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Wound Healing Activity of *Murraya Koenigii* in High Fat Diet And Streptozotocin Treated Type-2 Diabetic Rats

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ABSTRACT

Murraya koenigii (Curry leaf tree) is a spicy traditional Indian medicinal plant native to Indo-China but grown mostly in the tropics for the medicinal and flavourant properties of the leaves. Wound healing activity of aqueous extract of leaves of *Murraya koenigii* was studied by excision and incision wound model in high fat diet and streptozotocin treated type-2 diabetic rats (diabetic in hyperlipidemic rats). **In the excision** wound models, animals treated with *Murraya koenigii* (oral administration of variable dosage level 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 the Incision** wound models, animals treated with *Murraya koenigii* (oral administration of variable dosage level 200mg/kg, 300mg/kg and 400mg/kg) leaves aqueous extract showed significant increasing the breaking strength of the wound when compared to the diabetic hyperlipidemic control group rats. In both excision and incision 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 results suggested that aqueous extract of *Murraya koenigii* possess significant wound healing potential in diabetic hyperlipidemic rats. Further studies may reveal the exact mechanisms of action responsible for the wound healing activity of *Murraya koenigii* leaves aqueous extract in diabetic hyperlipidemic condition.

KEYWORDS: *Murraya koenigii* (MK), Incision wound, Excision wound, Ciprofloxacin, STZ-Streptozotocin, DH-Diabetic hyperlipidemic.

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INTRODUCTION:

Natural products are a source of synthetic and traditional herbal medicine and are still the primary health care system¹. The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infective diseases and management of chronic wounds. Normal wound healing response begins the moment the tissue is injured. The healing cascade begins immediately following injury when the platelets come into contact with exposed collagen. As platelet aggregation proceeds, clotting factors are released resulting in the deposition of a fibrin clot at the site of injury. The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing². The inflammatory cells also arrive along with the platelets at the site of injury and they provide key signals are known as cytokines or growth factors³. The fibroblast is the connective tissue cell responsible for collagen deposition that is needed to repair the tissue injury. Collagen is the most abundant protein in the animal kingdom, accounting for 30% of the total protein in the human body⁴. In normal tissues collagen provides strength, integrity and structure. When tissues are disrupted following injury, collagen is needed to repair the defect and restore anatomic structure and function.

Diabetic wounds are slow, non-healing wounds that can last for weeks despite adequate and appropriate care. Such wounds are difficult and frustrating to manage.⁵ Diabetic wound healing is an enigmatic and debilitating complication and poses a serious challenge in clinical practice. The exact pathogenesis of the poor wound healing with the diabetic wound is not clearly understood, but evidence from studies involving both human and animal models reveal several abnormalities in the various phases of wound healing process.^{6,7}

Murraya koenigii, belonging to the family Rutaceae, commonly known as curry-leaf tree, is a native of India, Srilanka and other south Asian countries. Leaves are rich in minerals, vitamin A, vitamin B, and are a rich source of carbohydrates, proteins, amino acids and alkaloids.^{8,9}

The plant has also been used in traditional Indian medicine systems for a variety of ailments.^{10, 11} It was found that reduction in total serum cholesterol and an increase in the HDL and lower release of lipoproteins into the circulation take place when rats were fed with a standard diet along with curry leaves.¹² 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.¹³ Hypoglycemic & lipid lowering activity of *Murraya koenigii* on normal and diabetic rats was found.¹⁴⁻¹⁶ Significant wound healing property is also reported in normal rats.¹⁷ There is no scientific report on wound healing effect of *Murraya koenigii* leaves extract on diabetic hyperlipidemic rats. The present study has been

undertaken to examine the wound healing potential of *Murraya koenigii* leaves aqueous extract in diabetic hyperlipidemic wistar rats.

