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Research Article

**SYNERGISTIC ACTIVITY OF *FICUS BENGALENSIS* AND
TRIGONELLA FOENUM-GRÆCUM IN ALLOXAN INDUCED
DIABETIC MALE ALBINO WISTAR RAT MODEL****Srinivasulu. P*, Vijetha. P, T. N. V. Ganesh Kumar, D. Suryanarayana Raju,
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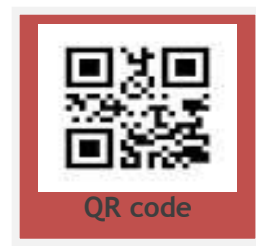
Abstract:

The antidiabetic activity is determined by estimating blood glucose levels and body weights in diabetic rats. Wound healing activity was determined by excision method. There is significant reduction of blood glucose levels from 1 week to 4 weeks in ethanolic extract treated group compared to the control group. The body weights are also increase in ethanolic extract treated group when compared to control group. The ethanolic extract ointment treated group was shown significant wound healing activity in diabetic rats when compared to the control and is almost near to standard group. Here we conclude this combination of ethanolic extract of *Ficus bengalensis* and *Trigonella foenum-graecum* was showed significant antidiabetic, weight gain and wound healing activity in alloxan induced diabetic rat model.

Key words: Diabetes mellitus, *Ficus bengalensis*, *Trigonella foenum-graecum*, Antidiabetic, Ethanolic extract.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder that is due either inhibition of insulin secretion or decrease of insulin sensitivity resulting hyperglycemia.[1,2]The hyperglycemia is due to increase of glucose uptake, glycogen synthesis, and decrease of glycogenolysis, gluconeogenesis. The symptoms of hyperglycemia include increased hunger and thirst, frequent urination. Diabetes mellitus are classified into three type's include type-I (i.e. insulin dependent diabetes mellitus), type-II (non-insulin dependent diabetes mellitus), and gestational diabetes. As per international diabetes federation 387 million people have diabetes worldwide, 179 million people were undiagnosed with diabetes, 4.9 million deaths were reported in 2014, every 7 seconds a person dies from diabetes[3], about 90% cases belong to type 2 diabetes[4].

For treating diabetes mellitus several drugs are available currently in the market, the targeted drugs like insulin secretagogues i.e. sulphonyl ureas (glibenclamide and gliclazide etc.), (repaginate and nateglinide), insulin sensitizer (biguanides), PPAR-gamma agonists (glitazones) [5, 6]. Few of above category drug had complication with liver and heart organs like liver toxicity (troglitazone), heart failure (rosiglitazone) and vitamin B12 deficiency (Metformin).

Literature review suggested that *Ficus bengalensis* and *Trigonella foenum-graecum* were having antidiabetic and wound healing activity. This is the present study, we aimed to see the effect of combination extracts as diabetic and wound healing with body weight effect.

Therefore tradition medicinal plants having active ingredients that having anti-diabetic activity without causing side effects, we studied antidiabetic activity using combination of two ethanolic extracts of *Ficus bengalensis* and *Trigonella foenum-graecum* in alloxan induced diabetes mellitus in albino male rats.

Wound healing is a complex biological process, initiated in response to an injury that restores the function and integrity of the damaged or injured tissues often terminated by a scar formation and has been the subject of intense research for a long time in modern biomedical sciences. When a wound occurs, body responds with a predictable series of events to repair the resulting damage, following processes are involved in wound repair that is haemostasis (coagulation, platelet and complement activation), inflammation phase (granulocytes, phagocytosis), cellular proliferation (fibroplasias, angiogenesis, re-epithelialization, extracellular matrix

(ECM) synthesis and fibronectin), remodeling (ECM synthesis, degradation and remodeling) [7].

Now a day's wound ulcer are very danger in diabetes patients, it is difficult for treating the patient. Therefore in the present study we determined to evaluate the antidiabetic activity with wound healing and body weight effect was determined in alloxan induced diabetic albino male rats. In the present study we were chosen two medicinal plants for this activity, the plants chosen for this study is *Ficus bengalensis* belongs to family Moraceae and *Trigonella foenum-graecum* belongs to family Fabaceae, leafs and seeds respectively. In this study aim is to evaluate the antidiabetic, wound healing activity and body weights effect of *Ficus bengalensis* and *Trigonella foenum-graecum* in alloxan induced diabetes albino rat model

MATERIALS AND METHODS

List of Chemicals Used

Ethanol (Changshu Yangyuan Chemical, China), Diethyl ether (Fischer Scientific, Mumbai), Surgical spirit (Krishna Pharma, Hyderabad), Emulsifying wax (Oxford Laboratory, Mumbai), White soft paraffin (Oxford Laboratory, India), Liquid paraffin (Fischer scientific, Mumbai), 1% Gum acacia (Oxford Laboratory, India).

