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COMPARATIVE STUDY OF HYPOGLYCEMIC AND HYPOLIPIDEMIC POTENCY OF *MURRAYA KOENIGII* FOR WOUND HEALING ACTIVITY IN TYPE-2 DIABETIC RATS

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ABSTRACT

In the present study we examined the role of 'Murraya koenigii' in diabetic wound healing, whether its hypolipidemic activity plays a vital role in cytokine production, as local cytokines production fluctuate during Cutaneous wound, diabetes and in hyperlipidemia. The activity of 'Murraya koenigii' was compared with Pravastatin and Glibenclamide. In blood parameter study Murraya koenigii aqueous extract showed significant ($p<0.001$) anti-hyperglycemic activity and anti-hyperlipidemic activity in diabetic hyperlipidemic rats when compared to the diabetic hyperlipidemic control group. Murraya koenigii was found to be more potent for its hypolipidemic activity as compare to hypoglycemic activity because the Murraya koenigii showed no significant ($p>0.05$) difference for its hypoglycemic activity (decreasing fasting blood glucose level) at dose level 400mg/kg in diabetic hyperlipidemic rats when compared to the normal control group rats and in the case of its hypolipidemic activity (decreasing lipid level) it showed no significant difference at dose level 300 mg/kg only. In the excision wound model, animals treated with Murraya koenigii (200mg/kg, 300mg/kg and 400mg/kg) leaves aqueous extract showed significant reduction in period of epithelisation and wound contraction 50% when compared to the diabetic hyperlipidemic control group rats. In this wound model very significant($p<0.001$) result was found with 300mg/kg dose level because the effect was dose dependent up to 300mg equivalent of extract. The groups treated with Murraya koenigii and Pravastatin showed more significant effect for excision wound healing than Glibenclamide treated group. These results clearly indicate that the hypolipidemic property of Murraya koenigii and Pravastatin may promote the epithelization and rate of wound contraction.

KEYWORDS: *Murraya koenigii (MK), Excision wound, Pravastatin, Glibenclamide, DH-Diabetic hyperlipedemic, HFD-High Fat Diet, STZ-Streptozotocin.*

INTRODUCTION

Wound has been defined as the disruption of anatomic or functional continuity of living tissue[1] produced by physical, chemical, electrical or microbial insult to the tissue and wound healing refer to the restoration of continuity of living tissues.Wound healing is a complex but orderly phenomenon involving a number of events[2].Induction of an acute inflammatory process, Regeneration of parenchymal cells, Migration and proliferation of both parenchymal and connective tissue cells, Synthesis of extra cellular matrix (ECM), Remodeling of connective tissue and parenchymal components and Collagenization and acquisition of wound strength. The process of healing follows a general scheme in which sequence of processes takes place an orderly way viz; inflammatory phase, proliferative phase and remodeling phase or maturation[3]. The inflammatory phase is marked by vasodilations, increased vascular permeability, platelet accumulation, coagulation and leucocytic migration [4].

While the proliferation phase is characterized by epithelization, fibroplasias and wound

contraction. Finally remodeling involves intermolecular cross-linking of collagen fibres[5].

Wound healing either by regeneration or repair has to restore continuity ultimately. So, it is said to be not complete until the disrupted surfaces are finally knit by collagen, until there is obliteration of dead spaces, until there is surface covering and until function has been restored to normal for a period of time [1]. Diabetes mellitus is one of the biggest causes of morbidity and mortality with an estimated 285 million people, corresponding to 6.4% of the world's adult population, which live with diabetes in 2010 and the number is expected to grow to 438 million by 2030. With an estimated 50.8 million people living with diabetes, India has the world's largest diabetes population, followed by China with 43.2 million [6]. It is a chronic disorder of carbohydrate, fat, and protein metabolism. A defective or deficient insulin secretary response, inducing metabolic abnormalities responsible for hyperlipidemia as well as hyperglycemia. Due to compromises in cellular migration, vascular proliferation, and extracellular matrix remodeling, make a negative impact on tensile strength, wound healing in such patients is fraught with complications [7]. Traditional Indian and Chinese medicines have been used successfully in diabetic hyperlipidemic complications because of their hypolipidemic activity. *Murraya koenigii* (Linn.) Sprend, popularly known as 'Curry leaf & Meetha Neem' have several pharmacological activities including hypolipidemic, hypoglycemic activity and wound healing activity [8-13]. Curry leaves also exhibited strong antioxidant property on liver and heart. It was found that phenolic antioxidant is present in *Murraya koenigii* and other herbs [14]. Hence our aim is to find out whether hypolipidemic action of Curry Leaves plays a vital role in healing diabetic wound in an experimental model of HFD-High Fat Diet and STZ-Streptozoin treated type II diabetic rats.

