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PHYTOCHEMICAL ANALYSIS AND ANTI-DIABETIC ACTIVITY OF *Jasminum sambac*

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Abstract:

The present study describes the anti-diabetic potential of ethanoloic extract of *Jasminum sambac Linn.* Leaves on streptozocin induced diabetic rats and excision wound model to substantiate its folklore claim. The ethanolic extracts at two doses 100 and 200 mg/kg, p.o. prevented diabetes by Glucose oxidase method further studying its lipid profiles and anti-oxidant effects in rats. The wound healing potential of diabetic rats were confirmed by the excision wound model studies with surface epithelization and wound contraction. Pretreatment with ethanolic extract of *Jasminum grandiflorum Linn.* leaves significantly ($P < 0.05$) increased the anti-oxidant enzymes and lipid peroxidation index. Further in wound healing activity the epithelialization period was significantly ($p<0.01$; $P<0.001$) lower in 10% and 5 % ointment of EEJG as that wound induced group. The results showed that ethanolic extract of *Jasminum grandiflorum Linn.* Leaves had significant anti-diabetic and wound healing effects.

Keywords: Anti-diabetic activity, Wound healing activity, ethanol, streptozocin Lipid profile studies, *Jasminum sambac* Linn. Leaves.

Introduction

Diabetes is a metabolic disorder which is consequential to high blood glucose level, either because pancreas does not generate adequate amount of insulin or cells do not act in response to that insulin. The sedentary life style and obesity is the best known reason for diabetes. It becomes pandemic and the best known cause of mortality and morbidity (Leitner et al. 2017). Basically three types, i.e. type 1, type 2 and type 3 (gestational) of diabetes exist which occurs during pregnancy and engrosses threat mutually for the mother and child. The World Health Organization (WHO) states that there are 366 million people who are diagnosed with diabetes in 2011 and it will rise to 552 million by 2030. The estimated worldwide prevalence of diabetics in 2000 was 2.8% and it is projected to 5.4% in 2025 (Rao et al. 2010).

Wound healing is the process of regeneration of ruptured tissue after injury to the skin or other soft tissue. Following injury to the skin, the inflammatory responses begin, and cells below the dermis begin to generate collagen (connective tissue) production. The inflammatory responses involve phagocytosis of foreign protein. Then, epithelial cells are regenerated and differentiated into skin layers (Lakshmi et al., 2011). In diabetic patients, endothelial nitric oxide synthase (eNOS) activation is reduced and thus blocks the movement of Enothelial progenitor cells (EPCs) from bone marrow into circulation. It has also been shown that SDF-1α expression is decreased in epithelial cells and myofibroblasts in diabetic wounds, which prevents EPCs from homing to wounds and therefore limits wound healing.

Jasminum sambac Linn. is a plant of Oleaceae family. Its flowers and leaves are widely used in folk medicine to prevent and treat breast cancers. Flowers are also useful in uterine bleeding when brewed as tonic (Mishra et al., 2010). J. sambac Linn. contains triterpenoids, alkaloids, tannins, flavonoid, steroid, glycoside, terpenes, resins and salicylic acid (Nayak and Krishna, 2007; Patil and Saini, 2012). Moreover, flavonoids and triterpenoids are known to promote the wound-healing process due to their astringent and antimicrobial properties (Villegas et al., 1997; Tsuchiya et al., 1996). However, the effect of Jasminum sambac in the prevention of diabetic complications has not

been reported. The present study is focused on to evaluate the wound healing potential of Jasminum sambac Linn. Leaves in streptozocin induced diabetic rats.

Materials and Methods

Drugs and chemicals

The following drugs were used: Streptozocin, Ethanol was obtained from Sigma Chemical Co. (India). All drugs were prepared immediately before use. All other used reagents and solvents were of analytical grade.

Plant material

The whole plants of *Jasminum sambac Linn. leaves* (Oleaceae) were collected and authenticated by KCP science college, Botany Dept. Vijayapur, Karnataka, has been preserved in Department for the future reference.

Extract preparation

The whole plant (198 g) of *Jasminum sambac Linn. leaves*was air dried (7 days at 40°C) and powdered. The powdered plant was exhaustively extracted with ethanol in soxhlet apparatus and the extract was concentrated in vacuum to yield 20 g of residue.

Phytochemical screening

On preliminary screening, the ethanolic extract of *Jasminum sambac. leaves* showed positive reaction for alkaloid, flavonoids, tanninsand saponins.

