



Protective Role of *Terminalia chebula* in Streptozotocin-induced Diabetic Mice for Wound Healing Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Author AS designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors RS and AKP managed the analyses of the study and protocol. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diabetes is a chronic disease characterized by high level of glucose in the blood. Wound healing becomes a challenging position to biomedical science when associated with diabetic peoples. It becomes delay in diabetic conditions. *Terminalia chebula* seeds may be a best alternative for the treatment of wound healing & antidiabetic activity.

Objective: The objective of the present study was to evaluate the protective role of seeds of *Terminalia chebula* in Streptozotocin-Induced Diabetic Mice for Wound Healing Activity.

Materials and Methods: The rate of wound contraction and estimation of various biochemical parameters such as superoxide dismutase, lipid peroxidation and nitric oxide levels in the granulation tissue of diabetic and non-diabetic mice were considered. The extract of *Terminalia chebula* with the concentration of 200 mg/kg and 400 mg/kg body weight was induced through intraperitoneal in diabetic and non-diabetic mice.

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Results: The results showed that seeds of *Terminalia chebula* were a potent source of antioxidative phenolic compounds that counteract with reactive oxygen species responsible for delayed wound healing. The seeds of *Terminalia chebula* significantly increased the level of superoxide dismutase, nitric oxide and decreased lipid peroxidation in granuloma tissue of diabetic mice.

Conclusions: The methanolic extract of leaves of *Terminalia chebula* increases the rate of angiogenesis and improves antioxidant enzymes status that eventually leads to faster wound healing in diabetic condition. However, further studies are needed to explore the biomolecules present in the leaves of *Terminalia chebula* that lead to faster wound healing.

Keywords: Antidiabetic; wound healing; herbal medicine; *Terminalia chebula*.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, altered metabolism of lipids, carbohydrate and proteins and on increased risk of complication like delayed wound healing. The administration of plant extracts based on traditional practices since ancient times more than 500 medicinal plants were used for treating the diabetes.

Currently available drugs for the treatment of Diabetes mellitus have a number of limitations, such as adverse effects and a high rate of secondary failure. As there is a growing trend towards using natural remedies adjunct to conventional therapy, traditionally used plants might provide a useful source of new hypoglycemic compounds. A number of plants have been reported to possess hypoglycemic effects and the possible mechanism suggested for such hypoglycemic actions could be through the increased insulin secretion from β -cells of islets of Langerhans [1].

In diabetic conditions, the level of lipid peroxide increased as per the increase in concentration of blood glucose. The increase lipid peroxidation in the hyperglycemic condition may be explained, as the superoxide dismutase enzyme which is antioxidant becomes inactive due to the formation of superoxide radical within the cell. Maximum lipid peroxidation leads to the damage of the tissue and organs which results into complication in diabetic patients. Insulin affects many sites of lipid metabolism. It stimulates synthesis of fatty acid in liver adipose tissue and in the intestine. The insulin has also been reported to increase the cholesterol synthesis. So in the case of Diabetes mellitus the carbohydrate and fat metabolism is impaired. The level of lipids, lipoprotein and lipid peroxidase in plasma increased due to the abnormal lipid metabolism in the lack of insulin hormone.

Medicinal plants plays a key role in the indigenous system of medicine for diabetes and in the related complications wound healing due to presence of natural antioxidant [2]. These medicinal plants contain a major group of antioxidative compounds phenolic glycosides, alkaloids, flavonoids, tannin, and steroids. Phenolic glycosides significantly inhibit the generation of reactive oxygen species such as superoxide anion and hydrogen peroxide (H_2O_2) that promote the delayed wound healing in diabetic condition. The constituents such as alkaloids, triterpenoides and tannins of the medicinal plants may play a major role in the process of wound healing in diabetic mice. Tannins and triterpenoides are also known to promote the wound healing process mainly due to their astringent and antimicrobial property [3], which seems to be responsible for wound contraction and increased rate of re-epithelization.

