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SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLE OF GINGER RHIZOME (ZINGIBER OFFICINALE) EXTRACT: SYNTHESIS, CHARACTERIZATION AND ANTI DIABETIC ACTIVITY IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT
In the current work, the antidiabetic potentials of silver nanoparticle of ethanolic extaract of ginger rhizome (Zingiber officinale) (SNEG) were studied in rats. Now a days a vast research is going in the field of metal and semiconductor nanoparticles as they have very potent application in the development of novel delivery system, In present work a economic and very favourable method is described for the synthesis of silver nanoparticles and ethanolic extract of *Zingiber officinale* rhizome through silver nitrate solution is used for the evaluation of antidiabetic activity. Various analytical methods such as Atomic Force Microscopy, X-ray Diffraction and Fourier Transform Infrared Spectroscopy were employed for monitoring the formation of nanoparticles. Presence of silver nanoparticles with an average size of 123.8 nm was revealed by Atomic Force Microscopy. The crystalline nature of synthesized nanoparticles was done by X-ray diffraction. This work shows that silver nanoparticles have good antidiabetic activity in comparison with standard hypoglycaemic agents, hence more work and investigation for clinical application is necessary.

KEYWORDS: Antidiabetic activity, Fourier Transform Infra Red Spectroscopy, Silver nanoparticles, X-Ray Diffraction, *Zingiber officinale.*

1. INTRODUCTION
Nanotechnology can be defined as the formation, development, enhancement and exploration of nano sized materials having size range of (1-100nm). It works with the substances which have specific properties such as physical, chemical, and biological. Now a days nanotechnology works on the design and development of many novel formulations for the prevention, treatment and diagnosis of many critical diseases[1] like cancer, T.B. and cardiovascular diseases etc. Because of their specific characteristics, silver nanoparticles (AgNP) may used as catalysts[2] in spectrally selective coatings for absorption of solar energy as optical sensors[3] in fabric tailoring as well as in electronics devices[4] and in various therapeutic activity of bactericidal agents.[5, 6] AgNP have a important role in prohibiting microbial (bacterial) growth in solid and liquid culture media because of its high reactivity.[7] AgNP prepared by green synthesis phenomena is effective in biological environmental systems.[8]

Ginger (Zingiber officinale) is a herbaceous plant having green grass shaped leaves and yellowish green flowers with purple marking. It is cultivated for the rhizomes which is used due to its culinary and medicinal importance.[9, 10] Ginger possesses various properties including carminative aromatic, and absorbent characters.[11, 12] Ginger is abundantly used as spice and functional food. For decades ginger has been an important ingredient in various alternative methods of treatments such as Chinese, Ayurvedic and Tibb-Unani herbal medicine. The therapeutic properties of ginger include anti-arthritic[13,14], anti-migraine[15], anti-thrombotic[16], anti-inflammatory[16], hypolipidaemic[16,17,18], hypo-cholesterolaemic[18] and anti-nausea properties.[10] Ginger have a number of potentially bioactive phytoconstituents, mainly gingerols and their related dehydration constituents, and various volatile oils including sesquiterpenes, like (-)zingiberene and b-bisabolene, and monoterpenes, mostly neral and geranial.[19,20] In which, gingerols prohibit both leukotriene and prostaglandin biosynthesis[21] and

angiogenesis.[22] In addition, several ginger components have serotonin receptor-blocking activity[23]

Some isolated work about the hypoglycaemic properties of ginger in animals model have shown variable results [24]. studies shows a little but notable blood glucose-lowering effect of ginger juice in diabetic and non-diabetic animals[25], also observed that ginger juice have hypoglycaemic effect in both normal and streptozotocin (STZ)-induced diabetic animal (rats). Mascolo et al., 1989, provided a important hypoglycaemic activity in normal rabbits at different times after a variety of administration schedules and doses.[26] Weidner and Sigwart, 2000, reported that an ethanolic extract of ginger had shown no activity on blood glucose levels in normal rats.[27] In addition, Singhal and Joshi, 2003, in their study reported an increase of blood sugar in normal rats given a dose of 30% ginger powder.[28] As different ginger preparations have used there is variation of the results in the studies.