List of Drugs Used

Glibenclamide (Gift sample from Medreich, Bangalore). Povidine-Iodine ointment USP (Betadine, 5% w/w, Win-Medicare, New Delhi).

Plant Materials

Leafs of *Ficus bengalensis*, seed of *Trigonella foenum-graecum* were collected locally and botanical identification were authenticated at department of botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Animals

Healthy Male Albino Wistar rats weighing 150-180 g with almost the same size and no prior drug treatment were used *in-vivo* studies. These rats were procured from Mahaveer enterprises, Hyderabad and were acclimatized to laboratory condition at Animal House, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, with room temperature $23\pm 5^{\circ}\text{C}$, 12h light/dark cycle and relative humidity $55\pm 10\%$ for a period of 7 days prior to the experimental period.

The Institutional Animal Ethical Committee reviewed the animal protocol prior to the experiment. All rats were treated in accordance with the guideline for the

care and use of laboratory animals (NIH Publication N0.86-23, revised 1985) with the permission of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences Animal Ethical Committee (1529/PO/a/11/CPCSEA./CHIPS/IAEC3/PRO-12/2014-15)

Preparation of Plant Extract

The collected fresh Leaves of *Ficus bengalensis* and seeds of *Trigonella foenum-graecum* were cleaned washed with distilled water to remove any kind of dust particles and dried in shade separately. The dried material was grinded separately and the powders obtained was passed through 14 mesh sieve to get the required particles size. The leaf and seeds were extracted in individual soxhlet extraction apparatus using absolute ethanol as a solvent for 72 hours, after that concentrated extract was obtained by using distillation. The concentrated extracts of *Ficus bengalensis* (500mg) and *Trigonella foenum-graecum* (1500mg) was dissolved in 0.5% HydroxyPropyl Methyl Cellulose (HPMC). The plant extract formulation was given orally to diabetic group as per body weights up to 28 days.

Induction of Diabetic Mellitus

The male, wistar albino rats, weighing 150-180g were fasted for 24hrs before alloxan administration. The rats were injected alloxan monohydrate in 0.9% saline at the dose of 150mg/kg intraperitoneally as per body weight of animals in a single dose. Water and food were given to the rats after 40 minutes of drug administration [8, 9, 10]. The blood glucose levels were measured after 48hrs of alloxan treatment. The blood glucose levels were measured by using glucometer. The blood glucose levels which are ≥ 250 mg/dl were considered to be diabetic. These were selected and divided into four groups consisting of 6 animals each [11].

Study Design

In this experiment a total 24 rats were used, in that 18 animals were diabetic remain are sham i.e. normal group, each group consist of 6 animals. The grouping was made as follows and

Group I: Sham

Group II: Control treated with 0.5% HPMC up to 28 days

Group III: Test group were treated with *Ficus bengalensis* and *Trigonella foenum-graecum* (500mg: 1500mg) up to 28 days

Group IV: Standard group were treated with glibenclamide 2 mg/kg up to 28 days

Measuring Blood Glucose Levels

Blood glucose levels were taken on 0, 7, 14, 21, 28 days by using glucometer.

Preparation of Ointment

Ethanolic extracts of *Ficus bengalensis* (500mg) and *Trigonella foenum-graecum* (1500mg) were mixed with the ointment base (bees wax, cetostearyl alcohol) and made upto 100gm with base and formulation was prepared by trituration.

Wound healing activity

The activity was performed in the diabetogenic animals as we used for diabetic model.

Study Design

In this experiment a total 18 rats were used, each group consists of 6 animals. The grouping were divided as follows

Group I: Control group treated with ointment base up to 30 days.

Group II: Test group were treated with *Ficus bengalensis* and *Trigonella foenum-graecum* ointment (1:3) up to 30 days.

Group III: Standard group were treated with Povidine iodine 5% up to 30 days.