The phytoconstituents derived from plants are important source of antioxidant like glycosides, alkaloids, steroids, flavonoids and tannins. The phytoconstituents greatly influence metabolic activities which directly affect glucose level in the body. The natural antioxidants plays a significant role to counteract reactive oxygen species (ROS). Reactive oxygen species produced inside the human body as a result of unbalance in metabolic processes and infectious diseases [4]. So natural antioxidants due to their radical scavenging ability are considered as possible protection against many chronic diseases [5,6]. This research work explores the antidiabetic and wound healing activity of the seeds of *Terminalia chebula* with their recent advancement in treating chronic wounds in diabetic condition.

In the several studies it has been reported that the seeds of *Terminalia chebula* is a potent source of alkaloids, carbohydrates, flavonoids,

triterpenoids, tannins, coumarin, quinone and phenolic compounds. The seeds have been known to contain 150 useful alkaloids among other pharmacologically active compounds. It shows antibacterial and antidiabetic activity [7]. The present research work was designed to evaluate the antidiabetic and wound healing activity of methanolic extract of the seeds of *Terminalia chebula* in STZ induced diabetic mice.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The seeds of *Terminalia chebula* were collected from the Faculty of Ayurveda, Banaras Hindu University, Varanasi. The plant was identified and authenticated by the Department of Botany, Banaras Hindu University, Varanasi. The material was shade dried, pulverized and preserved in air tight containers.

2.2 Extraction of Plant Material

The methanolic extraction of dried powder (1 kg) of the seeds was done by Soxhlet apparatus at 65°C. The extracted materials was then kept in water bath to evaporate solvent totally and then kept on a rotary shaker at 190-220 r/min for 6 hours and then stored at 4°C in airtight bottles. The yield of the extract was 5.6%.

2.3 Phytochemical Analysis

Qualitative analysis of phytochemicals such as alkaloids (Dragendorff test & Mayer's test), flavonoids [8], glycosides (Fehling's test), steroids (H₂SO₄ test), saponins (Foam test), proteins (Ninhydrin test), terpenes (Lieberman-Burchard) and tannins [9] were performed. Quantitative analysis of various phytochemicals such as alkaloids [10], flavonoids [11], phenols [10,12], saponin [12] and tannins [13] of seeds of *Terminalia chebula* were performed.

2.4 Wound Creation and Drug Administration

2.4.1 Animals

All experiment were performed on 7 to 8 week old male Swiss albino mice with an average weight of (25±1) g. The animals were individually kept under laboratory conditions at the Department of Biochemistry, Banaras Hindu University, Varanasi. The mice were divided into

five groups, comprising five animals in each groups. These groups were considered as diabetic and assigned as DC, DM, DM+E₁, D+E₁ and D+E₂.

2.4.2 Drugs administration

The DC (Control), DM (60 mg/kg body weight in 200 µl ddH₂O), DM+E₁ (30 mg/kg body weight Metformin in 100 µl ddH₂O + 100 mg/kg body weight plant extract in 100 µl ddH₂O), D+E₁ (200 mg/kg body weight plant extract in 200 µl ddH₂O), D+E₂ (400 mg/kg body weight plant extract in 200 µl ddH₂O) were injected for 14 consecutive days starting from day zero through intra peritoneal tube. The level of glucose, SOD, LPO and NO were measured on 3rd, 7th, and 13th day post wounding of both diabetic and non-diabetic mice.

2.4.3 Induction of diabetes mellitus

The mice were fasted for 12 h (overnight) and diabetes was induced by giving intraperitoneal streptozotocin (STZ) injection (50 mg/kg body weight) in cold 0.1M Na-citrate buffer, pH 4.5 for five consecutive days in the diabetic group of mice. The animals were confirmed for diabetes before the start of experiment. The fasting serum glucose level was measured by glucose oxidase-peroxidase method using glucose test kit (Span diagnostics Ltd., India). Only mice with fasting blood glucose level of 200 mg/dl and above were considered as diabetic and those with blood sugar level 130 mg/dl and below were considered as non-diabetic. These mice were used for the experiment further.

