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Evaluation of Pharmacological Effect of Curry leaves on Streptozotocin Induced Diabetic Foot Ulcer in Albino Wistar Rats

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Abstract:

Diabetic Foot Ulcer is a chronic disease associated with Diabetes Mellitus or hyperglycemia. Globally around 35% of Diabetic patient are at a risk of developing DFU, out of which 50% are at high risk of reoccurrence of the disease. It is the most prominent reason for amputation of lower limb, high rate of hospitalization and mortality in diabetic patient. It is increasing burden on health economy and lifestyle of diabetic person. The current research focuses on effect of curry leaves (*Murraya koenigii*), a widely used plant in Indian culinary on Streptozotocin induced diabetic foot ulcer in Albino Wistar Rats. Ethanolic and Hydroalcoholic extract of curry leaves are prepared and given orally for 21 days of study period. The histopathological and other laboratory analysis evidence shows that the hydroalcoholic extract of curry leaf enhance diabetic foot ulcer healing. Hence the study concluded that curry leaves has therapeutic potential to be used in the treatment of Diabetic Foot Ulcer.

Keywords- Diabetes Mellitus, Neuropathy, Curry Leaves, Amputation

Introduction:

Chronic diseases are long term conditions that progress slowly. Efforts can be done to manage such diseases but these diseases are often not cured. One such disease is diabetes mellitus. It is one of the major endocrine disorders which occur due to insufficiency, inability of pancreatic islets to secrete insulin hormone or insulin resistance in the body [1]. The disease generally characterized by hyperglycemia i.e. high rise in blood sugar level. Diabetes mellitus gives rise to various complications including- Retinopathy, Neuropathy, Cardiovascular diseases, Amputation etc [2]. These underlying complications can cause diabetic foot ulcer or DFU which is the major cause of economical burden, disability, hospitalization and mortality in diabetic patient [3]. According to World Health Organization, DFU is defined as the development of wound on the foot area of diabetic patient that is caused because of poor circulation of blood and nerve damage in diabetic patient Fig.1 [4]. These wounds are complicated to heal, course of treatment is complex & long term and the wounds often gets unnoticed due to sensory loss in diabetic patient, all of these factors makes the treatment burdensome and costly [5]. To make DFU therapy easy and economical researcher are investigating herbs and herbal formulation for treatment. Various herbs found to be beneficial in managing DFU which include- Neem, Ginger, and Turmeric etc [6, 7, and 8].

The current study focuses on curry leaves, a well known plant in every Indian household with various health benefits like wound healing, anti-inflammatory, hypoglycemic, antimicrobial etc [9, 10, 11 and 12]. The literature survey on the phytoconstituents present in curry leaves provides a solid foundation for preclinical investigation to assess its pharmacological effect on DFU.

Fig. 1 Diabetic Foot Ulcer [4]

Authentication- The Curry leaves (*Murraya koenigii*) are collected from Suddowala region of Dehradun, India 248015 in the month of October. A herbarium was prepared and authenticated by Botanical Survey of India Kaulagarh Road, Dehradun Acc No- 1521.

Fig.2 Curry Leaves Plant

Taxonomical Classification [13]:

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliospida
Order	Sapindales
Family	Rutaceae
Genus	*Murraya*
Species	*Koenigii*
Vernacular Name [14 and 15]	Karii patta in Hindi, Barsunga in Bengali, Hoja in Spanish, Curryblatter in German

Phytochemicals Present in Curry Leaves- Alkaloids, Glycosides, Terpenoids, Steroids, Essential Oils etc [16 and 17].

Materials and Methods:

Preparation of extract: Curry leaves are shade dried and coarsely powdered in Bajaj Mixer Grinder of Model No-GX 16 (410533)

Fig.2: Preparation of Curry leaves extract

1. **Preparation of Ethanolic extract -** 50 gram of leaf powder was taken in a beaker with 500ml of ethanol. The leaves powder was covered and macerated for 48 hours. Supernatant liquid was collected and filtered. The solvent was evaporated at room temperature for 2 days. After which it was transferred to a porcelain dish and evaporated over a laboratory water bath at temperature below 40℃ until a solid mass formed [18].
2. **Preparation of Hydroalcoholic extract-** 10 gram of leaves powder was taken in a beaker with 100ml of ethanol and water mixture (5:5). The leaves powder was covered and macerated for 48 hours. Supernatant liquid was collected and filtered. The solvent was evaporated at room temperature for 2 days. After which it was transferred to a porcelain dish and evaporated over a laboratory water bath at temperature below 40℃ until a solid mass formed [19].

