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RESEARCH ARTICLE

Protective effect of Gymnemic acid isolated from *Gymnema sylvestre* leaves coated Chitosan reduced gold nanoparticles in hyperlipedimia and Diabetes Induced vascular tissue damage in Rats

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ABSTRACT:

Objective: To synthesis chitosan reduced gold particle by using gymnemic acid isolated from *Gymnema sylvestre* leaves and to evaluate the antidiabetic activity against streptozocin and highfat diet treated rats. **Methods:** In this research paper, the synthesis gold particle by using gymnemic acid isolated from *Gymnema sylvestre* leaves with chitosan reducer. The synthesized AuNps were determined by UV–vis spectrum, high resolution transmission electron microscopy, X-ray diffraction, and Fourier transmission infrared spectroscopy analysis. The synthesized gymnemic acid coated chitosan reduced gold nanoparticles is subjected to evaluate its antidiabetic activity using high fat diet fed streptozotocin induced type 2 diabetes mellitus rats. **Results:** UV–vis spectrum showed a peak at 520 nm due to excitation of surface Plasmon vibrations. Fourier transmission infrared spectroscopy showed that nanoparticles were coated with plant secondary metabolites. HR-TEM and AFM images of synthesized AuNPS are spherical in shape and HR-TEM pictures evidently confirms the prepared AuNPS have the uniformed spherical shape with diameter of 14±3.69 nm in size respectively. The result of antidiabetic activity, chitosan reduced gold nano particles shows significant in both *invitro* and *invivo* models in high fat diet fed streptozotocin induced type 2 diabetes mellitus

KEYWORDS: Gymnemic acid, Gold Nanoparticles, GLUT IV, PPar γ and diabetes.

1. INTRODUCTION:

Nanomaterials have innumerable applications in variety of industries[1]. In general, noble metal nanoparticles are utilized for various biomedical applications and significant results have been obtained in the recent past[2]. Particularly, gold nanoparticles (AuNPs) are employed for the treatment of various diseases[3] due to their unique optical, chemical and biological properties[4]. Though variety of protocols is in practice[5], preparation of AuNPs by biosynthesis process has been the most preferred methodology for its facile and environment-friendly approach.

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However, a major drawback of biosynthesis includes agglomeration, uncontrolled size, shape, instability etc.

Currently employed physical, chemical and biological methods have associated with certain problems like toxicity, uncontrolled nucleation and polydispersity; therefore, a proper stabilizing as well as reducing agent is indispensable for the synthesis of monodispersive nanoparticles which is a major challenge to attain desired relevance in the field of Nanomedicine. Consequently AuNPs formation using biopolymers and biomolecules have attracted much attention and recently emerged as an exciting area of research in nanotechnology field.

Gymnema sylvestre R. Br is a woody, climbing plant that grows in the tropical forests of central and southern India and in parts of Asia[6]. It is a pubescent shrub with young stems and branches, and has a distichous phyllotactic

opposite arrangement pattern, which are 2.5-6 cm long and are usually ovate or elliptical, the flowers are small, yellow, in umbellate cymes and follicles are terete, lanceolate, up to 3 inches in length.

Gymnema sylvestre has been used in the treatment of diabetes since ages in folk, ayurvedic and homeopathic systems of medicine. It is also used in the treatment of asthma, eye complaints, family planning, snakebite, urinary complaints, stomach ailments, piles, chronic cough, breathing troubles, colic pain, cardiopathy, constipation, dyspepsia and hemorrhoids, hepato splenomegally. In addition, it also possesses antimicrobial, antitumor, obesity, anti-Inflammatory, and Antihyperglycemic Activity[7]. It has been extensively studied for its antihyperglycemic effect contains several active compounds useful for reducing the blood glucose levels in the case of diabetes mellitus. Gymnemic triacetate isolated from *G. sylvestre* was tested for its antidiabetic activity on streptozotocin-induced diabetic model. Similarly, extensive studies have been carried out for antidiabetes and its related complications using various medicinal plants. In the present investigation, biosynthesis of chitosan reduced AuNPs using antidiabetic potent gymnemic acid fraction from *G.sylvestre* R. Br. and its effect on high fat diet fed and streptozotocin induced type 2 model is discussed.

2. MATERIALS AND METHODS:

2.1 Collection and authentication:

Gymnema sylvestre leaves were collected from Anna Herbal Garden, Chennai, Tamil Nadu. It was identified by Botanist, Plant Anatomy Research Centre (PARC), Tambaram, Chennai, Tamil Nadu and specimen voucher (**PARC/2012/1279**) are kept at Department of Pharmacognoscy, Vels College of Pharmacy, Chennai. The leaves of *Gymnema sylvestre* were shade dried grounded and stored dry until extraction.