2.4.4 Wound creation

To develop wounds, a single full thickness 1.0 cm diameter superficial excision was made on the mid-dorsum of each diabetic and non-diabetic mice at day 0. The measurements of the wound diameter were taken on 3rd, 7th and 13th days by using transparency paper and permanent marker.

2.4.5 Determination of LPO and SOD activity

The levels of malondialdehyde (MDA) for LPO and SOD were estimated in the supernatant of wound tissue homogenates. To obtain supernatant, the homogenized wound tissues were centrifuged at 5,000 rpm for 10 min to remove the cell debris. The level of MDA was estimated according to the method of [14] and was expressed as nanomole per milliliter of MDA

conjugate formed in the reaction. The estimation of SOD was done by the method of [15].

2.4.6 Determination of NO_x activity

The levels of NO_x were estimated by the method of [16] and [17]. A total of 100 µl of tissue supernatant was mixed with Griess reagent [1% sulfanilamide, 0.1% N-(1-naphthyl) ethylene diamine with 5% phosphoric acid] and OD was taken at 570 nm.

2.5 Statistical Analysis

The data were analyzed by one way analysis of variance (ANOVA) using SNK test (Students-Newmann-Keuls) with sigma state 3.5. The p-value less than 0.05 were considered to be significant (Level of significance P<0.05=*, P<0.01=**, P<0.001=***). Data were represented as mean ± standard deviation. All the studies were performed in quadruplet.

3. RESULTS

3.1 Qualitative and Quantitative Analysis of the Phytochemicals in the Seeds of *Terminalia chebula*

Qualitative and quantitative analysis of the seeds of *Terminalia chebula* revealed the presence of secondary metabolites flavonoids, phenols, saponins, alkaloids, tannins and primary metabolites carbohydrates, proteins, lipids. Quantitative estimations of bioactive constituents are summarized in Table 1. *Terminalia chebula* has been tremendously used in the treatment of several diseases.

3.2 Antidiabetic Activity of the Seeds of *Terminalia chebula*

Administration of *Terminalia chebula* seeds as drugs resulted in a significant decrease (P<0.001) in serum glucose level on 3rd, 7th and 13th day in case of diabetic mice as compared to the diabetic controls. A less significant decrease (P<0.05) was observed in case of mice treated with metformin on day 7 but on day 13 this decrease was highly significant (P<0.001) mice (Table 2).

3.3 Wound Healing Activity of the Seeds of *Terminalia chebula*

The measurements of the wound diameter were taken on the day 3rd, 7th and 13th using

transparency paper and a permanent marker. The wound areas were recorded and measured on graph paper (Fig. 1). The leaves of *Terminalia chebula* increased the rate of wound healing in the diabetic mice. The wound closure was optimal in the diabetic group of D+E₂ (Fig. 2).

Table 1. Qualitative and quantitative analysis of the seeds of *Terminalia chebula*

Bioactive constituents	Presence	Quantity in gram % (w/w)
Carbohydrate	+	0.29±0.02
Alkaloids	+	2.31 ± 0.32
Steroids	—	—
Saponins	+	4.01±0.02
Tannins	+	1.23±0.03
Flavonoids	+	2.03±0.41
Phenols	+	1.75±0.33
Lipids	+	3.08±0.09
Proteins	+	0.72±0.01

*Results are mean ± SD of quadruplet determination on the basis of dry weight

3.4 Levels of SOD in Wound Tissue after Drugs Administration

The wound tissue from diabetic mice showed decreased extra cellular SOD activity as compared to non-diabetic mice as shown in (Fig. 3).

After administration of *Terminalia chebula* seeds as drugs, the SOD activity was found significantly increased on both 7th and 13th day in all diabetic groups injected with plant extract (p<0.05). However no significant changes observed in non-diabetic groups.