Wound Model

Excision Wound

Rats were anaesthetized with diethyl ether and an area of about 350 mm² was marked on the back of the Diabetogenic rat by standard ring. Full thickness of the marked skin was removed carefully. Epithelization period was observed. Wound healing rate was monitored by measuring the wound area on 0, 5, 10, 15, 20, 25 and 30 days. This was achieved by tracing the wound on a graph paper. Reduction in the wound area was expressed as percentage of the original size [6,12,13,14,15,16].

Statistical Analysis

The statistical treatment was applied through One way ANOVA followed by Dunnett test. The statistical significant was $P < 0.05$. All the data values are expressed as Mean \pm SEM.

RESULTS

The present study results were clearly indicated that there were dramatic increases of body weights in extracts and standard drug treated groups when compared to that of control group. The blood glucose levels of ethanolic extracts of *Ficus bengalensis* and *Trigonella foenum-graecum* treated groups were almost near to the normal but not up to the mark of standard drug, there was a profound decrease of

blood glucose levels than they used alone. So combination of these two extracts had a great effect

on blood glucose levels were tabulated in table no 1, 2 and 3 and figures no 1,2 and 3.

Table 1: Blood Glucose Levels of *Ficus bengalensis* and *Trigonella foenum-graecum* Combination

Blood Glucose Levels	Sham (Mean ± SEM)	Control (Mean ± SEM)	<i>Ficusbengalensis</i> And <i>Trigonellafoenum-graecum</i> (Mean ± SEM)	Glib @ 2mg (Mean ± SEM)
0 Day	97.83±0.79	284.33±1.60	287±1.63	292.5±1.47
7 Day	97.5±0.99	286.83±0.79	246.66±1.45	226.5±1.52
14 Day	97±0.96	293±1.93	216.66±1.62	194.16±1.24
21 Day	97.66±0.88	302.5±1.56	173.16±1.53	140±0.68
28 Day	98.3±0.55	315.33±2.04	108.33±1.62	98.5±1.97

Table 2: Body Weights of *Ficus bengalensis* and *Trigonella foenum-graecum* Combination.

Body weights	Sham (Mean ± SEM)	Control (Mean ± SEM)	<i>Ficusbengalensis</i> And <i>Trigonellafoenum-graecum</i> (Mean ± SEM)	Glibenclamide @ 2mg (Mean ± SEM)
0 Day	242.33 ±0.80	250.33±0.61	251.8±0.79	250±0.57
7 Day	251.83±0.79	241.5±0.76	265.16±0.40	270.16±0.47
14 Day	263.66±1.08	237.66±1.08	279.66±1.28	287.33±0.76
21 Day	265.83±1.04	228.33±1.33	292.5±1.78	307.5±0.84
28 Day	272.5±1.05	204.83±1.24	322.83±1.75	327.83±1.81

Table 3: Period of Epithelization of *Ficus bengalensis* and *Trigonella foenum-graecum* Combination.

Wound healing (period of epithelization)	Control (mm ²) (Mean ± SEM) (% inhibition)	Povidine @ 5 % (mm ²) (Mean ± SEM) (% inhibition)	<i>Ficusbengalensis</i> And <i>Trigonellafoenum-graecum</i> (mm ²) (Mean ± SEM) (% inhibition)
0 Day	350 ±0.25	352±0.93	350.83±0.65
5 Day	337.16 ±1.79 (3.66%)	322.6±61.22 (7.8%)	324.33±1.56 (7.33%)
10 Day	305.66 ±1.89 (12.66%)	247.66±1.72 (29.23%)	260.66±0.61 (25.52%)
15 Day	261±1.77 (25.42%)	122.83±1.62 (64.90%)	132.33±1.85 (62.19%)
20 Day	144±1.65 (58.85%)	14±1.29 (96%)	34.83±1.85 (90.04%)
25 Day	85.66±1.99 (75.52%)	0 (100%)	6.83±1.40 (98.04%)
30 day	50.66±1.99 (75.52%)	0 (100%)	0 (100%)