2.2 Extraction of Gymnemic acid by Hoopers method[8]:

Step 1: Extraction with petroleum ether (Defatting process):

I kg of dried *Gymnema sylvestre* dry leaf powder was packed into a clean soxhlet extraction unit. Seven litres of petroleum ether (60-80°C) was added and extracted for 24-36 hrs till all components are soluble in petroleum. petroleum ether extract is collected and distilled. Then a net of 240gm of petroleum extracts was obtained. petroleum ether extracts was obtained.

Step 2: Extraction with 90% methanol:

The plant material is then extracted with 90% methanol. 90% methanol is added to the marc and the extraction was continued up to 24-36hrs till total methanol soluble extracts was obtained. Then methanol soluble extract was distilled and finally 185gm of thick paste were obtained.

Step 3: Isolation of pure gymnemic acid from methanol extract:

175gm thick paste of methanol soluble extract was dissolved in 1% aq.KOH solution on continuously stirring for 45 min to 1hr.The solution is then filtered through filter paper to separate the undissolved particles. Diluted HCL was added slowly under constant stirring, during which the gymnemic acids were precipitated. Precipitated solution was filtered under suction and precipitate was dried. The pure gymnemic acid was obtained. Crude gymnemic acid fraction toal yield was found to be 29.6%. The isolated gymnemic acid fraction was subjected to qualitative chemical test and thin layer studies and positive tests for steroids, terpenoids and glycosides. For further studies the gymnemic acid fraction was dissolved in ethanol and used.

2.3 Chemicals:

Gold (III) chloride trihydrate ($HAuCl_4$.3H2O, 99.99%), Gymnemic acid, nitric acid (HNO_3) and hydrochloric acid (HCL) were purchased from Sigma-Aldrich. DMEM without FCS medium, penicillin/streptomycin and glutamine, Foetal calf serum was purchased from sigma aldrich. Streptozotocin from Sigma, USA. Cholic acid is purchased from Loba Chemie. Pvt. Ltd' Mumbai. Cholesterol was purchased from SISCO Research Laboratories Pvt Ltd, Mumbai, India. Egg yolk powder was purchased from Himedia laboratories Pvt. Ltd. Mumbai., Adrenaline bi tartrate was purchased from Sisco Research Laboratories Pvt Ltd, Mumbai. All chemicals were of analytical grade and used as received without any further purification.

2.4 Synthesis of GNPs using chitosan Biopolymer[9]:

All glassware and magnetic stir bars used in the synthesis and storage of AUNPs were thoroughly washed in aqua regia (HCL: HNO_3 [3:1] v/v) to dissolve any residual metallic particles, that may interfere with the synthesis followed by rinsed in Milli-Q water and allowed to oven dry, to avoid unwanted nucleation and aggregation during the synthesis and storage. Before the preparation of AUNPs, working solution of chitosan were prepared by dissolving a definite amount of stock chitosan (v/v).

AuNPS were synthesized by well-known wet-chemical reduction method using chitosan biopolymer with some changes as described earlier.16 Before the preparation of gold nanoparticles, acetic acid was diluted to 1% aqueous solution and the stock solutions of medium molecular weight chitosan biopolymer were prepared by dissolving various amount of chitosan (0.2–1.0%) in 1% acetic acid solution (v/v). Due to the condensed

solubility of polymer chain of chitosan, the mixture was sonicated about 30 min and allowed to retain for about 1 week at room temperature to obtain clear solution. Followed by 3 ml of HAuCl4 and 1 ml of 1.0% chitosan solutions were taken then the mixture was allowed to boil in water bath at 80°C for 1 h under reflux. After synthesis, AuNPs was confirmed by the color change from pale yellow to dark ruby red color. The synthesized AuNPs were further puri- fied by centrifugation at 13, 000 rpm for 15 min at room temperature and redispersed in Milli-Q water for further analysis.

Loading of gymnemic acid fraction on to chitosan reduced gold nanoparticles:
A calculated amount of gymnemic acid fraction was added to dispersion of gold nanoparticles reduced using 1% chitosan to yield an gymnemic acid fraction concentration of 10mg/ml in solution. The dispersion was incubated for 16hrs at 2-8°C followed by ultracentrifugation at 30, 000 rpm for 30mts. The pellet thus obtained was separated from the supernatant solution and redispersed in MilliQ water prior to further characterization.

Loading Efficiency:

$$\phi\% = ([GA]_{Tot} - [GA]_{free}) / [GA]_{Tot} \times 100 = 60 \pm 4.9\ \%$$

2.5 Characterization of gymnemic acid coated chitosan reduced gold nanoparticles:
The size and surface morphology of as synthesized AuNPs were analyzed using high-resolution transmission electron microscopy (H-TEM, JOEL 2010) operated at 200 kV. The number of AuNS per Liter was calculated according to the number of atoms present in each AuNPs. The size and surface charge of the synthesized AuNPs at different concentrations were examined using (Malvern Zetasizer, Nano ZS90) instrument (5 mW HeNe laser _ = 632 nm). The sample was taken in a cuvette of 1 cm path length with an equilibration time of 60 S at 25 _C. Particle size of the sample was measured as such without dilution. The optical absorption spectrum in the wavelength of 200–800 nm was measured using spectrophotometer in a 2 ml glass cuvette.