3.5 Levels of LPO in Wound Tissue after Drugs Administration

The estimated levels of lipid oxidation in wound tissue supernatant from various diabetic and non-diabetic plant drugs treated groups of mice were as depicted in (Fig. 4). In diabetic wounds, the level of LPO in terms of MDA (nmol/ml) was increased as compared to the non-diabetic wounds. The administration of *Terminalia chebula* seeds as drugs was effective in preventing lipid peroxidation as observed by decrease in the level of malondialdehyde content in diabetic groups. The decrease in the level of LPO in non-diabetic was not significant.

3.6 Levels of NO in Wound Tissue after Drug Administration

The wound tissue from diabetic mice showed decreased nitric oxide level as compared

to non-diabetic mice (Fig. 5). After administration of *Terminalia chebula* seeds as drugs, the NO was found increased on both 7th and 13th day in all diabetic groups beings significant ($p < 0.05$).

Table 2. Effect of *Terminalia chebula* seeds on the level of serum glucose (mg/dl) in various diabetic and non-diabetic groups of mice

Groups	Level of serum glucose (mg/dl)			
	0 th Day	3 rd Day	7 th Day	13 th Day
DC	219±2.21	230±2.11	238±2.34	256±2.12
DM	199±2.32	187±2.04	149±2.12	139±2.18
DM+E ₁	208±2.45	183±2.33	149±2.03	122±2.14
D+E ₁	211±2.75	179±2.18	131±2.39	116±2.32
D+E ₂	219±2.34	161±2.02	142±2.22	105±2.41

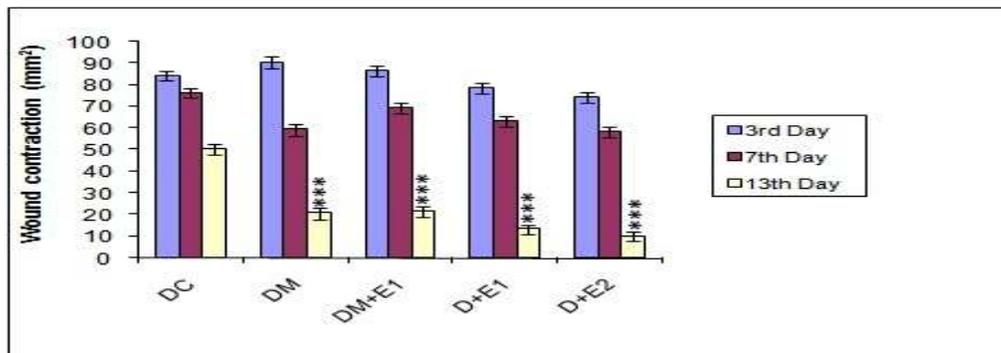


Fig. 1. Wound diameter (mm²) in different groups of diabetic and non-diabetic mice after the administration of *Terminalia chebula* seeds as antidiabetic drugs

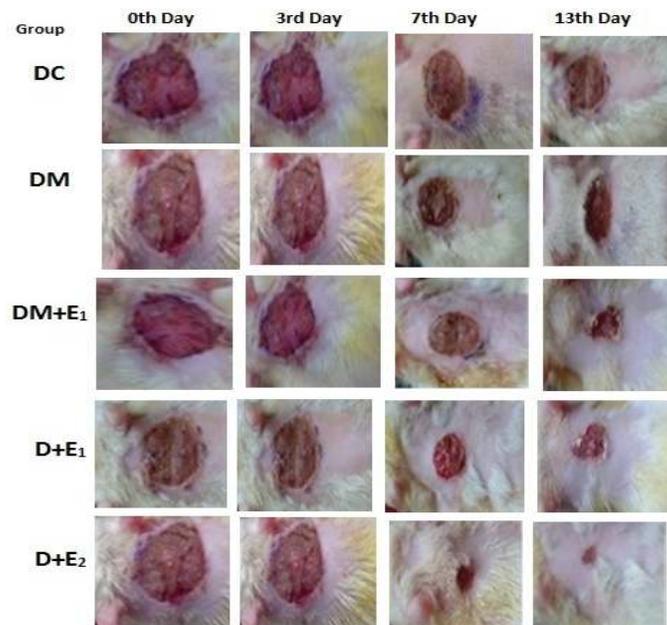


Fig. 2. Photograph showing various stages of wound healing activity after administration of *Terminalia chebula* seeds extract in different group of diabetic mice

