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PHARMACOLOGICAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING *STEMODIA VISCOSA* AQUEOUS EXTRACT

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Keywords:

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ABSTRACT: The Pharmacological effects of silver nanoparticle of *Stemodia viscosa* aqueous plant extract on albino wistar rats was explored**.** The plant extract 40 ml was added to 10 ml of 10mM silver nitrate aqueous solution and kept at room temperature for synthesizing silver nanoparticle. Diabetes was induced in overnight fasted mice by single intra-peritoneal injection of 55 mg/kg of Streptozotozin (STZ), freshly dissolved in 0.1M cold citrate buffer, pH 4.5. After five days of STZ administration, blood was collected and plasma glucose levels were determined. For wound healing activity, a control ointment base was formulated without any drug content. Two creams were formulated by using 5% extract were incorporated in 100gm of cream base. The observation of the percentage wound contraction were made on 4^{th} , 8^{th} , 12^{th} , 16^{th} and 22^{nd} day of post wounding days. All the values were statistically analyzed by unpaired student test comparing with control. The administrations of plant extract to STZ induce diabetic mice, significantly lowered the plasma glucose level as compared to diabetic control. The study is further, extended to analyze lipid profile which was significant that Streptozotocin induced mice was found to be higher with 67.05 mg/dl than the normal control mice. The ointment formation were prepared from the plant extract the results given in that wound contracting ability of *Stemodia viscosa* ointment (5% w/w) was found significant than the control. *Stemodia viscosa* exhibit a remarkable wound healing and anti-lipedmic activity.

INTRODUCTION: The number of chronic wounds will increase worldwide due to the increase in age-related conditions and pathologies such as diabetes, obesity, and cardiovascular diseases. An estimated excess of US$25 billion is spent annually on treatment of chronic wounds, and the burden is rapidly growing due to increasing healthcare costs, an aging population, and a sharp rise in the incidence of diabetes and obesity worldwide.

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While current therapeutic agents have generally inadequate efficacy and number of serious adverse effects, the medicinal plants have been used in medicine since ancient times and are well known for their abilities to promote wound healing and prevent infection without grave side effects. Thus, herbal therapy may be an alternative strategy for treatment of wounds [1]. Wound healing is a complex process encompasses migration to the wounded area, proliferation, deposition and remodeling of extracellular matrix [2, 3, 4].

A variety of materials for wound dressing, skin substitutes and recombinant growth factors entered into the markets which are shown to enhance the healing process entered into the market with therapeutic efficiency [5].

Many natural products and extracts are also known to posses wound healing properties [6, 7]. Hence, there is a great interest to find new wound healing products. Wound healing is a complex series of interrelated events that are mediated through the phases by a wide range of chemically co-ordinate cellular processes as well as hormonal influences [8]. Medicinal plants have been shown to possess wound healing activity in animal studies [9, 10].

The recent emergence of nanotechnology has provided a new therapeutic modality in silver nanoparticles for use in wounds. Nonetheless, the beneficial effects of silver nanoparticles on wound healing remain unknown [11].

Nowadays, silver-based topical dressings have been widely used as a treatment for infections in open wounds and chronic ulcers. Silver ions (Ag^+) loaded zirconium phosphate nanoparticles is a novel nanosized and highly crystalline antibacterial agent which carries (Ag^+) ions by ion exchanging. It can also protect the host material from oxidation and discoloration and have been often used as additive for wound healing [12].

Diabetes Mellitus (DM) is one of the most prevalent metabolic disorders characterized by increased blood glucose level and improper primary metabolism resulting from the defects in insulin secretion, insulin action, or both. It is one of the most common health problem worldwide, and the prevalence of this disease is rapidly increasing, leading to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complication [13, 14]. Reactive oxygen species (ROS) produced during hyperglycemia, which plays a major role in complications of diabetes. Antioxidants from the natural sources act as free radical scavengers and lower the risk of diabetic complication [15, 16].