MATERIAL AND METHODS

Plant material:

Fresh leaves of *Murraya koenigii* were collected from medicinal garden of Sri Balaji College of Pharmacy, Jaipur and were authenticated by Prof. K.P. Sharma, Herbarium Incharge in Department of Botany, University of Rajasthan, Jaipur and voucher specimen (No. RUBL21109) has kept in Herbarium of Botany department and leaves were shade dried and powdered to course powder size.

Extraction:

The powder was extracted with distilled water using soxhelt at boiling temperature (100 ◦C) up to 10 h. Adark brown colour extract is obtained. This dark brown extract was cooled and filtered to remove the residue. The extract was concentrated on rotavapour under reduced pressure and then finally lyophilized to get a powder weighing about 75g. [11].

Preliminary Phytochemical Studies:

The extract was then subjected to qualitative phytochemical screening for the identification of the phytoconstituents [15].

Acute toxicity:

The acute toxicity study was done by **"fixed dose"** method in healthy adult female albino Wistar rats according to CPCSEA recommended "OECD guidelines 420 [16].

Animal:

Adult male and female albino Wistar Rats (250-300g) were obtained from animal house facility of Sri Balaji College of Pharmacy, Jaipur. Animal House Facility of this division is approved by Govt. of India under the Ministry of Environment & forest (Reg. No. 1212/ac/08/CPCSEA). Then all the animals were acclimatized at least under standard husbandry condition i.e. room temperature 24±1oc; relative humidity 45-55% and 12:12 hr light/dark cycle. The animal had free access to standard laboratory chow diet with water supplied ad libitum under strict hygienic condition. The rats were anaesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anesthesia (10 mg/kg body weight of an animal). Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study.The approval of the Institutional Animal Ethical Committee (IAEC) of Sri Balaji College of

Pharmacy, Jaipur was taken prior to start of experiments. All the protocol and experiment were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA).

Induction of diabetic hyperlipidemia (DH): [17, 18] (Development of High Fat Diet-fed and Streptozotocin-treated type 2 diabetic rat's model)

The rats were allocated into two dietary regimens normal pellet diet NPD and high fat diet HFD (2% cholesterol, 30% dalda and 68% of pellet chow) ad libitum, respectively, for the initial period of 2 weeks [17]. After the 2 weeks of dietary manipulation on the confirmation of hyperlipidemia in rats , a subset of the overnight fasted rats from each dietary group was injected intraperitoneally (i.p.) with low dose of STZ (35 mg/kg) while the respective control rats were given vehicle 0.1M citrate buffer (pH 4.5) in a dose volume of 1ml/kg, i.p, respectively [18]. The body weight and biochemical estimations (plasma glucose (PGL), triglycerides (PTG), total cholesterol (PTC), and LDL & HDL) were carried out just before and 7 days after the vehicle or STZ injection, i.e., on 3 weeks of dietary manipulation in rats. The rats with the fasting PGL of ≥200 mg/dl were considered diabetic and selected for further pharmacological wound healing studies. The rats were allowed to continue to feed on their respective diets until the end of the study and blood plasma related biochemical estimations were also carried out on 6^{th}, 12^{th}, 18^{th} and 24^{th} days in the excision wound model. The treatments of drugs were started after 7 days STZ injection on the confirmation of hyperglycemia in diabetic hyperlipidemic rats, it was considered as day 0 for further pharmacological activity.

Estimation of biochemical parameters:

Before the experiment, food was withheld overnight, with free access to water. Rats were anaesthetized with 1 ml of intravenous ketamine hydrochloride (10 mg/kg body weight) and blood was collected by tail vein. The blood was centrifuged and the collected serum sample was subjected for the estimation of glucose(Glucose diagnostic kit- Span diagnostic Ltd. Surat),total cholesterol(Serum total cholesterol diagnostic kit- Span diagnostics Ltd, Surat),High Density Lipoprotein(HDL)(Serum HDL cholesterol diagnostic kit- Span diagnostics Ltd, Surat),Low Density Lipoprotein (LDL) by using Friede Wald formula[23] and triglyceride(Triglycerides diagnostic kit- Span diagnostic Ltd. Surat). The other reagents and chemicals were used of Regular laboratory grade [19-23].