Animals - 36 Female Wistar rats are procured from Lala Lajput Rai University of Veterinary and Animal Sciences, Hisar by the approval of Institutional Animal Ethical Committee (Proposal no-SIP/IAEC/PCOL/08/2023). All the animals are quarantine for 1 week with enough water and food supply, 12:12 hour of day and night cycle.

Induction of Diabetes- Body weight of animals was measured and then animals are divided into 6 groups (6 rats in each group)**.** Animals were fasted overnight then a single dose of 70mg/kg dose of STZ prepared in 50mM citrate buffer was introduced in the intraperitoneal cavity of rats from Group 2, Group 3, Group 4, Group 5 and Group 6. After 4 days Blood Glucose Level of each rat was measured using Glucometer. More than 80% of rats were found to have Blood Glucose Level above 200mg/dL [20].

Induction of Foot Ulcer- Rats were anesthetized using 75mg/kg Ketamine and 10mg/kg Xylazine and a rectangular excision wound of size 2×5mm is created on right hind footpad [20].

Animals Group:

Group 1- Normal Control

Group 2- Disease Control (No treatment)

Group 3- Rats received 100mg/kg of Ethanolic curry leaves extract for 21 days [21]

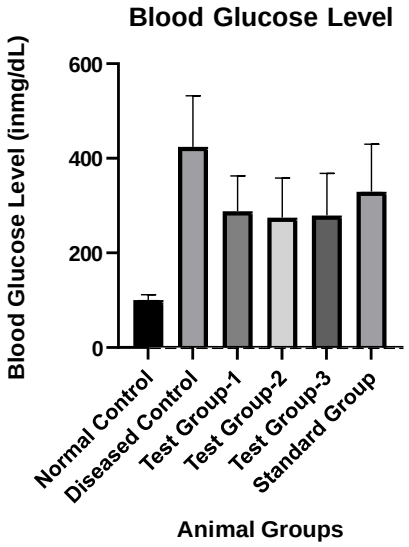
Group 4- Rats received 200mg/kg of Ethanolic curyy leaves extract for 21 days [22]

Group 5- Rats received 100mg/kg of Hydroalcoholic curry leaves extract for 21 days [21]

Group 6- 10% of Povidone Iodine applied to foot for 21 days [23, 24, 25]

Mortality- No mortality occurs in any group during the treatment period.
Inclusion Criteria- Before inducing diabetes using STZ all the rats must have normal BGL 1.e. below 100mg/dl
Exclusion Criteria- Rats with pre-existing skin lesion or ulcers on their feet before study should be excluded. Pregnant female rats, very young and very old rats were also excluded.
Results:

1. **Statistical Analysis-** Analysis of blood glucose level (BGL) and C-reactive protein (CRP) were done with one way ANOVA technique using Graph Pad Prism Software.



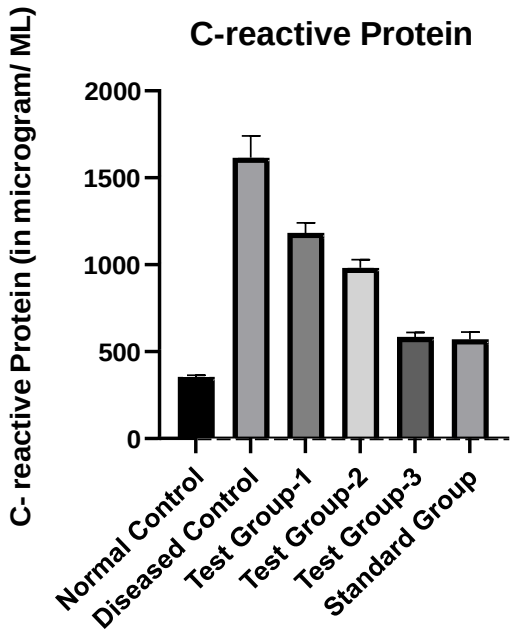
Graph No.1: Action of Curry leaves extract on Blood Glucose Level of Rats