Though antidiabetic drugs are available for long term therapy are found to be associated with various toxicities and none of them gives long duration glycaemic control without causing any adverse side effects. Thus there is a growing interest in using natural plant sources having minimal side effects for the treatment of DM [17, 18]. *Stemodia viscosa*, is a genus of about 40 species belonging to the family Scrophulariaceae occurring in tropical and subtropical regions of the world. The chemical investigation of this genus is restricted to five species from which flavanoids, diterpenes, and diterpenes derivatives with a rare tetracyclic skeletal, named stemodane, were isolated [19]. *Stemodia viscosa* Roxb. an aromatic weed found in cultivated fields of India, is also referred to as sticky blue rod, Pintye *etc*.

It is an erect, aromatic, viscidly pubescent herb with quadrangular stem reaching up to 60 cm high. Leaves are 4 cm long, sessile, oblong and amplexicaul. Flowers axillary, violet in colour and bilipped. The fragment leaves of this herb are placed in pillows to induce a restful sleep, or crushed and mixed with fat to make rubbing medicine to treat cold and flu symptoms [20].

The present review describes currently available topical wound healing medications and their drawbacks. We describe the properties of silver nanoparticles to aid wound healing and anti-inflammatory effects; diminished resistance of bacteria to silver nanoparticles; and silver nanoparticles, mechanism of action. In addition, dressings impregnated with silver nanoparticles, the role of silver nanoparticles in impaired wound healing, and silver nanoparticles, mechanism of action in wound healing are discussed.

However very little information has been published on this plant and there is no scientifically proven data to show whether the silver nanoparaticle of *Stemodia viscosa* aqueous plant extract has pharmacologic activity or not. Therefore we have undertaken the present study to explore the effects of the above extract on wound healing and antilipedmic activity in diabetic mice.

MATERIALS AND METHOD:

Collection of Sample: For the synthesis of silver nanoparticles, *Stemodia viscosa* was collected from the village Mallampatti, Madurai District, Tamil Nadu, India during March 2016 and was authenticated by Dr. Karunai selvi, Assistant Professor, Department of Botany, V. V. Vanniaperumal College for women, Virudhunagar, Tamil Nadu, India. Silver nitrate ($AgNO_3$) was purchased from Merck Limited, India. The nutrient media used was supplied by Hi–Media Laboratories.

Preparation of the Plant Extract: Extract have been prepared by using fresh plant of *Stemodia viscosa*, weighing 20 grams. The plant was washed thoroughly thrice in distilled water and it was cut into fine pieces, transferred into a 500 ml Erlenmeyer flask with 100 ml of distilled water and boiled for 10 minutes. It was then filtered to obtain the plant extract.

Synthesis of Silver Nanoparticles: In a typical synthesis of silver (Ag) nanoparticles, the plant extract 40 ml was added to 10 ml of 10mM silver nitrate aqueous solution and kept at room temperature. The experiment was done in triplicate for reproducibility. After 1 hour the color of the extract changed from colorless to honey brown indicating the formation of silver nanoparticles.

Ointment Formulation: A control ointment base was formulated without any drug content. Two creams were formulated by using 5% extract (5% of aqueous extract of *Stemodia viscosa* were incorporated in 100 gm of cream base). The standard drug for screening wound healing activity is Petroleum jelly ointment (5% w/w) which was brought commercially.

Experimental Animals: Albino rats of either sex (130 - 180 gm) were procured from animal house, Venture Institute of Biotechnology, Madurai, Tamil Nadu, India used for the present study. They were maintained under standard conditions (24 - 28 ^{o}C) and fed a standard diet for mice and given water. The care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India.

Wound Healing Activity: [21] The hairs were removed from the dorsal thoracic region of the rats using depilitor and veet hair removing cream. A full thickness incision wound of circular area of 500 mm² and 2 mm in depth was created along markings under mild anaesthesia.