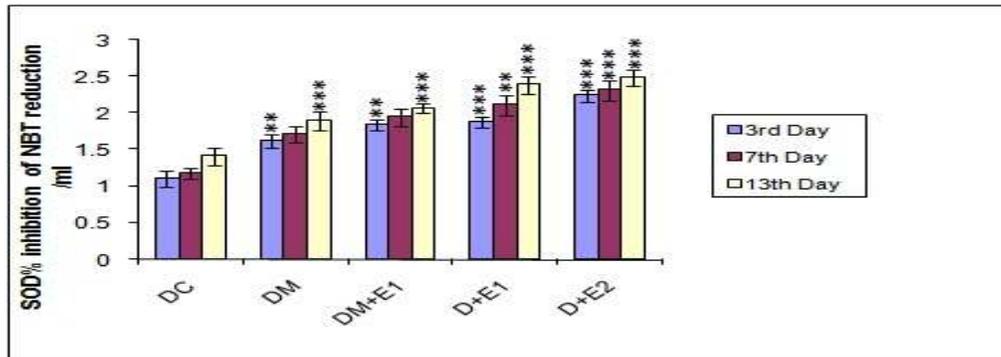


Fig. 3. Level of SOD (% inhibition of NBT reduction/ml of wound tissue supernatant) in various diabetic and non-diabetic groups of mice after administration of *Terminalia chebula* seeds

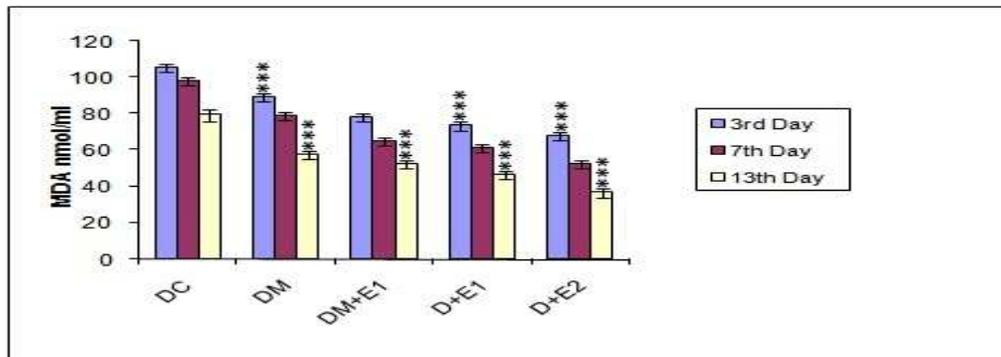


Fig. 4. Level of lipid peroxidation in terms of malondialdehyde (nmol/ml of wound tissue supernatant) in various diabetic and non-diabetic groups of mice after administration of *Terminalia chebula*

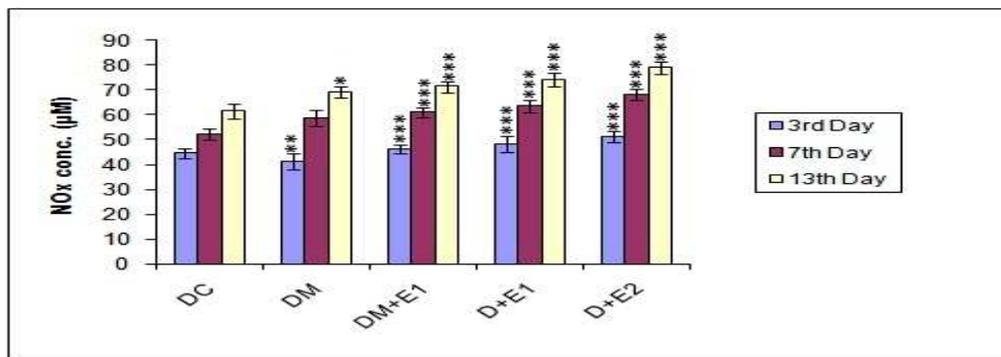


Fig. 5. Level of total nitric oxide content (µM) in wound tissue supernatant in various diabetic and non-diabetic groups of mice after administration of *Terminalia chebula*