Wound Model: Excision Wound

Group-I: Normal control group receive oral 5ml/kg of 0.5% CMC with 0.1% Tween 80(Vehicle)

Group-II: DH control group receive oral 5ml/kg of 0.5% CMC with 0.1% Tween 80 (Vehicle)

Group-III: DH test group receive oral 5ml/kg of 200mg/kg *Murraya koenigii* extract.

Group-IV: DH test group receive oral 5ml/kg of 300mg/kg *Murraya koenigii* extract.

Group-V: DH test group receive oral 5ml/kg of 400mg/kg *Murraya koenigii* extract

Group-VI: DH standard group receive oral 5ml/kg of Glibenclamide 0.6mg/kg

Group-VII: DH standard group receive oral 5ml/kg of Pravastatin 10mg/kg

Albino Wistar rats were divided into seven groups as above, each containing six animals. All the rats were anesthetized with 1 ml of intravenous ketamine hydrochloride (10 mg/kg body weight) and impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The particular skin area was shaved one day prior to the experiment. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The animals were treated with drugs daily and treatment was continued for 21 days. Wound area was measured by tracing the wound on a millimeter scale graph paper on predetermined days i.e., 2, 4, 6, 8, 10, 12 and 14 (until 50% wound contraction) days post-wounding. The wound contraction-50% (days) was determined by plotting the wound area vs days on a graph paper. Falling of scab leaving no

raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelization [24, 25].

RESULTS

The freshly prepared aqueous extract was subjected to preliminary phyto-chemical screening test for various constituents. This revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids and steroids. In acute toxicity studies, the extract in doses up to 2000mg did not produce any signs of toxicity and mortality. The animals were physically active and were consuming food and water in a regular way. No abnormal behavior was noticed. As no mortality was recorded within 24 hours during the acute toxicity test, LD50 could not be calculated.

Effect of *Murraya koenigii* aqueous extract on Fasting blood glucose level (mg/dl) in diabetic hyperlipidemic rats:

In this study data observation on 18th & 24th day, diabetic hyperlipidemic rats treated with test drug *Murraya koenigii* aqueous extract (oral administration of variable dosage level MK-200mg/kg, MK-300mg/kg and MK-400mg/kg) and standard drug glibenclamide 0.6mg/kg showed significant ($p<0.001$) decreasing the fasting blood glucose levels when compared to the diabetic hyperlipidemic control group rats. Animals treated with standard drug Pravastatin 10mg/kg showed no significant ($p>0.05$) difference in fasting blood glucose levels when compared to the diabetic hyperlipidemic control group rats. In this study very significant($p<0.001$) result was found with MK-300mg/kg dose level because the effect was dose dependent up to MK-300mg equivalent of extract and there was no significant ($p>0.05$) difference in fasting blood glucose level of animal treated with MK-400 mg/kg when compared with animal treated with MK-300 mg/kg. Diabetic hyperlipidemic control group showed significant ($p<0.001$) increasing in fasting blood glucose level when compare to the normal control group rats and diabetic hyperlipidemic animals treated with MK-400 & Glibenclamide 0.6mg/kg groups showed no significant ($p<0.001$) difference in fasting blood glucose levels when compare to the normal control group rats and diabetic hyperlipidemic animals treated with MK-200, MK-300, and Pravastatin 10mg/kg showed significant ($p<0.05$) increasing in fasting blood glucose level when compare to the normal control group rats.(**Table:1**)

Table 1: Effect of *Murraya koenigii* aqueous extract on Fasting blood glucose level (mg/dl) in diabetic hyperlipidemic rats.

Treatment	Fasting Blood Glucose (FBG) Level mg/dl				
	Day-0	Day-6	Day-12	Day-18	Day-24
Normal control	86.9±12.24	88.92±10.01	87.65±9.06	85.55±8.16^{a1}	86.05±8.69^{a1}
DH control	352.62±10.77	358.50±10.71	364.75±13.34	368.78±12.78	372.98±11.26
MK-200mg/kg	366.28±11.98	348.93±9.59	296.05±12.22	258.50±12.97^{a1,b1}	242.30±11.23^{a1,b1}
MK-300mg/kg	364.62±11.12	214.47±11.54	156.95±10.97	114.067±9.26^{a1,b2}	110.95±11.68^{a1,b3}
MK-400mg/kg	372.93±9.89	188.27±10.77	143.82±9.05	105.73±9.59^{a1,b,c}	98.68±13.45^{a1,b,c}
Glibenclamide 0.6mg/kg	360.20±10.72	175.25±11.01	136.86±11.22	100.35±12.05^{a1,b}	94.48±11.02^{a1,b}
Pravastatin 10mg/kg	356.61±9.65	359.55±9.20	363.18±11.45	365.15±12.56^{a,b1}	369.92±13.39^{a,b1}

Values are expressed as Mean ± SD; n=6,
a1 P<0.001,a2 P<0.01 & a3 P<0.05 & 'a'- no significant' when compared with Diabetic hyperlidemic (DH) control group-II;
b1 P<0.001,b2 P<0.01 & b3 P<0.05 & 'b'- no significant' when compared with normal control group-I;
c1 P<0.001,c2 P<0.01 & c3 P<0.05 & 'c'- no significant when compare to MK-300 (group-IV), using one way ANOVA followed by Tukey-Kramer multiple comparison test.