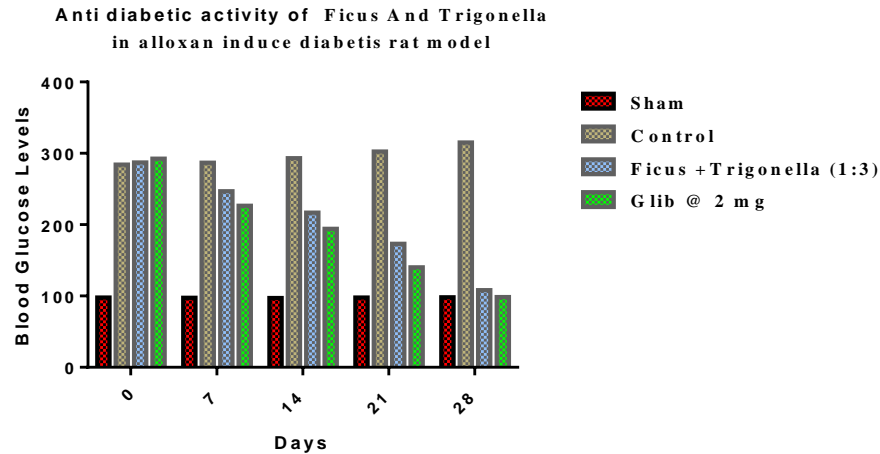


Fig 1: Anti diabetic activity of *Ficus bengalensis* and *Trigonella foenum-graecum* in alloxan induce diabetic rat model. Ficus: *Ficus bengalensis*; Trigonella: *Trigonella foenum-graecum*.

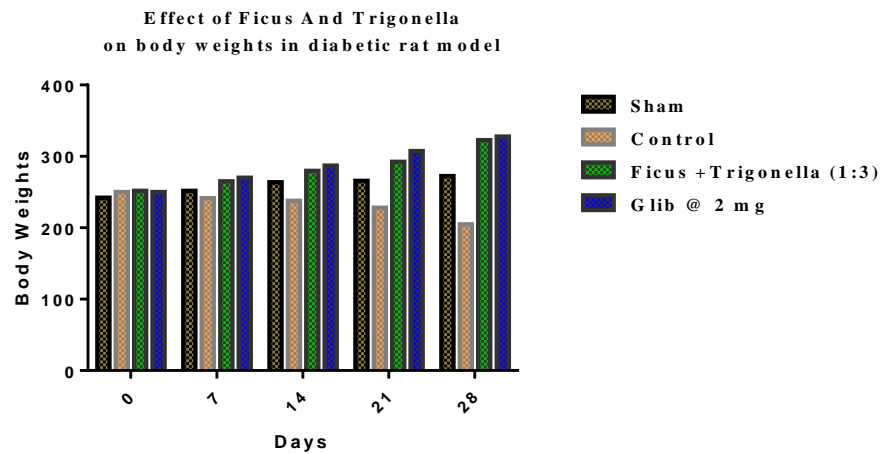


Fig 2: Effect of *Ficus bengalensis* and *Trigonella foenum-graecum* on body weights diabetic rats. Ficus: *Ficus bengalensis*; Trigonella: *Trigonella foenum-graecum*.

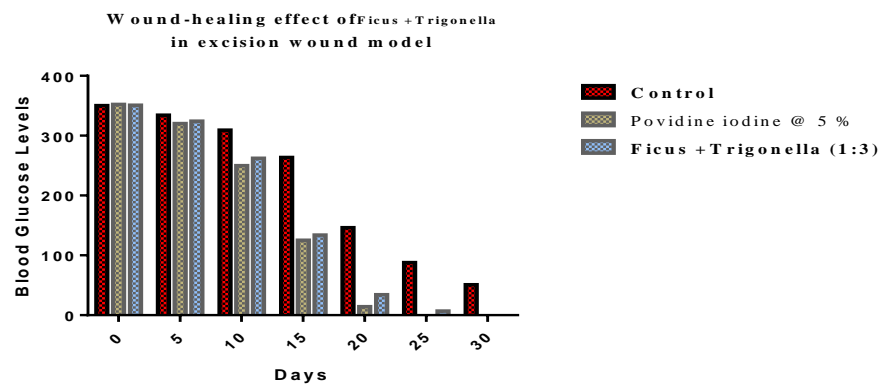


Fig 3: Wound healing effect of *Ficus bengalensis* and *Trigonella foenum-graecum* in excision wound model. Ficus: *Ficus bengalensis*; Trigonella: *Trigonella foenum-graecum*.

DISCUSSION

The well-known from those natural plants would not show any kind of side effect made us to find a plant source which can be used to treat antidiabetes. In the meanwhile, path- physiology of this disorder shows that wound healing is major side effect in patient having diabetes. So our concern was also to cure wounds parallelly. This led us to find another source from plants which has potential wound healing effects. This we have chosen *Ficus bengalensis* and *Trigonella foenum-graecum* for this study [17,18,19].