Animals

The study was conducted on Albino Wistar rats (150±10 g) of either sex Animals were obtained from the BLDE SSM College of Pharmacy and Research Center, Vijayapura. Karnataka.. Animals were fed with commercially available standard rat pelleted feed (M/s Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and water was provided *ad libitum*.The rats were housed under conditions of controlled temperature (25±2 ° C) and were acclimatized to 12-h light: 12-h dark cycles. Experimental animals were used after obtaining prior permission and handled according to the University and institutional legislation as regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Acute toxicity studies

The acute toxicity studies for methanolic (EEJS) extract of *Jasminum sambac* were done according to the OECD guidelines No. 423. The extract did not produce any signs of toxicity when given in doses up to 2000 mg/kg by an oral route. Hence, for further studies 100 & 200 mg/kg dose of the extract were selected.

Streptozocin induced diabetes

A freshly prepared solution of STZ (60 mg/kg in 0.1 M citrate buffer, pH 4.5) was injected intra peritoneal to overnight-fasted rats The rats exhibited hyperglycemia within 48 h of STZ administration (Vogel et al., 2002). The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.

Study design

The experiment was carried out in five groups of six rats each:
Group I- Normal control rats received saline.

Group II- Diabetic control rats received streptozocin (45mg/kg)
Group III- Diabetic rats treated with standard drug, Metformin (250 mg/kg).
Group IV- Diabetic rats treated with EEJS (100 mg/kg in 0.5% CMC).
Group V- Diabetic rats treated with EEJS (200 mg/kg 0.5% CMC).
Serum glucose was estimated on day 1, 7, 14 and 21 by Glucose oxidase method and the absorbance was measured at 505 nm by UV-Spectrophotometer (ELICO-Sl-159). On the 22nd day the animals were sacrificed and the liver was removed and homogenized for the estimation of antioxidants parameters.

Preparation of liver homogenate
The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenized in chilled Tris–HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5000 rpm for 10 min, supernatant was collected and used for the estimation of antioxidants **Estimation of antioxidants**
The hepatic levels of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA), an index of lipid peroxidation was estimated by standard kits.

Estimation of lipid profiles
The serum levels of total cholesterol, LDL, HDL. VLDL and triglycerides were estimated by standard biochemical kits.

Wound healing activity (Morton and Malone, 1972)
Animals were under light ether anaesthesia through the surgical procedure. An impression of 2.5 cm diameter (500 sq mm) as described by Morton and Malone was made after leaving at least 5 mm complete space from the ears. The skin of the impressed area was excised carefully to the complete thickness and a wound of 500 sq mm was formed. Homeostasis was achieved by application of normal saline solution. The rats were kept individually in separate cages. The physical attributes of wound healing viz wound closure (contraction) and epithelization were recorded. The wound contraction was studied by tracing the raw wound area on a transparent paper on 0,3rd, 6th, 9th, 12th, 15th and 18th day. The criterion for complete epithelization was fixed as formation of scar with absence of raw wound area. The animals were grouped as follows,
Group I: Control, applied topically (0.5 g), simple ointment.
Group II: Standard, applied topically (0.5g), 5% w/w Povidone ointment.
Group III: Treated with EEJS 5% w/w ointment (0.5g), topically.
Group IV: Treated with EEJS 10% w/w ointment (0.5g), topically.

Statistical analysis
The data were expressed as mean ± SEM. The results were analyzed by SPSS version 19 using one-way analysis of variance. The differences between mean values were considered significant at $P < 0.05$.

Results
Acute toxicity studies
Acute oral toxicity studies revealed the non toxic nature of the EEJS. There was no lethality observed and any profound toxic reactions found at a dose of 2000mg/kg b.wt. p.o. which indirectly pronounces the safety profile of the plant extract.

Antidiabetic activity of *Jasminum grandiflorum* on STZ induced diabetes

In the study, single intraperitoneal injection of streptozocin (60mg/kg; b.wt) had displayed noxious biochemical changes. Whilst, treatment with EEJS significantly attenuated the toxic manifestation and thus inhibited the state of diabetes. In the present study, STZ intoxicated rats displayed significant ($p<0.001$) elevation of blood glucose level on 1st, 7th, 14th and 21st day as that of the control rats. Meanwhile, EEJS at the doses of 100mg/kg had not display any significant alteration in the blood glucose level as that of the diabetic control. However, treatment with EEJS at the dose of 200 mg/kg showed significant ($p<0.001$) reduction in the blood glucose level on 7th, 14th and 21st day as compared to the diabetic control (Table 1)

Effect of *Jasminum grandiflorum* on antioxidant enzymes and lipid peroxidation in STZ induced diabetic rats

In the present study, STZ intoxicated rats displayed significant ($p<0.001$) reduction in the level of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) and increase in the MDA in the liver tissue of diabetic rats. However, treatment with EEJG at the dose of 100mg/kg had not displayed effects, but at the dose of 200mg/kg there was an significant increase in the level of antioxidants and decrease in lipid peroxidation(Table 2).