Effect of *Murraya koenigii* aqueous extract on Plasma serum Total Cholesterol, Triglycerides, HDL-c and LDL-C levels (mg/dl) in diabetic hyperlipidemic rats:

In this study data observation on 18th & 24th day, diabetic hyperlipidemic rats treated with test drug *Murraya koenigii* aqueous extract (oral administration of variable dosage level MK-200mg/kg, MK-300mg/kg and MK-400mg/kg) and standard drug Pravastatin 10 mg/kg showed significant ($p<0.001$) decreasing plasma serum total cholesterol, Triglycerides, LDL-c levels and significant ($p<0.0010$ increasing HDL-c levels when compared to the diabetic hyperlipidemic control group rats. Animals treated with standard drug Glibenclamide 0.6 mg/kg showed no significant ($p>0.05$) difference in plasma serum total cholesterol, Triglycerides, HDL-c and LDL-c levels when compared to the diabetic hyperlipidemic control group rats. In this study very significant ($p<0.001$) result was found with MK-300mg/kg dose level because the effect was dose dependent up to MK-300mg equivalent of extract and there was no significant ($p>0.05$) difference in plasma serum total cholesterol, Triglycerides, HDL-c, and LDL-c level of animal treated with MK-400 mg/kg when compared with animal treated with MK-300 mg/kg. Diabetic hyperlipidemic control group showed significant ($p<0.001$) increasing in plasma serum total cholesterol, Triglycerides, LDL-c level and significant ($p<0.001$) decreasing HDL-c when compare to the normal control group rats and diabetic hyperlipidemic animals treated with MK-300, MK-400 & Pravastatin 10 mg/kg groups showed no significant ($p<0.001$) difference in plasma serum Total cholesterol, Triglycerides, HDL-c, and LDL-c levels when compare to the normal control group rats and diabetic hyperlipidemic animals treated with MK-200, and Glibenclamide 0.6 mg/kg showed significant ($p<0.001$) increasing in plasma serum Total cholesterol, Triglycerides, LDL-c level and significant ($p<0.001$) decreasing HDL-c level when compare to the normal control group rats. (**Tables: 2-5**).

Table 2: Effect of *Murraya koenigii* aqueous extract on plasma serum Total Cholesterol level (mg/dl) in diabetic hyperlipidemic rats.

Treatment	Total Cholesterol (TC) Level mg/dl				
	Day-0	Day-6	Day-12	Day-18	Day-24
Normal control	104.82±10.27	105.60±8.49	108.68±11.39	107.63±9.01^{a1}	106.47±11.10^{a1}
DH control	215.63±9.01	221.33±11.44	226.60±12.34	230.75±11.30	234.12±12.75
MK-200mg/kg	213.68±11.39	198.87±11.04	186.10±13.43	164.55±11.15^{a1,b1}	155.63±12.99^{a1,b1}
MK-300mg/kg	218.95±11.21	182.63±12.13	157.52±10.58	126.83±12.46^{a1,b}	118.65±8.90^{a1,b}
MK-400mg/kg	217.35±8.13	175.70±10.42	148.43±12.37	122.27±13.97^{a1,b,c}	115.68±11.12^{a1,b,c}
Glibenclamide 0.6mg/kg	212.88±7.60	215.02±11.97	217.97±9.61	219.57±12.15^{a,b1}	220.77±11.96^{a,b1}
Pravastatin 10mg/kg	220.55±11.29	167.93±12.24	143.60±13.56	120.98±10.62^{a1,b}	112.45±10.96^{a1,b}

Values are expressed as Mean ± SD; n=6,
a1 $P<0.001$,a2 $P<0.01$ & a3 $P<0.05$ & 'a'- no significant' when compared with Diabetic hyperlidemic (DH) control group-II;
b1 $P<0.001$,b2 $P<0.01$ & b3 $P<0.05$ & 'b'- no significant' when compared with normal control group-I;
c1 $P<0.001$,c2 $P<0.01$ & c3 $P<0.05$ & 'c'- no significant when compare to MK-300 (group-IV), using one way ANOVA followed by Tukey-Kramer multiple comparison test