Now days so many synthetic formulations are available in the present market that having well anti diabetic activity but simultaneously that has producing different side effects like hypoglycemia.

For reduction of those side effects along with decreasing of blood glucose levels we screen out anti diabetic, wound healing activity and body weight of two medicinal plants i.e. *Ficus bengalensis* and *Trigonella foenum-graecum* in alloxan induced diabetic albino male rats models.

In the present study alloxan 120mg/kg was given intraperitoneally. Along with we made a wound by using excision method in diabetogenic albino male rats.

The mechanism of decreasing blood glucose levels in *Ficus bengalensis* is thought due to presence of a glycoside called leucopelargonidin in leaves, wound healing activity is due to enhanced wound contraction it would have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited in the wound area, In *Trigonella foenum-graecum* foenum- fracecum is thought due to presence of insulin secretion stimulating compound 4- hydroxy isoleucine in seeds which decreasing blood glucose levels and wound healing activity is due to releases its anti-inflammatory properties and works to maintain the healing process and reduce the inflammation that is no longer needed as the wounds heals. Fenugreek seeds contain fatty acids which build collagen that is promoted wound healing and maintain skin elasticity [20,21].

The present study indicates synergistic activity of this combination i.e. *Ficus bengalensis* (1) and *Trigonella foenum-graecum* (3) in the ratio of 1gm: 1.5 gm/kg. For this study we made suspension and ointment of *Ficus bengalensis* and *Trigonella foenum-graecum* in the ratio of 1:3 and administered the suspension daily up to 28 days, we took body weight of diabetic animals along with blood glucose levels on 7,14,21,28 days respectively, whereas ointment applied daily for up to epithelialization i.e. 30 days and measured the wound diameter on 0, 5, 10, 15, 20, 25, 30 consequent days.

In diabetes induced diabetic rats, we found that there is a decrease of blood glucose levels from 287 ± 1.63 to 108.33 ± 1.62 on 0 to 28 day respectively as compared to that of single use, whereas standard drug i.e. 292.5 ± 1.47 to 98.5 ± 1.97 on 0 to 28th day. Here we observed hypoglycemic effect for standard drug but this combination doesn't cause hypoglycemia that is one of the beneficial effects of this combination.

In excision wound model, the *Ficus bengalensis* and *Trigonella foenum-graecum* combination was able to period of epithelization significantly by 30th day, it appears that this combination was able to promote epithelization either by proliferation of epithelial cell or by anti-inflammatory activity, whereas here standard drug showed period of epithelization by 30th day.

Wound healing was increased with extracts treated group than the control group but near to the standard drug, here wound healing activity were taken 30 days that is due to diabetic mellitus that might be the reason.

In diabetic model there is significant elevation of body weight that is due to elevation of insulin (i.e. insulin is an anabolic hormone that is due to increased glycogen, lipid, esterification of fatty acids and decreased proteolysis, lipolysis, gluconeogenesis) during the study.

Finally we expressing that there is reduction of blood glucose levels, elevation of body weight and increased period of epithelization in alloxan induced diabetes in albino male rats by using this combination of *Ficus bengalensis* and *Trigonella foenum-graecum* as synergistic action this might be helpful in type-II diabetes mellitus patient without any observable side effects as compared with that of synthetic drug molecules which had severe hypoglycemic effect, it may leads to even coma.

CONCLUSION

From foregoing it could be concluded that *Ficus bengalensis* and *Trigonella foenum-graecum* plants are important in medicine for treating diabetes with wound ulcers. The present study has demonstrate that ethanolic extracts of *Ficus bengalensis* leaves and *Trigonella foenum-graecum* seeds has properties that decreasing the blood glucose levels, increased body weights and wound healing activity when compared with normal control. Here the synergistic activity of this combination is due presence of leucopelargonidin a glycoside in *Ficus bengalensis* and 4-hydroxyisoleucine is the insulin stimulation active compound present in *Trigonella foenum-graecum*.

On the basis of study carried out it was found that the ethanolic extracts of leaves, seeds of *Ficus*

bengalensis and *Trigonella foenum-graecum* respectively are much safe and effective in type-II diabetic patients. Our study proved that synergistic effects of this combination might helpful for controlling blood glucose levels as well as wound healing affect in diabetic with foot ulcers patients much beneficial.

Yet there is much work to be carried out to find the actual chemical constituent of these plants which had shown activity, its isolation and characteristics. There is also a scope to find the scope in humans to treat this disorder.

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