The motive of the present study was to know the efficacy of an aqueous extract of raw ginger in maintaining serum sugar, cholesterol and triacylglycerol levels in Streptozotocin induced diabetic rats was treated routinely intraperitoneally (IP) for a period of 7 weeks. As there have been many different reports about the use of different preparations of ginger, aqueous extracts of raw ginger were used in the current study. The mode of administration was taken to be IP as we have previously reported similar actions with IP and per-oral administration of ginger drug in normal animal (rats)[16], and IP administration was shown to be less stressful for the animals. Other than this, the chosen dosage of 500 mg raw ginger extract/kg body weight was previously shown to be effective and non-toxic in rats while a dosage of 50 mg/kg gave different results.[16]

2. MATERIAL AND METHODS

2.1 Material

Zingiber officinale rhizomes were collected from Botanical garden, Guru Ramdas Khalsa Institute of Science and Technology (Pharmacy), Jabalpur, MP, India. Streptozotocin and Metformin hydrochloride was obtained from as a generous gift sample from Micro Lab Ltd, Bangalore. India. $AgNO_3$, ethanol and other chemicals was used analytical reagent grade.

2.2 Extract preparation

Zingiber officinale rhizomes were collected from Botanical garden, Guru Ramdas Khalsa Institute of Science and Technology (Pharmacy), Jabalpur, MP, India. The collected rhizomes were milled to fine powder and were stored at room temperature. The rhizome was dried and ground into fine powder using a blender. Than powder (200g) was homogenized in ethanol (95%; 500 ml) and left in a laboratory conical flask at normal room temperature for 4 days. After that, the mixture was filtered through a fine muslin cloth and a filter paper (Whatman filter paper). Then the mixture was heated for 5 minutes using the vaccum rotary evaporator the extract became concentrated. The extract was then used further as ginger rhizome broth for the experiment.

2.3 Synthesis of silver nanoparticle

A 0.017gm of silver nitrate was accurately weighed and dissolved in 100 ml of double distilled water and stored in amber coloured bottle until further use**.** A 100 ml of 1mM silver nitrate ($AgNO_3$) was heated to boiling. To this appropriate solution, 10 ml of ginger rhizome broth was poured for reduction of silver ions (Ag+), then kept at room temperature for 1 hour, the change in colour of solution was examined timely. The color change from yellow to yellowish brown indicated the formation of silver nanoparticle. It observed that the aqueous silver ions could be reduced by the ethanolic extract of ginger officinale to generate very stable silver nanoparticles. The prepared silver nanoparticle was lyopillized for further use.

2.4 FTIR analysis of SNEG

FTIR Spectra of AgNP formed with ginger rhizome broth were determined with a FT-IR spectrophotometer (Cary- 630 FTIR, Agilent Technologies).

2.5 Particle Size Determination

The mean particle size and zeta potential of silver nanoparticle of ethanolic extract of *Ginger officinale* (SNEG) was determined using particle size analysis instrument (Malvern Instrument, United Kingdom).

2.6 Morphology

2.6.1 Atomic Force Microscopic analysis

The structure and surface characterstics of SNEG was estimated by AFM (AIST-NT Smart SPM 1000, CA). AFM of the nanoparticles was accomplished at glass substrate in AC mode.

2.7 XRD analysis of SNEG

Crystalline nature of silver nanoparticle of ethanolic extract of ginger officinale was confirmed by X-ray diffractometer (Bruker, Munich, Germany). The 124 scanning rate was 2θ/min over a 2θ range of 0–40º and with an interval of 0.02º.

2.8 Anti diabetic activity

2.8.1 Pharmacological screening of antidiabetic activity Animals: Wistar albino rats weighing about 150-200 gms of either sex were taken for the pharmacological studies. The animals were kept under standard conditions, room temperature (25°C), 45-65% relative humidity, standard pellet diet and water was provided. The animals were housed for one week in cages prior to the experiments to make them to laboratory conditions. It is normally distributed into three different groups with six animals in each group under equal conditions throughout the experiments. The experimental protocol was approved Institutional Animal Ethics Committee of the Guru Ramdas Khalsa Institute of Science and Technology (Pharmacy), Jabalpur (MP)

India (Registration Number 1471/PO/a/11/CPCSEA, India). To induce diabetes initially all the animals were treated with streptozotocin 150mg/kg body weight through intraperitonially. Animals were divided into three groups having eight rats in each group. Group first serves as control, Group second serves as standard and treated with Metformin hydrochloride (10 mg/kg of body weight), and group third serves as test and treated with SNEG (200mg/kg of body weight).

