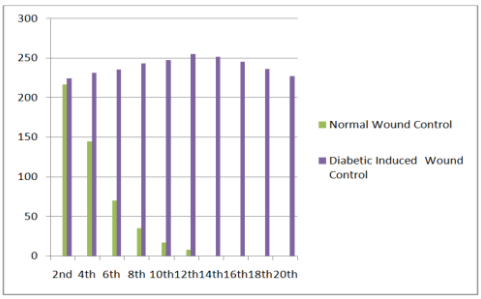

Figure 1: The Wound Area (Mm²) Normal wound control compared with Diabetic Induced Wound Control.

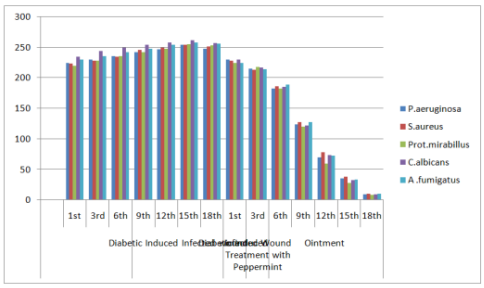

Figure 2:The Wound Area (Mm²) Streptozotocin Induced Infected Wound Microorganism Treatment With Topical Peppermint Ointment.

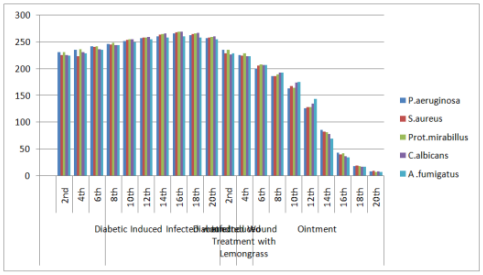

Figure 3: The Wound Area (Mm²) Streptozotocin Induced Infected Micro Organism Wound Healing Treatment With Topical Lemongrass Ointment.

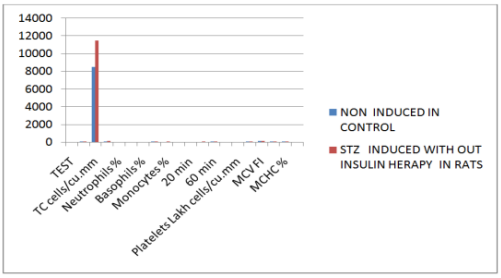

Figure 4: Diabetic Induced Rats and Normal Control Rats Compared In Hematological parameter

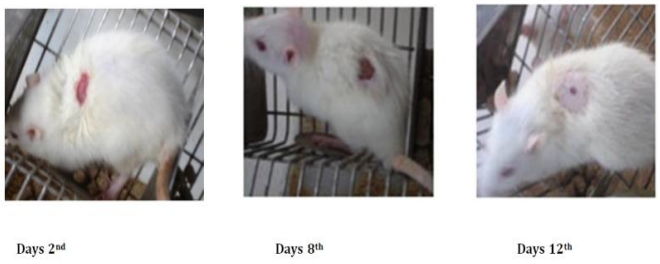

Plate 1: Normal Wound Healing Rats

Plate 2:Streptozotocin Induced Wound Control.

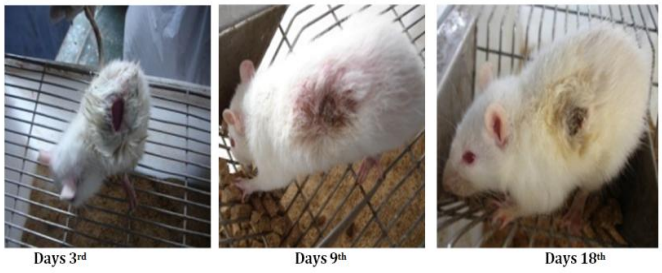

Plate 3: Streptozotocin Induced Infected Micro Organism Wound Control.

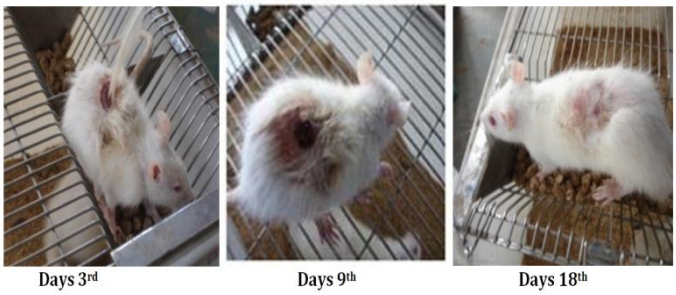

Plate 4:Streptozotocin Induced Infected Micro Organism Treatment With Peppermint Ointment.