Table 3: Effect of *Murraya koenigii* aqueous extract on plasma serum Triglycerides level (mg/dl) in diabetic hyperlipidemic rats

Treatment	Triglycerides (TG) Level mg/dl				
	Day-0	Day-6	Day-12	Day-18	Day-24
Normal control	108.60±11.07	110.23±7.76	117.87±10.57	111.50±10.71^{a1}	113.95±7.74^{a1}
DH control	220.83±10.00	224.13±11.78	227.86±10.54	229.78±11.96	231.95±9.87
MK-200mg/kg	219.05±9.63	204.72±10.48	193.62±12.82	172.15±10.44^{a1,b1}	164.38±12.08^{a1,b1}
MK-300mg/kg	121.55±8.00	182.60±12.02	154.25±12.26	125.73±12.85^{a1,b}	118.45±11.51^{a1,b}
MK-400mg/kg	216.25±9.21	175.20±12.23	149.50±10.39	121.86±10.54^{a1,b,c}	115.52±12.96^{a1,b,c}
Glibenclamide 0.6mg/kg	218.50±11.77	222.63±11.99	224.88±14.06	225.73±12.91^{a,b1}	226.42±11.30^{a,b1}
Pravastatin 10mg/kg	223.87±10.43	172.47±10.90	148.70±12.21	125.23±11.17^{a1,b}	120.20±12.78^{a1,b}

Values are expressed as Mean ± SD; n=6,
a1 P<0.001,a2 P<0.01 & a3 P<0.05 & 'a'- no significant' when compared with Diabetic hyperlidemic (DH) control group-II;
b1 P<0.001,b2 P<0.01 & b3 P<0.05 & 'b'- no significant' when compared with normal control group-I;
c1 P<0.001,c2 P<0.01 & c3 P<0.05 & 'c'- no significant when compare to MK-300 (group-IV), using one way ANOVA followed by Tukey-Kramer multiple comparison test

Table 4: Effect of *Murraya koenigii* aqueous extract on plasma serum HDL-c level (mg/dl) in diabetic hyperlipidemic rats.

Treatment	HDL-c Level mg/dl				
	Day-0	Day-6	Day-12	Day-18	Day-24
Normal control	52.82±5.31	50.65±5.33	49.70±4.62	55.18±5.07^{a1}	53.16±5.65^{a1}
DH control	26.35±4.35	23.23±4.68	21.20±5.45	19.12±5.09	18.47±4.90
MK-200mg/kg	25.85±5.76	30.05±5.53	34.98±4.99	36.95±3.94^{a1,b1}	38.18±5.68^{a1,b1}
MK-300mg/kg	22.72±5.48	33.12±5.63	40.35±4.78	46.48±5.43^{a1,b}	51.18±5.79^{a1,b}
MK-400mg/kg	23.53±5.12	36.50±4.64	43.80±5.39	50.48±4.58^{a1,b,c}	55.53±5.15^{a1,b,c}
Glibenclamide 0.6mg/kg	25.07±5.23	24.52±4.30	23.62±4.54	22.85±4.61^{a,b1}	22.02±4.79^{a,b1}
Pravastatin 10mg/kg	24.60±5.49	34.52±5.46	42.60±4.97	48.46±5.79^{a1,b}	52.55±4.81^{a1,b}

Values are expressed as Mean ± SD; n=6,
a1 P<0.001,a2 P<0.01 & a3 P<0.05 & 'a'- no significant' when compared with Diabetic hyperlidemic (DH) control group-II;
b1 P<0.001,b2 P<0.01 & b3 P<0.05 & 'b'- no significant' when compared with normal control group-I;
c1 P<0.001,c2 P<0.01 & c3 P<0.05 & 'c'- no significant when compare to MK-300 (group-IV), using one way ANOVA followed by Tukey-Kramer multiple comparison test

Table 5: Effect of *Murraya koenigii* aqueous extract on plasma serum LDL-c level (mg/dl) in diabetic hyperlipidemic rats.