Each group contain three animal group I served as control, group II as standard control and group III as treated group; simple ointment and ointment containing the extracts were applied everyday topically from 0 to 20 day post wounding or number of days required for falling of the escher without any residual raw wound gave the period of epithelization, starting from the day of the excision. Change in wound areas were calculated giving an indication of the rate of wound contraction. The areas of the wounds were measured by tracing the wounds on to a graph paper on the day of wounding and subsequently on 0, 4th, 8th, 12th, 16th and 20th day of post wounding.

The number of days required for falling of the scar without any residual raw wound, gave the period of epithelization. The observation of the percentage wound contraction were made on 4th, 8th, 12th, 16th and 20th day of post wounding days.

Antilipdemic Effect in Diabetic Mice:

Induction of Diabetes and Experimental Design: Diabetes was induced in overnight fasted mice by single intra-peritoneal injection of 55 mg/kg of Streptozotozin (STZ), freshly dissolved in 0.1M cold citrate buffer, pH 4.5. After five days of STZ administration, blood was collected and plasma glucose levels were determined. The animals confirmed as diabetic by the elevated plasma glucose levels (> 200 mg/dl) were used for the experiment.

Biochemical Analysis: Serum glucose was measured by using a glucometer (Accu - Chek Active, India). Serum total cholesterol [22], total triglyceride [23], LDL-C, VLDL-C [24] and HDL-C [25] were estimated using standard Enzymatic (Span Diagnostic, India).

RESULTS:

Wound Healing Activity: Table 1 depict the effect of *Stemodia viscosa* as ointment on the wound in mice model.

TABLE 1: EFFECT OF SILVER NANOPARTICLES OF *STEMODIA VISCOSA* ON EXCISION WOUND MODEL

Parameter	Wound area (mm^2)					
	0 day	4th day	8th day	12th day	16th day	20th day
Group I Control	525±1.3	490±1.5	380±2.5	260±2.59	175±1.7	125±2.8
Group II Standard	535±2.0	320±2.5	190±2.0	100±2.3	40±1.3	0
Group III-5% nanoparaticle extract ointment	530±1.5	350±2.0	200±1.19	111±1.12	45±1.03	0

Values are expressed as mean ± SE, n=3

The ointment formation were prepared from the plant extract the results given in that wound contracting ability of *Stemodia viscosa* ointment (5% w/w) was found significant than the control.

Antilipdemic Effect in Diabetic Mice: In animals especially mice diabetic can be induced by administration of diabetogenic trease such as Streptozotozin. The result indicate Streptozotozin induced mice led to significant elevation in blood glucose level during 15 days of treatment when compared with that of normal control mice. Further, plants extract *Stemodia viscosa* lead to significant reduction in blood glucose level when compared to Streptozotozin treated experimental mice. Effect of plant extract *Stemodia viscosa* on lipid profile in Streptozotozin induced diabetic mice. **Table 2** depict lipid profile of total cholesterol, HDL cholesterol, LDL cholesterol, and Triglycerides. Streptozotozin treatment resulted in significant increase of total cholesterol with 105.2 mg/dl was found to be elevated high where as the normal control mice revealed 75.2 mg/dl where as plant extract and Streptozotozin treated mice found to exhibit 75.5 mg/dl. The effect of Streptozotozin induced mice on HDL cholesterol was found to be decreased to 18.5 rather compared to the normal mice which exhibited 40.2. On analysis of plant extract with Streptozotozin revealed 34.07 ± 1.12 mg/dl.

The study is further extended to analyse LDL cholesterol in which Streptozotozin induced mice revealed 67.05 mg/dl followed by the normal control mice which exhibited 30.5 mg/dl. Similarly analysis of plant extract along with Streptozotozin induced mice revealed 52.3 ± 2.8 mg/dl respectively. Similarly the normal control mice for triglycerides level exhibited 71.9 mg/dl followed by the drastic increase of triglycerides content with 90.2 ± 2.3. Simultaneously, Streptozotozin along with the plant extract revealed 68.5 ± 5.7 respectively.