MATERIAL AND METHODS:

Freshly collected leaves of *Murraya koenigii* from medicinal garden of Sri Balaji College of Pharmacy, Jaipur, after authentication 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.¹⁵

PRELIMINARY PHYTOCHEMICAL STUDIES:

The extract was then subjected to qualitative phytochemical screening for the identification of the phytoconstituents.¹⁸

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.”¹⁹

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 (Letter No. SBCEP/IAEC/10-464). 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 (Reg. No. 1212/ac/08/CPCSEA).

INDUCTION OF DIABETIC HYPERLIPIDEMIA (DH):

(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.²⁰

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.²¹

The body weight and biochemical estimations (plasma glucose (PGL), triglycerides (PTG), total cholesterol (PTC), and LDL-c & HDL-c) 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 feed and water intake of the animals were also measured. The rats were allowed to continue to feed on their respective diets until the end of the study.

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.

EXPERIMENTAL DESIGN FOR WOUND HEALING STUDY

WOUND MODELS: 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 Ciprofloxacin 10mg/kg

Wistar rats were divided into six 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 mm². 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.²²⁻²³

INCISION 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 Ciprofloxacin 10mg/kg

Wistar rats were divided into six groups as above, each containing six animals and anesthetized with 1 ml of intravenous ketamine hydrochloride (10 mg/kg body weight) and shaved on para vertebral side of the back with an electric clipper. Para vertebral straight incisions of 6 cm length each were made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete haemostasis the wound were closed by means of interrupted sutures placed at equidistance points about 1 cm apart. Animals were treated daily with drugs, from 0 day to 9th post-wounding day the wound breaking strength were estimated on 10th day of wounding by continuous, constant water flow technique. Allis forceps were firmly applied on either side of incision wound 3 mm away from wound margin on adjacent normal skin. The forceps on one side was hooked to a fixed metal rod while the other forcep was attached to a thread suspended by weights running over a pulley. As soon as gapping of the wound occurred, addition of weights was stopped and simultaneously the weights were lifted so as to avoid opening of the entire wound. The weights required to produce gapping were noted.²⁴⁻²⁵

STATISTICAL ANALYSIS:

Results were expressed as Mean \pm Standard Deviation (SD). The data was statistically analyzed using the one-way ANOVA followed by Tukey-Kramer multiple comparison test to determine whether

results in a particular group were significantly different from those in the corresponding control groups. Results were statistically significant when P values are less than 0.05 ($P < 0.05$).

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.

In the excision wound models, animals treated with test drug *Murraya koenigii* (oral administration of variable dosage level 200mg/kg, 300mg/kg and 400mg/kg) leaves aqueous extract showed significant ($P < 0.01$) reduction in period of epithelisation and wound contraction 50% when compared to the diabetic hyperlipidemic control group rats and standard Ciprofloxacin (10mg/kg) treated group showed significant ($P < 0.05$) reduction in period of epithelization but not wound contraction 50% when compared to the diabetic hyperlipidemic control group rats.

The period of epithelisation and wound contraction 50% of the wound was significantly ($P < 0.001$) increased in diabetic hyperlipidemic group rats when compared with the normal control (Table 1 & Figure 1).

In the Incision wound models, animals treated with *Murraya koenigii* (single dose, oral administration of variable dosage level 200mg/kg, 300mg/kg and 400mg/kg) leaves aqueous extract and standard Ciprofloxacin (10mg/kg) showed significant ($P < 0.05$) increasing the breaking strength of the wound when compared to the diabetic hyperlipidemic control group rats.

The breaking strength of the wound was significantly ($P < 0.001$) decreased in diabetic hyperlipidemic group rats when compared with the normal control. (Table 2 & Figure 2).

In both excision and incision wound model very significant ($p < 0.001$) result was found with 300mg/kg dose level because there was no significant difference in 300 & 400 mg/kg so the effect was dose dependent up to 300mg equivalent of extract.

Table 1: Effect of *Murraya koenigii* on period of epithelization and wound contraction 50 % (days) in Excision wound model.