Treatment	LDL-c Level mg/dl				
	Day-0	Day-6	Day-12	Day-18	Day-24
Normal control	30.28±7.78	32.90±10.46	35.41±10.77	30.15±5.99a1	30.51±7.93a1
DH control	145.12±10.00	153.27±14.07	159.83±12.42	165.68±10.21	169.26±17.51
MK-200mg/kg	144.02±12.22	127.87±15.64	112.39±17.53	93.17±12.79a1,b1	84.57±11.15a1,b1
MK-300mg/kg	171.92±9.55	112.99±15.59	86.32±9.92	55.20±9.49a1,b2	43.78±10.26a1,b
MK-400mg/kg	150.57±12.27	104.16±10.97	74.73±11.82	47.41±14.69a1,b,c	37.05±14.48a1,b,c
Glibenclamide 0.6mg/kg	144.12±10.63	145.97±11.00	149.37±11.41	151.57±12.64a,b1	153.47±15.21a,b1
Pravastatin 10mg/kg	151.18±12.00	98.92±9.23	71.26±6.94	47.47±12.42a1,b	35.86±15.03a1,b

Values are expressed as Mean ± SD; n=6,
a1 P<0.001,a2 P<0.01 & a3 P<0.05 & 'a'- no significant' when compared with Diabetic hyperlidemic (DH) control group-II;
b1 P<0.001,b2 P<0.01 & b3 P<0.05 & 'b'- no significant' when compared with normal control group-I;
c1 P<0.001,c2 P<0.01 & c3 P<0.05 & 'c'- no significant when compare to MK-300 (group-IV), using one way ANOVA followed by Tukey-Kramer multiple comparison test

These results suggest that the *Murraya koenigii* aqueous extract & Pravastatin produced the anti-hyperlipidemic activity in diabetic hyperlipidemic rats and Glibenclamide did not shown anti-hyperlipidemic activity to reduce the lipid levels in diabetic hyperlipidemic rats and diabetic hyperlipidemic control group showed hyperlipidemic as compare to normal control group rats.

Effect of *Murraya koenigii* aqueous extract on epithelization period and wound contraction 50% in excision wound model:

On Epithelization period (days):

In the Excision wound model, animals treated with *Murraya koenigii* aqueous extract (oral administration of variable dosage level MK-200mg/kg, MK-300mg/kg and MK-400mg/kg and theirs mean epithelization periods (days) are 20.83±1.07, 17.17±0.69 and 16.67±1.11 respectively) showed significant (p<0.01) decreasing the epithelization period of the excision wound when compared to the diabetic hyperlipidemic control group (mean epithelization period 23.33±1.25) rats. Animals treated with standard drugs Pravastatin 10mg/kg and Glibenclamide 0.6mg/kg (theirs mean epithelization periods are 20.83±1.07 and 21.17±0.90 respectively) showed significant (p<0.01, and p<0.05 respectively) decreasing the epithelization period of the excision wound when compared to the diabetic hyperlipidemic control group rats. In this study very significant(p<0.001) result was found with MK-300mg/kg dose level because the effect was dose dependent up to MK-300mg equivalent of extract and there was no significant (p>0.05) decreasing the epithelization period of the excision wound on animal treated with MK-400 mg/kg when compared with animal treated with MK-300 mg/kg. Diabetic hyperlipidemic control group showed significant (p<0.001) increasing in epithelization period of the excision wound when compare to the normal control group (mean epithelization period 20.5±0.96) rats and animals treated with MK-300 & MK-400 mg/kg groups showed significant (p<0.001) decreasing in the epithelization period of the excision wound when compare to the normal control group rats and animals treated with MK-200, Pravastatin 10mg/kg and Glibenclamide 0.6mg/kg showed no significant (p>0.05) difference in epithelization period of excision wound when compare to the normal control group rats.(**Table:6**)

Table 6: Effect of *Murraya koenigii* aqueous extract on 50%-wound contraction period and Epithelization period of excision wound

Animal Group	Treatment	50%-wound contraction (days)	Epithelization period (days)
Group-I	Normal control	11.13±0.47^{a1}	20.5±0.96^{a1}
Group-II	DH control	13.05±0.61	23.33±1.25
Group-III	MK-200mg/kg	10.95±0.40^{a1,b}	20.83±1.07^{a2,b}
Group-IV	MK-300mg/kg	9.08±0.32^{a1,b1}	17.17±0.69^{a1,b1}
Group-V	MK-400mg/kg	8.92±0.23^{a1,b1,c}	16.67±1.11^{a1,b1,c}
Group-VI	Glibenclamide 0.6mg/kg	12.17±0.40^{a2,b2}	21.17±0.90^{a3,b}
Group-VII	Pravastatin 10mg/kg	11.03±0.37^{a1,b}	20.83±1.07^{a2,b}