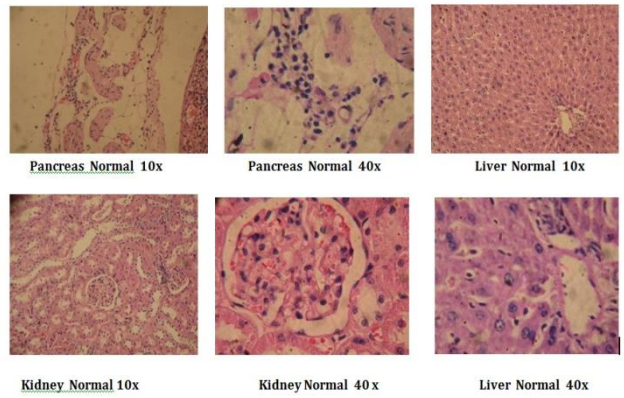

Plate 5: Histopathology Analysis of Normal Rat (No aggregation)

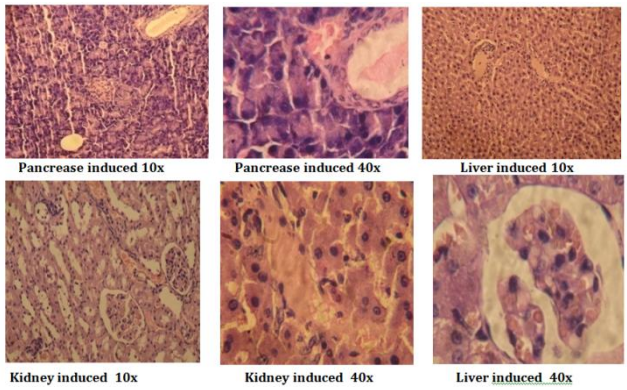

Plate 6:Histopathology Analysis of Streptozotocin Induced Infected Rat (With aggregation).

REFERENCES

1. Darby IA, Bisucci T, Hewitson TD, Maclellan DG., 1997. Apoptosis Is Increased In A Model Of Diabetes - Impaired Wound Healing In Genetically Diabetic Mice. Int J Biochem Cell Biol; 29: 191-200.
2. Gupta, A.K., 1995. Introduction To Pharmaceutics, Cbs Publishers, New Delhi.
3. Litchfield, J.T. And F.A. Wileoxaon, 1949. J. Pharmacol. Exp. Ther., 99; 95. C.F. S.N. Somayaji, A.P. Jacob And K.L. Bairy, 1995. Indian J. Experimental Biol., 33: 201-204.
4. Job Gopinath,N(2001).In:Bioaccumulation Of Chromium In Chromate Industrial Workers And Chromium Toxicity Studies In Rabbit Oryctolagus Cuniculus. Thesis Submitted To The University Of Madras,Chennai.
5. Mortan, J.J.P. And M.H. Malone, 1972. Arch. Int. Pharmacodynamics, 196: 117-126
6. Nikos GT, Costas DE, 2007. Antifungal activity of lemongrass (Cympopogon citratus L.) essential oil against key postharvest pathogens. Innovative Food Sci Emerg Technol 8(2); 253- 258.
7. Nagy S, Redei A, Karády S.,1961. Studies On Granulation Tissue Prodution In Alloxan- Diabetic Rats. J Endocrinol 22: 143-6.
8. Pillai, S.K. and Sinha, H.C. (1968). In:Statistical Methods For Biological Workers Pubs.Ramprasad And Sons. Agra, India.
9. Prakash A, Pandit Pn, Sharma Ls.,1974. Studies In Wound Healing In Experimental Diabetes. Intern Surg 59: 25-8.
10. Ramamurthy Ns, Zebrowski Ej, Golub Lm.,1973. Collagenolytic Activity Of Alloxan Diabetic Rat Gingivae. Diabetes 22: 272.
11. Saha, K. And P.K. Mukherjee, 1997. Wound Healing Activity Of Leucar Lavandeelaefolia Rees., J. Ethnopharmacol., 56: 139-144.
12. Vahlquist.R.(1950). Blood., 5:874.
13. Yadav, H., Jain, S., & Sinha, S. H. (2007). Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. Nutrition, 23, 62 - 68.