Group/Pamameter studied	Epithelization period (days)	Wound contraction 50% (days)
Group-I(Normal control)	20.5±0.96***	11.13±0.47***
Group-II (DH control)	23.33±1.25	13.05±0.61
Group-III (MK-200mg/kg)	20.83±1.07**	10.95±0.40***
Group-IV (MK-300mg/kg)	17.17±0.69***	9.08±0.32***
Group-V (MK-400mg/kg)	16.67±1.11***.b	8.92±0.23***.b
Group-VI (Ciprofloxacin 10mg/kg)	21.33±0.94*	12.28±0.42 ^a

Values are expressed as Mean ± SD; n=6,

***P<0.001, **P<0.01 & *P<0.05 & ‘a’- no significant’ when compared with Diabetic hyperlipidemic (DH) control group; ‘b’- no significant when compare to MK-300 (group-IV), using one way ANOVA followed by Tukey-Kramer multiple comparison test.

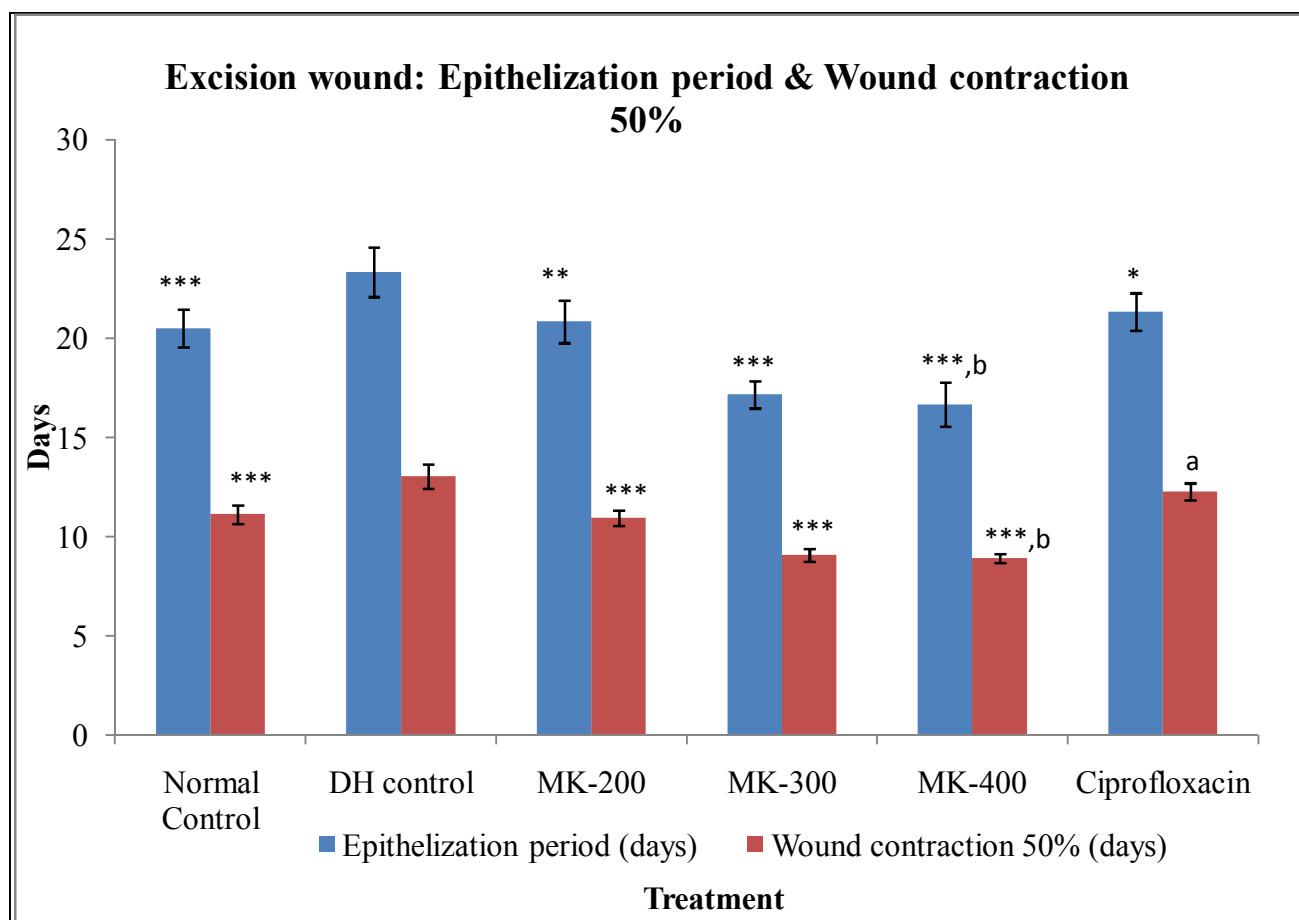


Figure 1: Effect of *Murraya koenigii* on period of epithelization and wound contraction 50%(days) in Excision wound model.

Table 2: Effect of *Murraya koenigii* on Breaking strength in Incision wound model.

Group/Parameter studied	Breaking strength(gm)
Group-I(Normal control)	202.83±13.37***
Group-II (DH control)	164.50±10.72
Group-III (MK-200mg/kg)	198.50.5±12.79**
Group-IV (MK-300mg/kg)	192.67±5.15.96***
Group-V (MK-400mg/kg)	310.67±13.24***,b
Group-VI (Ciprofloxacin 10mg/kg)	189.67±12.91*

Values are expressed as Mean ± SD; n=6,

***P<0.001, **P<0.01 & *P<0.05, compared with Diabetic hyperlipidemic (DH) control group; 'b'- no significant when compare to MK-300 (group-IV), using one way ANOVA followed by Tukey-Kramer multiple comparison test.