Values are expressed as Mean ± SD; n=6,
a1 P<0.001,a2 P<0.01 & a3 P<0.05 & 'a'- no significant' when compared with Diabetic hyperlidemic (DH) control group-II;
b1 P<0.001,b2 P<0.01 & b3 P<0.05 & 'b'- no significant' when compared with control group-I;
c1 P<0.001,c2 P<0.01 & c3 P<0.05 & 'c'- no significant when compare to MK-300 (group-IV), using one way ANOVA followed by Tukey-Kramer multiple comparison test

On 50%-Wound contraction period (days):

In the Excision wound model, animals treated with *Murraya koenigii* aqueous extract (oral administration of variable dosage level MK-200mg/kg, MK-300mg/kg and MK-400mg/kg and theirs mean 50%-wound contraction period (days) are 10.95±0.40, 9.08±0.32 and 8.92±0.23 respectively) showed significant (p<0.01) decreasing the 50%-wound contraction period of the excision wound when compared to the diabetic hyperlipidemic control group (mean 50%-wound contraction period 13.05±0.61) rats. Animals treated with standard drugs Pravastatin 10mg/kg and Glibenclamide 0.6mg/kg (theirs mean 50%-wound contraction period are 11.03±0.37 and 12.17±0.40 respectively) showed significant (p<0.001, and p<0.05 respectively) decreasing the 50%-wound contraction period of the excision wound when compared to the diabetic hyperlipidemic control group rats. In this study very significant(p<0.001) result was found with MK-300mg/kg dose level because the effect was dose dependent up to MK-300mg equivalent of extract and there was no significant (p>0.05) decreasing the 50%-wound contraction period of the excision wound on animal treated with MK-400 mg/kg when compared with animal treated with MK-300 mg/kg. Diabetic hyperlipidemic control group showed significant (p<0.001) increasing in 50%-wound contraction period of the excision wound when compare to the normal control group (mean 50%-wound contraction period 20.5±0.96) rats and animals treated with MK-300 & MK-400 mg/kg groups showed significant (p<0.001) decreasing in the 50%-wound contraction period of the excision wound when compare to the normal control group rats and animals treated with MK-200 and Pravastatin 10mg/kg showed no significant (p>0.05) difference in the 50%-wound contraction period of excision wound when compare to the normal control group rats and Glibenclamide 0.6mg/kg showed significant (p<0.01 respectively) increasing the 50%-wound contraction period of excision wound when compare to the normal control group rats.(**Table:6**)

These results suggest that the *Murraya koenigii* aqueous extract produced the excision wound healing activity in diabetic hyperlipidemic rats and Pravastatin showed more excision wound healing activity in diabetic hyperlipidemic rats as compare to Glibenclamide and diabetic hyperlipidemic control group showed delay in excision wound healing activity as compare to normal control group rats.

DISCUSSION & CONCLUSION

The present study was undertaken to evaluate whether *Murraya koenigii* leaves aqueous extract promote wound healing in experimentally induced wounds in diabetic hyperlipidemic albino Wistar rats. The results of the present study substantiate the use of *Murraya koenigii* leaves aqueous extract in folklore medicine for the treatment of wounds in diabetic hyperlipidemic condition.

The acute toxicity study was done by **"fixed dose"** method according "OECD guidelines 420. The extract in doses up to 2000mg did not produce any signs of toxicity and mortality. As no mortality was recorded within 24 hours during the acute toxicity test, LD50 could not be calculated. The non – toxic effect of the aqueous leaf extract of *Murraya koenigii* lend support to the widespread use of the plant as a spice for food flavoring.

During blood parameter study we found that *Murraya koenigii* aqueous extract & Glibenclamide produced the anti-hyperglycemic (decrease fasting blood glucose level) activity in diabetic hyperlipidemic rats and Pravastatin did not shown anti-hyperglycemic activity to reduce the blood glucose level in diabetic hyperlipidemic rats and diabetic hyperlipidemic control group showed hyperglycemic as compare to normal control group rats and *Murraya koenigii* aqueous extract & Pravastatin produced the anti-hyperlipidemic activity in diabetic hyperlipidemic rats and Glibenclamide did not shown anti-hyperlipidemic activity to reduce the lipid levels in diabetic hyperlipidemic rats and diabetic hyperlipidemic control group showed hyperlipidemic as compare to normal control group rats.

Murraya koenigii was found to be more potent for its hypolipidemic activity as compare to hypoglycemic activity because the *Murraya koenigii* showed no significant difference for its hypoglycemic activity (decreasing fasting blood glucose level) at dose level 400mg/kg in diabetic hyperlipidemic rats when compared to the normal control group rats and in the case of its hypolipidemic activity (decreasing lipid level) it showed no significant difference at dose level 300 mg/kg only.