Table No.1: Effect of various Pharmacological interventions on BGL
(Values are taken as Mean ± SEM, N=6), $p < 0.001$

S.No.	Group Name	Body Weight
1	**Normal Control**	100.7±11.02
2	**Disease Control**	424±108.4
3	**Test Group-1** (100mg/kg Curry leaves ethanolic extract)	288.2±74.46
4	**Test Group-2** (200mg/kg Curry leaves ethanolic extract)	274.8±83.24
5	**Test Group-3** (100mg/kg Curry leaves hydroalcoholic extract)	279.3±88.78
6	**Standard Group** (10% Povidone Iodine)	329.7±100.3

Table No. 2: Multiple Comparison of Blood Glucose level in Rats (Day 21)

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Adjusted P Value
Normal Control vs. Diseased Control	-323.3	-470.8 to -175.9	Yes	<.001
Normal Control vs. Test Group-1	-187.5	-334.9 to -40.07	Yes	.007
Normal Control vs. Test Group-2	-174.2	-321.6 to -26.73	Yes	.013
Normal Control vs. Test Group-3	-178.7	-326.1 to -31.23	Yes	.011
Normal Control vs. Standard Group	-229.0	-376.4 to -81.57	Yes	<.001
Diseased Control vs. Test Group-1	135.8	-11.60 to 283.3	No	.085
Diseased Control vs. Test Group-2	149.2	1.734 to 296.6	Yes	.046
Diseased Control vs. Test Group-3	144.7	-2.766 to 292.1	No	.057
Diseased Control vs. Standard Group	94.33	-53.10 to 241.8	No	.395
Test Group-1 vs. Test Group-2	13.33	-134.1 to 160.8	No	>.999
Test Group-1 vs. Test Group-3	8.833	-138.6 to 156.3	No	>.999
Test Group-1 vs. Standard Group	-41.50	-188.9 to 105.9	No	.954
Test Group-2 vs. Test Group-3	-4.500	-151.9 to 142.9	No	>.999
Test Group-2 vs. Standard Group	-54.83	-202.3 to 92.60	No	.864
Test Group-3 vs. Standard Group	-50.33	-197.8 to 97.10	No	.901



Graph No.2: Action of Curry leaves extract on C - reactive protein Level of Rats

Table No. 3: Effect of various Pharmacological interventions on level of C - reactive protein

(Values are taken as Mean ± SEM, N=6), $p < 0.001$

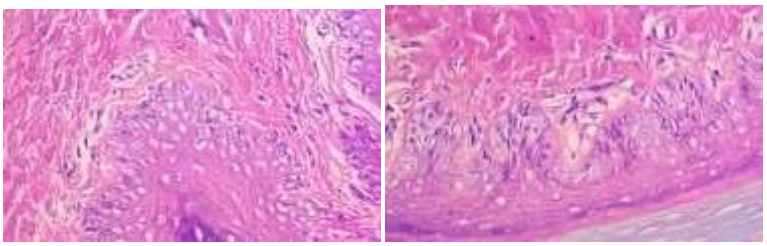
S.No.	Group Name	Body Weight
1	Normal Control	354±8.894
2	Disease Control	1617±124.9
3	Test Group-1	1183±58.83
4	Test Group-2	983±46.23
5	Test Group-3	583.5±27.50
6	Standard Control	571±40.82

Table No. 4: Multiple Comparison of CRP Level in rats

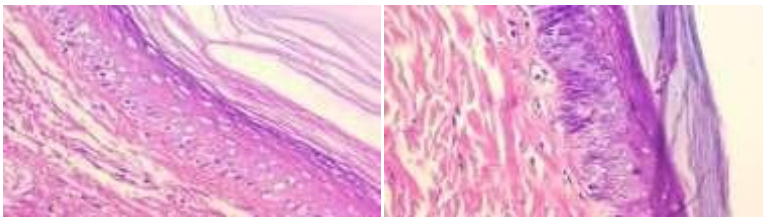
Multiple comparisons test (Tukey's)	Mean Difference	95.00% CI of difference	Below threshold?	Adjusted p value