Effect of *Jasminum grandiflorum* on lipid profiles in STZ induced diabetic rats

In this study, STZ intoxicated rats displayed significant ($p<0.001$) elevation in the level of total cholesterol, LDL, VLDL triglycerides and decrease in HDL in serum. Meanwhile, treatment with EEJG at the dose , 100 mg/kg displayed a minimal restoration of lipid profiles but it was statistically non significant. However, EEJG at the dose of 200mg/kg showed a significant ($p<0.001$) decrease in the level of total cholesterol, LDL, VLDL and triglycerides with an increase in HDL level and thus restored the lipid profiles to normalcy (Fig 1)

Wound healing activity of *Jasminum sambac* on excision wound models

On day 0 till day 3 there were no significant wound contractions observed in both control and wound induced and extracts treated experimental groups. On the 6th day, significant wound contraction process started in experimental animals treated with 5 and 10% ointment of EEJS. On the day 12, the wound contraction rate was rapid in 10% ointment of EEJS as that of the 5% ointment of EEJS. The complete healing was observed in 10% ointment of EEJS on 15th day, whilst in 5% ointment of EEJS the complete healing was observed only on 18th day (Fig 2)

Effect of *Jasminum grandiflorum* on period of epithelization on wound induced rats

In this study, the epithelialization period was significantly ($p<0.01$; $P<0.001$) lower in 10% and 5 % ointment of EEJS as that wound induced group (Table 3).

Discussion

Plants serve as an excellent source of various therapeutic agents. One of the major advantages of using plants is that they seldom show the deleterious side effects commonly associated with other allopathic drugs. This study investigated the ability of Jasminum grandiflorum leaves as potent antidiabetic agent in streptozocin induced diabetes in a murine model and in additional wound healing activity.

It is known that in STZ induced diabetes model, the drug STZ reaches the beta cells through a glucose transporter mechanism.STZ is reported to cause alkylation of DNA by liberating high levels of nitric oxide and nitrosourea, resulting into inhibition of aconitase (Kulkarni et al., 2012). In animal models of STZ the occurrence of insulin resistanceis dependent on several factors like dose of STZ, the age, and the strain of animals. Report published earlier report that an i.p. injection of STZ on the

second day of birth at dose level 90 mg/ kg developed insulin resistance (Patil et al.. 2014). Administration of *Jasminum grandiflorum* extracts to diabetic rats displayed significant reduction in the blood glucose levels to near normal. Albeit, the exact mechanism of action of the extract is unknown, the reduction in blood glucose level could be due to increased pancreatic insulin secretion from existing β-cell of the pancreas (Ghosh and Suryawanshi, 2001).

In diabetes, it is observed that an increase in levels of free fatty acids occurs. The circulating free fatty acids have deleterious effect on the endothelial functions by various pathways and mechanisms which include free radical production, protein kinase C activation and increase in severity of dyslipidemia (Inoguchi et al., 2000) Oxidative stress is caused by an increase in free radical production by the body and which lead to micro and macro vascular complication in diabetes. Oxidative stress has a strong interlink to the complications and progress of diabetes and insulin resistance. With an increase in oxidative stress, coupled with increase in free fatty acids and blood glucose level, the insulin activity and secretion levels are adversely affected (Rahimi et al., 2005). In this study, streptozocin intoxicated rats showed increased level of total cholesterol, LDL, VLDL and triglycerides and decreased level of HDL. However, treatment with *Jasminum sambac* significantly restored the lipid profiles level to normalcy.

Wound healing is a highly complex, but orchestrated cascade of events that can roughly be divided into three overlapping phases—inflammation, granulation tissue formation, and remodeling of the extracellular matrix. These events involve several cellular phenomena such as migration, proliferation, adhesion, phenotypic differentiation, etc. Immediately after injury, there is clot formation and the earlier phases of wound repair involve inflammation and synthesis of ground substance. The ground substance mainly consists of proteoglycans, which are heterogeneous, nonfibrillar components of the extracellular matrix. These complex macromolecules are made up of a protein core linked covalently to linear heteropolysaccharides, the glycosaminoglycans (GAGS). Proteoglycans and GAGS have been shown to play important roles in all the above-mentioned events of wound healing (Chitra et al., 1988)

Biological activities in skin are due to its interaction with various binding proteins. In the tissue repair process, inflammatory cells promote the migration and proliferation of endothelial cells leading to neovascularization of connective tissue cells which synthesize extracellular matrices including collagen resulting in re-epithelialization of wounded tissue (Agarwal et al., 2009) The increased tensile strength may be due to increased collagen concentration and stabilization of fibers.

The wound-healing property Jasminum grandiflorum 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.

Conclusion

These results demonstrate significant antidiabetic and wound healing potential of leaves of *Jasminum sambac*, justifying the use of plant in the indigenous system of medicine. Further studies for investigating the specific compound(s) responsible for such beneficial role in diabetes would open new outlook in the therapy of type 2 diabetes.