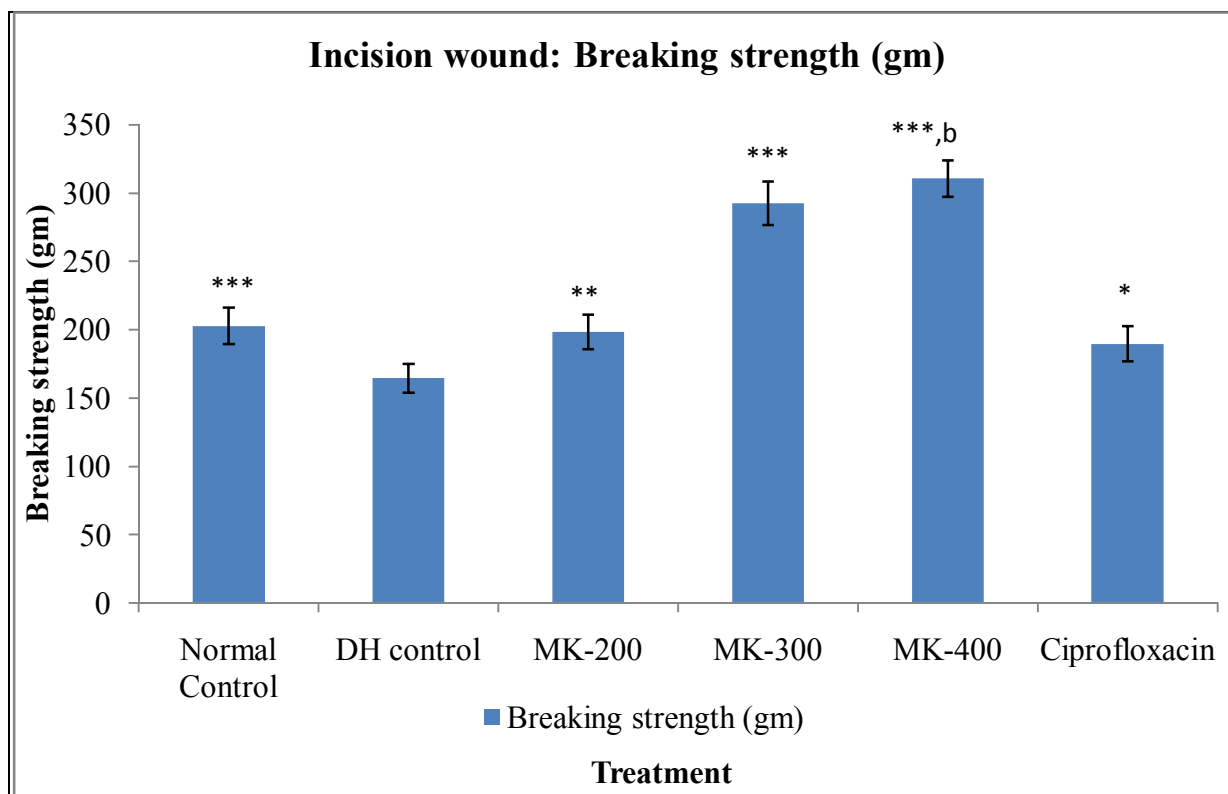


Figure 2: Effect of *Murraya koenigii* on Breaking strength in Incision wound model.

DISCUSSION & CONCLUSION:

In this study, the aqueous extract of leaves of *Murraya koenigii* was screened for wound healing activity on diabetic in hyperlipidemic albino wistar rats.

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.

Murraya koenigii extract variable dosage level and standard drug Ciprofloxacin (except in wound contraction 50%) show significant ($p<0.05$) wound healing activity in excision and incision wound model when compared to the diabetic hyperlipidemic control group rats. The 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.

Normal wound healing occurs in three stages: inflammation, proliferation and remodeling. The wound healing process depends on a given provision of local circulation, as well as the formation and deposition of collagen. The wound healing is impaired in the diabetic state because of, at least in part, low growth factors. Wound healing deficits in diabetes are diverse, multifactorial, complex and inter related²⁶ and are believed to be caused by impaired blood flow and oxygen release from increased blood sugar, decreased collagen and fibronectin synthesis from protein malnutrition, impaired local immune and cell defenses and decreased anabolic activity with decreased insulin and growth hormone. Collagen, fibrin and keratin accumulate advanced glycation Amadori end products which affect binding of regulatory molecules, susceptibility to proteolysis and decrease the ability for protein cross-linkage.²⁷ Di Girolamo et al postulated that defects in wound healing are caused by the hyperglycosylation of the locally synthesised cellular fibronectin.²⁸ Hyperglycaemia affects the whole range of neutrophil functions, including migration, chemotaxis, adherence and phagocytic and bactericidal activity.²⁹