TABLE 2: HYPOGLYCEMIC EFFECT OF SILVER NANOPARTICLES OF *STEMODIA VISCOSA* IN STREPTOZOTOCIN INDUCED DIABETIC MICE

S. no.	Treatment (groups)	Biochemical Parameters		LDL cholesterol mg/dl	Triglycerides mg/dl
		Total cholesterol mg/ dl	HDL Cholesterol mg/dl		
1.	Untreated normal control	75.52 ±7.5	40.2 ±1.67	30.5 ±0.67	71.9 ±4.10
2.	Streptozotocin Induced mice	105.2 ±5.2	18.5 ±1.53	67.05	90.2±2.3
3.	Plant extract + Streptozotocin Treated mice	77.5±5.76	34.07±1.12	52.3±2.8	68.5±5.7

Values are expressed as mean ± SE, n=3

DISCUSSION: The process of wound healing occurs in four phases 1) coagulation, which prevents blood loss, 2) inflammation and debridement of wound, 3) repair, including cellular proliferation and 4) tissue remodeling and collagen deposition. Any agent which accelerates the above process is a promoter of wound healing. Plant products have been shown to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing is due to the presence of active terpenes, alkaloids and flavanoids [26].

The wound healing properties of *Stemodia viscosa* appear to be due to the presence of its active principles which accelerates healing process and confer breaking strength to the healed wound. *Stemodia viscosa* shows a significant of wound healing activity as evidence by these corrections in the number of required, for falling of the wound contraction compared to the control. The changes in the wound area were measured at fixed time at 4^{th}, 8^{th}, and the 12^{th} day. The period of epithelization was 20 days for treatment groups. Simultaneously, where control groups were painterly, our results are in total conformity with the work of Panduraju *et al*., 2011 [27].

The hypoglycemic activity of the plant extract was persistant throughout the period almost in comparable to the normal control mice. Whereas the plant extract treated as the level of blood glucose was found to be gradually decrease which revealed anti-hypoglycemic potential of plant extract *Stemodia viscosa*. The antidiabetic effect of plant extract due to the action of one or more of

these components which stimulate the secretion of insulin to activation of enzymes. The present of tannins, alkaloids, and glycosides present in the plant could at independently in enhancing the activity of glycolytic enzymes [28]. 1-Heptadecanol with molecular mass of 256 was detected in the *Jatropha gossipifolia* extract. The same compound was also found in *Allamanda violacea* [29]. This compound was reported to demonstrate antioxidant, hypocholesterolemic, haemolytic, nematicidal and pesticidal activities [30]. Hypoglycemic activity of nanosynthesized plant extract was used to treat the diabetic patient.

The current study made with the plant extract of *Stemodia viscosa* was orally supplemented in mice model for a period of 15 days in order to reduce the diabetic condition. The results revealed the effect of plant extract on lipid profile in STZ induced diabetic mice revealed a significant increase of total cholesterol with 105.2 mg/dl was found elevated than the normal control mice. It has been shown that the administration of plant extract *Stemodia viscosa* to STZ induce diabetic mice, significantly lowered the plasma level as compared to diabetic control. Moreover, administration of extract of *Stemodia viscosa* was found effectively prevent increase in blood glucose level may be due to desideration of insulin. The study is further, extended to analyze LDL- cholesterol, and HDL-cholesterol which was significant that Streptozotozin induced mice was found to be higher with 67.05 mg/dl than the normal control mice.

CONCLUSION: From the above results, it was found out that *Stemodia viscosa* exhibit a remarkable wound healing and anti-lipidemic activity. Hence, *Stemodia viscosa* can be used for the treatment of various diseases like diabetics, anti-lipidemic and for wound healing purposes.

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CONFLICT OF INTEREST: Nil

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