References

1. Lakshmi, Y.M. Rao, C.H. Bhargavi, U. Seelam Antidiabetic and wound healing activity of various bark extracts of Polyalthia longifolia Asian J Pharm Clin Res, 4 (2011), pp. 109-113
2. H. Brem, M.T. Canic Cellular and molecular basis of wound healing in diabetes J Clin Invest, 117 (2007), pp. 1219-1222
3. I. Vogel H.G., Vogel W.H. 2nd ed. Springer Publication; New York: 2002. Chapter K: Anti-diabetic activity, Drug Discovery and Evaluation, Pharmacological Assays. (pp. 535–538)
4. Morton JJ, Malone MH. Evaluation of vulneray activity by an open wound procedure in rats.

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5. Arch Int Pharmacodyn Ther. 1972 Mar; 196(1):117-26.
6. C.P. Kulkarni, S.L. Bodhankar, A.K. Ghule, V. Mohan, P.A. ThakurdesaiAntidiabetic activity of trigonella foenumgraecum l. Seeds extract (ind01) in neonatal streptozotocin-induced (n-stz) rats
7. Diabetol Croat, 41 (2012), pp. 29-40
8. M.A. Patil, P. Suryanarayana, U.K. Putcha, M. Srinivas, G.B. Reddy Evaluation of neonatal streptozotocin induced diabetic rat model for the development of cataract Oxid Med Cell Longev (2014), Article 463264
9. Ghosh S1, Suryawanshi SA.Effect of Vinca rosea extracts in treatment of alloxan diabetes in male albino rats. Indian J Exp Biol. 2001 Aug;39(8):748-59.
10. T. Inoguchi, P. Li, F. Umeda, et al.High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cellsDiabetes, 49 (2000), pp. 1939-1945
11. R. Rahimi, S. Nikfar, B. Larijani, M. Abdollahi A review on the role of antioxidants in the management of diabetes and its complications Biomed Pharmacother, 59 (2005), pp. 365-373
12. Chithra P, Sajithlal GB, Chandrakasan G. Influence of *Aloe vera* on theglycosaminoglycans in the matrix of healing dermal wounds in rats. J Ethnopharmacol. 1998;59:179–86
13. Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD, Goel RK. Evaluation of wound healing activity of extracts of plantain banana (Musa sapientum var.paradisiaca) in rats. Indian J Exp Biol. 2009;47:32–40.

Table: 1. Qualitative phytochemical analysis of various extracts of *Jasminum sambac*

Type of Constituent	**Root**	**Leaves**
Alkaloids	-	-
Phenolic compounds and tannins	-	-
Flavonoids	-	+ +
Saponins	-	+
Steroids	+ +	+ + +
Triterpenoids	+ +	+ + +
Proteins	-	+ +
Carbohydrates	-	+
Glycosides	-	-

Table: 7.11 Effect of EEJS on liver glycogen content in STZ induced diabetic rats

Treatment	**Liver Glycogen content (mg/g tissue)**
Group I Control 0.5% CMC (1ml/kg; p.o)	54.43 ± 5.45
Group II Diabetic Control	22.12 ± 2.35***
Group III Metformin (250 mg/kg, b.wt; p.o)	53.75 ± 5.53***
Group IV EEJS leaves (50mg/kg, b.wt; p.o)	23.34±2.56
Group V EEJS leaves (100 mg/kg, b.wt; p.o)	25.56 ± 3.56
Group VI EEJS leaves (200 mg/kg, b.wt; p.o)	48.76 ± 4.27***
Group VII EEJS root (50mg/kg, b.wt; p.o)	24.56±2.56
Group VIII	26.57 ± 3.76

The values are expressed as Mean ± SEM, n=6.* p<0.05, ** p<0.01, *** p<0.001, extract treated groups were compared with diabetic control group. One way ANOVA by Tukey's multiple comparison test. *** p<0.001, diabetic control group was compared with the normal group.

Period of epithelialization

In this study, the epithelialization period was significantly (p<0.01; P<0.001) lower in 10% and 5 % ointment of EEJS leaves and roots as that wound induced group.

Groups	Period of epithelialization in days
Control (Ointment base)	21.56±0.57

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Excision wound	23.76±0.65
DC + EEJS leaves 2% ointment base	22.28±0.56
DC + EEJS leaves 5% ointment base	17.28±1.43 **
DC + EEJS leaves 10% ointment base	15.12±0.67***
DC + EEJS roots 2% ointment base	22.12±0.45
DC + EEJS roots 5% ointment base	16.24±0.56**
DC + EEJS roots 10% ointment base	14.65±0.87***
Standard Povidone iodine ointment	14.12±0.65***

The values are expressed as Mean ± SEM, n=6. ** $p<0.01$, *** $p<0.001$, extracts treated groups were compared with wound induced group. One way ANOVA by Tukey's multiple comparison test.