2.8.2 Method

The acclimatized animals were kept fasting for one day with water *ad libitum* and injected intraperitonially at a dose of 150mg/kg of body weight of streptozotocin freshly prepared in normal saline (0.9% w/v) solution. Before starting the experiment animals were grouped according to their weight of body. After 60 minutes of streptozotocin administration, animals were given feed according to their need and provided 1 ml of (100ml/dL) glucose I.P to battle encountering severe hypo glycaemia. After 72 hours of streptozotocin injection, the animals were examined for presence of diabetes by estimating their blood glucose level by using glucometer. The blood glucose levels were more than 140 mg/dl was criteria for diabetes according to world health organization. To the animals the SNEG (200 mg/kg of body weight, intra peritonially) and standard drug Metformin hydrochloride (10 mg/kg body weight) were administered by dissolving in 2% Tween 80, water and normal saline respectively. The blood glucose levels were monitored at interval of initial (zero hour), 1st, 3rd, 5th and 7th day of administration of unit dose for acute and controlled release action was studied respectively. The body weight of the animals from all the groups was recorded and all the parameters were tabulated.[29,30]

3. RESULT AND DISCUSSION

3.1 FTIR analysis of SNEG

FTIR analysis was done to measure the characterization and conjugation of potent biomolecules of ginger rhizome with silver nanoparticles. According to the FTIR spectra of SNEG (Fig 1) different peaks was obtained which indicates different functional groups and intermolecular bonding of SNEG. The spectra are obtained at 1384, 2923, 3419, 1648, 1163, 1376, 1059 and 1113 cm-1 indicates the presence of $NO3^-$, O-H carboxylic acid, O-H stretching H-bonded alcohols and phenols, N-H bonded primary amines, C-N stretching alcohols, C-N stretching of aromatic amine group, C-N stretching ester and ethers in the residual solution. The variety of functional groups indicated in FTIR spectra are mainly obtained from heterocyclic substances that are generally water soluble compounds of ginger rhizome. So it can be understood that various water soluble heterocyclic compounds like alkaloids and flavonoids works as the capping ligands for the formation of SNEG.[31]

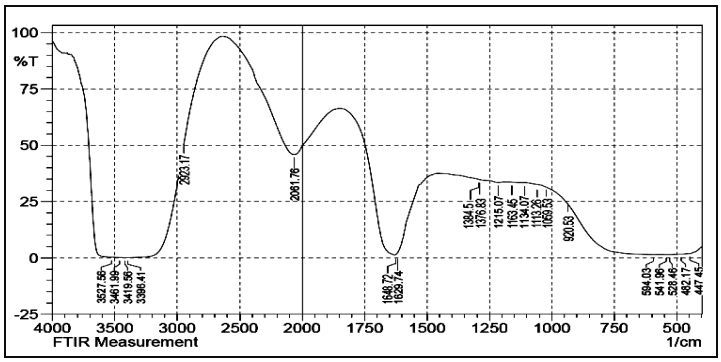

Fig 1: FTIR spectrum of the SNEG.

3.2 Particle Size Determination

The average particle size of SNEG were found to be 123.8±0.16 nm (Fig 2) and distribution of particles size of SNEG were found to be 72.3% of 126.31nm, 19.5% of 116.67nm and 8.2% of 124.5nm and zeta potential of SNEG was found to be -20.2 mV (Fig 3).

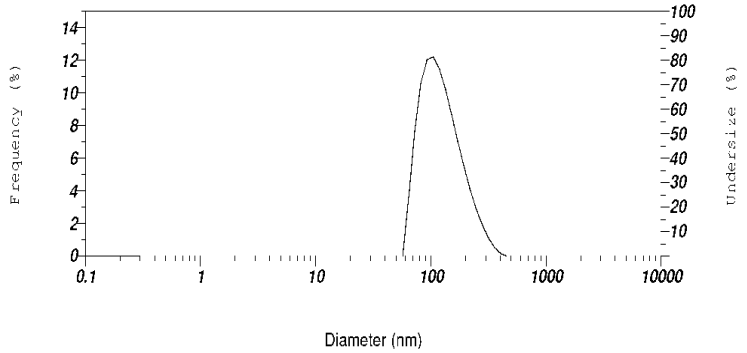

Fig 2: Particle size study of SNEG.