4. DISCUSSION

4.1 Qualitative and Quantitative Analysis of *Terminalia chebula*

The preliminary phytochemical analysis of the seed extract showed the presence of tannins,

triterpenoids and alkaloids. Any one of the observed phytochemical constituents present in *Terminalia chebula* may be responsible for the wound healing activity. Tannins promotes the wound healing through several cellular mechanism, chelating of the free radicals and reactive species of oxygen, promoting

contraction of wound and increase the formation of capillary vessels and fibroblast. Recent studies have shown that phytochemical constituents like flavanoids and triterpenoids are known to promote the wound-healing process mainly due to their astringent and antimicrobial properties, which appear to be responsible for wound contraction and increased rate of epithelialisation. The wound-healing property of *Terminalia chebula* may be attributed to the phytoconstituents present in the plant, and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. The early tissue approximation and increased tensile strength of the excision wound observed in our study may have been contributed by the tannin phytoconstituent of *Terminalia chebula* from the astringent effect which has been reported elsewhere [18]. Earlier studies showed the presence of triterpenoids which are responsible for the effective wound healing activity of *Cecropia peltata* and *Pentas lanceolata* [19].

4.2 Antidiabetic Activity of *Terminalia chebula*

Administration of *Terminalia chebula* seeds extract resulted in highly reduced glucose level on the seventh and thirteenth day of treatment. The effect of extract was more obvious and visible in diabetic mice as compared to the metformin treated diabetic mice. Besides, the addition of the *Terminalia chebula* seed extract of metformin as a therapeutic enhances the potential of the synthetic drug as shown in the results. Moreover, there is not much differences in the hypoglycemic effect when the dose is increased from 200 mg/kg to 400 mg/kg, suggesting that the 200 mg/kg may be given as the optimum dose. The hypoglycemic effect on this plant could be explained in term of its richness in secondary metabolites such as alkaloids, saponins, flavonoids, phenolic and tannins. Which have been proved to enhance insulin secretion and regeneration of β - cell of islets of langerhans in case of diabetic patients [20].

4.3 Wound Healing Activity of the Seeds of *Terminalia chebula*

Intraperitoneal injection of *Terminalia chebula* extract resulted in enhances wound healing in diabetic mice. In term of reduction of wound area in thirteen days as compare to control the wound

closure was found to be maximum in the mice treated with 400 mg/kg close to *Terminalia chebula* extract. The wound healing potential of this herbal extract could be attributed to the high content of alkaloids, flavonoids, and tannins which impart astringent and antiseptic properties responsible for increased rate of epithelialization and collagen synthesis which ultimately result in wound contraction [21].

The wound healing also correlated with the efficient management of oxidative stress by increased SOD and NO level due to *Terminalia chebula* extract. As a phytoconstituent (phenolic and tannins) to possess strong LPO inhibition and free radical scavenging abilities they increase the level of SOD and NO and help in prompt wound repair by enhancing the transport of oxygen towards the wound and by promoting various other cellular repair mechanisms. This might be the reason that the extract has been used since long time as a potent wound healing agent by the traditional and tribal communities.

5. CONCLUSIONS

The present study provides a scientific rationale for the traditional use of the seeds of *Terminalia chebula* in the treatment of diabetes and delay wound healing that effectively stimulate the secretion of insulin and promote wound contraction in diabetic mice as compared to control and other drug treated groups. This finding suggests that the extract of this plant can be further tested for antidiabetic and wound healing properties in human and can be further commercially used for managements of diabetic wounds on large scale.

CONSENT

Written consent was obtained from all participants.

ETHICAL APPROVAL

Ethical approval for the study was obtained by the Central Animal Ethical Committee of the Institute of Medical Science, Banaras Hindu University, Varanasi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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