Normal Control vs. Diseased Control	-1262	-1372 to -1152	Yes	<.001
Normal Control vs. Test Group-1	-828.0	-938.4 to -717.6	Yes	<.001
Normal Control vs. Test Group-2	-628.5	-738.9 to -518.1	Yes	<.001
Normal Control vs. Test Group-3	-229.0	-339.4 to -118.6	Yes	<.001
Normal Control vs. Standard Group	-216.5	-326.9 to -106.1	Yes	<.001
Diseased Control vs. Test Group-1	434.0	323.6 to 544.4	Yes	<.001
Diseased Control vs. Test Group-2	633.5	523.1 to 743.9	Yes	<.001
Diseased Control vs. Test Group-3	1033	922.6 to 1143	Yes	<.001
Diseased Control vs. Standard Group	1046	935.1 to 1156	Yes	<.001
Test Group-1 vs. Test Group-2	199.5	89.13 to 309.9	Yes	<.001
Test Group-1 vs. Test Group-3	599.0	488.6 to 709.4	Yes	<.001
Test Group-1 vs. Standard Group	611.5	501.1 to 721.9	Yes	<.001
Test Group-2 vs. Test Group-3	399.5	289.1 to 509.9	Yes	<.001
Test Group-2 vs. Standard Group	412.0	301.6 to 522.4	Yes	<.001
Test Group-3 vs. Standard Group	12.50	-97.87 to 122.9	No	>.999
Normal Control vs. Diseased Control	-1262	-1372 to -1152	Yes	<.001
Normal Control vs. Test Group-1	-828.0	-938.4 to -717.6	Yes	<.001

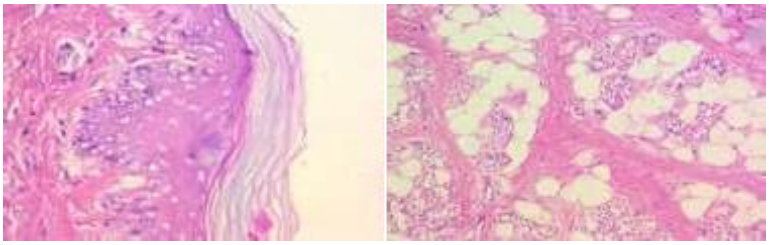
2. **Histopathology-** The ulcer tissues of rats from various groups are collected and histopathology is done.



(Group 1 and Group 2)



(Group 3 and Group 4)



(Group 5 and Group 6)

Fig. 3: Histopathology of Foot Tissue

Conclusion- It is concluded from various observation and laboratory data that curry leaves extract shows potential of healing DFU in rats by decreasing BGL, inflammatory markers and promote fast recovery of wound. On comparison between ethanolic extract and hydroalcoholic extract it was observed that rats receiving 100mg/kg of hydroalcoholic extract shows more significant result. The study opens a new perspective for researchers to evaluate potential benefit of curry leaves to be used in the treatment of DFU.

Future Prospective- Curry Leaves **(***Murrya koenigii***)** plant is widely used in Asian food has enormous health benefits. As the study suggest its diabetic foot ulcer healing potential so more clinical studies needs to be done for proving its pharmacological activity. Cultivation of plant as medicinal crop should be promoted globally to reduce the burden on healthcare system and financial status of patient's suffering with DFU.

Conflict of Interest- We declare that we have no conflict of interest.

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Ethical approval

The animals were procured from Lala Lajput Rai University of Veterinary and Animal Sciences, Hisar India by the approval of Institutional Animal Ethical Committee (Proposal no-SIP/IAEC/PCOL/08/2023).

Abbreviations- DFU- Diabetic Foot Ulcer, STZ- Streptozotocin, BGL- Blood Glucose level, CRP- C reactive protein

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