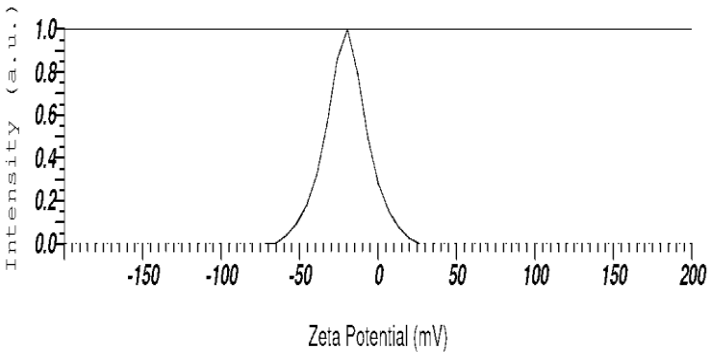

Fig 3: Zeta potential study of SNEG.

3.3 Morphology

3.3.1 Atomic Force Microscopic analysis

The structure and surface characterstics of SNEG was characterized by atomic force microscope and image was obtained. The SNEG was visullized in spherical shaped and in nanometric size range (128nm) as estimated by AFM image (Fig 4).

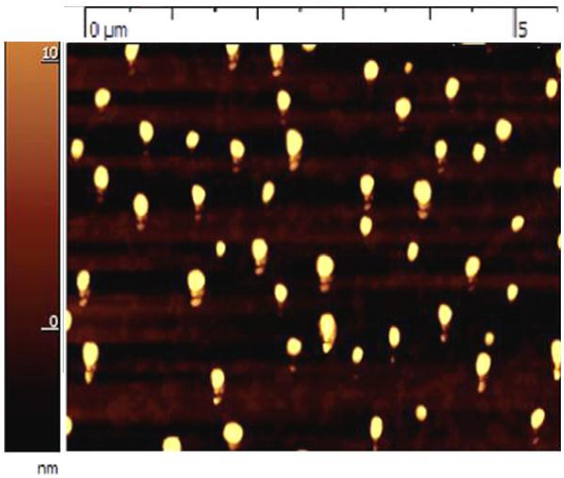

Fig 4: AFM image of SNEG.

3.4 XRD analysis of SNEG

XRD is a very potent technique that is normally used to analyze the characteristics and details of structure nanoparticles. The XRD patterns are observed by measuring the angles at which an X-ray beam is diffracted by the crystalline phases in the object. The XRD pattern of synthesized Ag-NPs is shown in fig 5, the following prominent peaks at 18°, 28°, 34°, 38°, 42°, 45°, 59°, 64° and 78° (2θ) indicated the crystalline property of silver nanoparticles, from the XRD pattern it is known that Ag-NPs formed using ginger rhizome broth were essentially crystalline in nature.

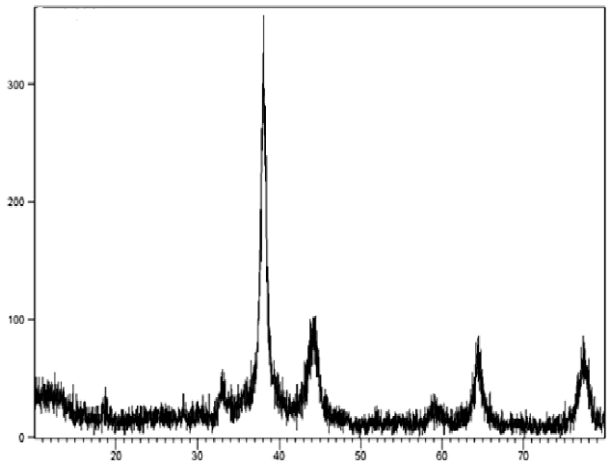

Fig: 5. XRD spectrum of green synthesized of SNEG.

3.5 Anti diabetic activity

The data of the blood glucose level of rats treated with streptozotocin (150mg/kg body eight) produced diabetes within 72 hours. After 72 hours of streptozotocin administered the blood glucose levels of rats were observed above 150 mg/dl. (Table 1) According to the world health organization the blood glucose level above 150 mg/dl indicates the hyperglycaemic action. Extreme thirst, downfall in body weight were also noticed (Table 2). The administration of *Zingiber officinale* ethanolic extract at a dose of 200 mg/kg body weight showed significant antihyperglycaemic effect which was evident from the 1st day. The reduction in blood glucose was highly significant on the 7th day. The antihyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats is shown in Table 1 and Fig 6, the fasting blood sugar level of diabetic animal notabaly reduced from 242 ± 1.5 mg/dl to 86 ±0.91 mg/dl on 7th day after the administration of the SNEG. The decreasing blood glucose levels are comparable to that of 10 mg/kg of Metformin hydrochloride. The present study was undertaken to evaluate the antidiabetic properties of an ethanolic extract of ginger in streptozotocin induced diabetic rats. Streptozotocin damage to the islet tissue was confined to the insulin secreting beeta cell of pancrease (*Dunn Js kekpatric*) through a direct action (*Hellman Diderholn*). The alpha cells being resistant to streptozotocin (*Dunnis Js*