In the excision wound model the epithelization & 50%-wound contraction period (days) of the excision wound was significantly increased in diabetic hyperlipidemic rats (DHR) when compared with the normal control. This confirms the fact that the wound healing is delayed in diabetic complication.

Murraya koenigii (MK-300) aqueous extract showed very significant ($p<0.001$) decreasing the epithelization period (17.17±0.69) and 50%-wound contraction period (9.08±0.32) of the excision wound in diabetic hyperlipidemic rats when compared to the normal control group (epithelization period 20.5±0.96 & 50%-wound contraction period 11.13±0.47 respectively) and diabetic hyperlipidemic control group (epithelization period 23.33±1.25 & 50%-wound contraction 13.05±0.61 respectively) rats. Pravastatin showed slightly more decreasing the epithelization period (20.83±1.07) and 50%-wound contraction period (11.03±0.37) of the excision wound in diabetic hyperlipidemic rats as compare to Glibenclamide (epithelization period 21.17±0.90 & 50%-wound contraction period 12.17±0.40 respectively). In this study very significant ($p<0.001$) result was found with MK-300mg/kg dose level because the effect was dose dependent up to MK-300mg equivalent of extract and there was no significant ($p>0.05$) decreasing the epithelization period & 50%-wound contraction period of the excision wound of animal treated with MK-400 mg/kg when compared with animal treated with MK-300 mg/kg.

In case of epithelization period data indicate that oxidative stress is an early event in the evolution of hyperlipidemia, and appropriate support for enhancing antioxidant supply in higher lipid subjects may help prevent the course of the disease. This oxidative stress may cause damage to the growing tissue (collagen and epithelium) at the repair site. The groups treated with *Murraya koenigii* and Pravastatin showed much more significant effect than Glibenclamide treated groups. This result clearly indicates that

the hypolipidemic property of *Murraya koenigii* and Pravastatin may promote the epithelization.

In case of Wound contraction-50% migration of fibroblasts into and through the extracellular matrix during the initial phase of wound healing appears to be a fundamental component of wound contraction. Migration of fibroblasts to the site of injury and their subsequent proliferation are triggered by a number of growth factors, including TGF, PDGF, EGF, FGF, and the so called fibrogenic cytokines, interleukin-a (IL-1), and TNF-a. the growth factors involved in inflammatory fibrosis. Delay in diabetic wound healing is due to interruption of cytokine release from macrophages, which is due to diabetic hyperlipidemic condition. This indicates that the agents, which have hypolipidemic nature, will increase the rate of wound contraction. *Murraya koenigii* and Pravastatin showed a very significant increase in the rate of wound contraction, which indicates due to its hypolipidemic nature. The researchers showed that the combination of transforming growth factor–β1 and fibroblast growth factor had marked positive effects on biochemical parameters of wound healing and reversed the tensile strength deficit of diabetic wounds [26]. Low dose of streptozotocin injection caused diabetes mellitus type-II, probably due to partial destruction of the β-cells of the Islets of Langerhans of the pancreas [27]. This results in over-production of glucose and decreased utilization by the tissues, forming the basis of hyperglycemia in diabetes mellitus [28]. The delay in diabetic wound healing, is due to interruption of cytokine release from macrophages, which may be due to the fundamental diabetic-hyperlipidemic condition. The preliminary phytochemical screening of extract of *Murraya koenigii* shows presence of mucilage, proteins, sterols and Triterpenoids, alkaloids, tannins, flavonoids, phenolic compounds. This wound healing activity of the extract observed might be due to the presence of phytochemicals present in the plant extract. Tannins [29] and triterpenoids [30] are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation. Importantly, it was clearly observed that the aqueous extract of *Murraya koenigii*, possessed a definite prohealing action in normal healing in diabetic condition, observed by a significant increase in the rate of wound contraction, breaking strength and epithelization period,which may be due to a increase in collagen concentration and stabilization of fibers. This indicates that the antioxidant property[14] of leaves of *Murraya koenigii* may promote epithelization by controlling oxidative stress and its hypolipidemic nature, will increase the rate of wound contraction.

CONCLUSION

In conclusion, these preliminary investigation and data obtained from this study demonstrated that, the prohealing mechanism on diabetic wound is only because of the more hypolipidemic property as compare to hypoglycemic property of *Murraya koenigii* in high fat diet and streptozotocin treated type-2 diabetic rats. However, identification and elucidation of the active constituents in this plant may provide useful leads to the development of new and effective drugs for improved wounds healing in diabetic conditions.

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