We have demonstrated wound healing properties of *Murraya koenigii* application in streptozotocin induced diabetic and high cholesterol diet induced hyperlipidemia in rats.

The breaking strength of the wound was significantly decreased in diabetic hyperlipidemic rats (DHR) when compared with the normal control. The DHR took more number of days for epithelization and wound contraction when compared with normal control group rats.

The researchers showed that the combination of transforming growth factor- β 1 and fibroblast growth factor had marked positive effects on biochemical parameters of wound healing and reversed the tensile strength deficit of diabetic wounds.³⁰

Low dose of streptozotocin injection caused diabetes mellitus type-II, probably due to partial destruction of the cells of the Islets of Langerhans of the pancreas.³¹ This results in over-production of glucose and decreased utilization by the tissues, forming the basis of hyperglycemia in diabetes mellitus.³² 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.

Tannins³³ and triterpenoids³⁴ 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 property¹³ of leaves of *Murraya koenigii* may promote epithelization by controlling oxidative stress and its hypolipidemic nature, will increase the rate of wound contraction.

In conclusion, these preliminary investigations and data obtained from this study demonstrated that effect of herbal plant *Murraya koenigii* leaves aqueous extract have good wound healing activity in high fat diet and streptozotocin treated type-2 diabetic rats (diabetic in hyperlipidemic rats).

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