Duffing). Thus Streptozotocin proved to be a suitable compound for inducing experimental diabetes with specific symptoms such as given above. Watching the complications associated with marketed antidiabetic drugs, health care personnels are searching for safe alternatives for them**.** Recently various herbal medications with different mode of action have been found to have antidiabetic effect. The blood glucose levels of the hypoglycaemic activity of streptozotocin induced diabetic rats and effect of SNEG on diabetic rats were given in Table 1 and 2 respectively. It shows the reduction in blood glucose levels and effect on body weight. Based on our recent results**,** it looks like that ethanolic extract of *Zingiber officinale* shows notable blood glucose – lowering in Streptozotocin induced diabetic rats. Further studies are necessary to find the exact mode of action of the hypoglycaemic activity of SNEG**.**

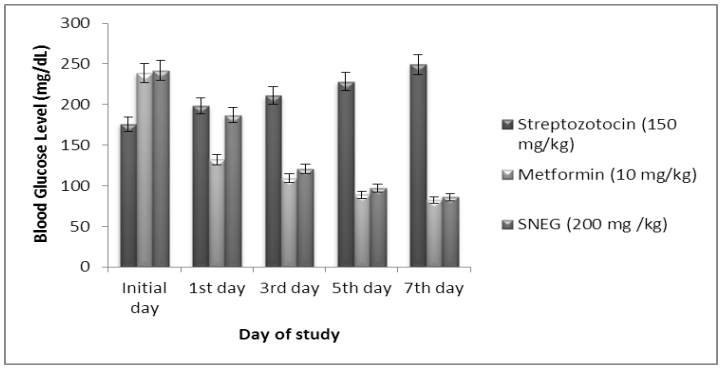

Fig 6: Effect of SNEG on fasting blood glucose levels.

Table 1: Effect of SNEG on fasting blood glucose level in Streptozotocin induced diabetic rats.

Treatment	Dose	Blood Glucose Level (mg/dL)					
		Normal	After Streptozotocin Induction	1st day	3rd day	5th day	7th Day
Control	Streptozotocin (150 mg/kg)	82 ± 1.15	176 ± 0.5	198 ± 1.13	211 ± 1.18	228 ± 1.5	249 ± 0.67
Standard	Metformin hydrochloride (10 mg/kg)	78 ± 0.85	239 ± 1.5	132 ± 1.8	109 ± 1.17	89 ± 0.74	82 ± 1.9
Test	SNEG (200 mg /kg)	77 ± 1.15	242 ± 1.5	187 ± 0.98	121 ± 0.32	97 ±1.19	86 ±0.91

n ± eight animals in each group.

Table 2: Effect of SNEG on body weight in Streptozotocin induced diabetic rats.

Treatment	Dose	Initial body weight	7th Day Body weight
Control	Streptozotocin (150 mg/kg)	170 ± 1.5	171 ± 0.68
Standard	Metformin hydrochloride (10 mg/kg)	171 ± 0.5	169 ± 1.1
Test	SNEG (200 mg /kg)	173 ± 1.19	171 ± 1.15

n ± eight animals in each group.

4. CONCLUSION

Plants and their extracts can be effectively used in the synthesis of silver nanoparticles. The shape and size of nanoparticles can be very easily controlled with the use of plants. In the present study we observed that ginger rhizome may be be good source for the synthesis of AgNP. This approach for synthesis of AgNP of ethanolic sxtract of *Ginger rhizome* has many advantages including the ease with which the process can be scaled up and its economic viability. The use of such eco-friendly nanoparticles as a antidiabetic agent in medical and electronic applications, renders this method potentially exciting for a large-scale synthesis of other inorganic materials (nanomaterials). The effect of antidiabetic activity of ethanolic extract of ginger rhizome can be enhanced via formation of silver nanparticle by nanotechnology process. Results revealed that SNEG showed effective antidiabetic activity towards Streptozotocin induced diabetic rat. Hence it can be concluded that nanosize particle ginger rhizome was more effective in diabetes inhibitory better than the larger size particles.

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