2.6 Cell culture:
L6 cells (from ATCC) were grown in DMEM (4.5 g/liter glucose) supplemented with 10% (vol/vol) fetal bovine serum, 10 mM HEPES buffer, 2 mM L-Glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin at 37°C with 8% CO_2. Upon reaching confluence, differentiation was induced by media containing 2% (v/v) fetal bovine serum for 7 days.

The cells were grown in monolayers at 37 °C and 5% CO_2. For individual experiments, cells were seeded into six-well plates at a density of 1×10^5 cells per well for L6 cells in complete DMEM media for 24 h. After incubation, remove the medium from the wells for MTT assay. In each well wash with MEM (w/o) FCS. And add 200µl of MTT concentration of (5mg/ml). And incubate for 6-7hrs in 5% CO_2 incubator. After incubation 1ml of DMSO was added in each well and mix by pipette and leave for 45 seconds and it shows the purple color formation. The suspension is transferred in to the cuvette of spectrophotometer and O.D values is read at 595nm and % of cell viability was calculated using the formula.[10,11,12]

Graph was plotted using the % of cell viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control was included in each assay to compare the full cell viability in cytotoxicity assessments.

(OD of sample/OD of cell control)*100 = %cell viability

Quantification of cell death:
Routine measurement of cell viability was determined by the ability of the cells to exclude trypan blue. After incubation, all cells (adhered and detached) were collected, centrifuged at 200 *g* for 5 min and resuspended in complete DMEM containing trypan blue (0.4% in PBS) in 1:1 ratio. Viable and dead cells were counted using a haemocytometer and the dead cells were expressed as the percentage of the total cells for each condition. Over the course of the present experiments, the extent of cell death recorded in untreated L6 cells averaged 9.8±1.1%.

Caspase-3 activity measurement:
Caspase-3 activity was measured using a western blotting method. Cell and islet extracts were separated on NuPage SDS-PAGE gels (Invitrogen) and transferred to polyvinylidine difluoride membranes. Equal loading of protein between lanes was confirmed by Coomassie staining and subsequent β-actin immunoblots.

Glucose uptake assay[13]:
Cells were cultured on 6 well plates and incubated for 48 h at 37°C in a CO_2 incubator. When semi confluent monolayer was formed, the culture was renewed with serum free DMEM containing 0.2% BSA and incubated for 18 h at 37°C in the CO_2 incubator. After 18 h, the media was discarded and cells were washed with KRP buffer once. The cells were treated with Insulin, standard drug and plant extract and added glucose (1M) and incubated for half an hour. The supernatant was collected for glucose estimation and glucose uptake was

terminated by washing the cells thrice with 1 ml ice-cold KRP buffer. Cells were subsequently lysed by freezing and thawing thrice. Cell lysate was collected for glucose estimation.

Glucose uptake was calculated as the difference between the initial and final glucose content in the incubated medium by GOD-POD method as follows:

Mix 10 µl of sample and 1 ml of reagent, incubate for 25 min at 15-25°C or 10 min at 37°C. Measure the absorbance of the standard ($A_{standard}$) and the sample (A_{sample}) against the reagent blank within 60 min, the time interval from sample addition to read time must be exactly the same for standard/control and sample.

$$\text{Glucose concentration} \quad \text{mmol} /1 = \frac{A_{sample}}{A_{standard}} \times 55.5$$

$$\text{mg/dl} = \frac{A_{sample}}{A_{standard}} \times 100$$

PPAR gamma was measured by using PPAR gamma Transcription Factor Assay Kit

2.7 Animals:

Male wistar rats (150-200g) were used in this investigation. Animals were maintained under standard environmental conditions and had free access to feed and water *ad libitum*. Experimentation on animals has approved by CPCSEA and Institutional Animals Ethics Committee (IAEC). The approval no is XV/VELS/PCOG/04/2000/CPCSEA/IAEC/30.10.13.

2.8 Induction hyperlipedemia with Type-2 diabetes:

Animals are treated with modified high fat diet every day for 30 days. The high fat diet is freshly prepared every day and the method of preparation was described earlier by Devi *et al.,* (2004). Control animals are provided with normal pellet chow (Lipton, India) and water *ad libitum.* After 1 week on high fat diet, animals were fasted overnight and Diabetes was induced by injecting streptozotocin (STZ) (Subdiabetogenic dose – 30mg/kg in 0.1mol/L citrate buffered saline, pH 4.5) in to the tail vein via (Yong *et al.,* 2005). [14]

2.9 Animal Grouping and drug administration:

Animals are divided in five groups. Group 1 (n=6) served as control animal treated with 0.9% saline. Group 2 (n=6) served as high fat diet fed diabetic animal treated with 0.9% saline. Group 3 (n=6) served as high fat diet fed diabetic animals treated with Metformin 200mg/kg. Group 4(n=6) served as high fat diet fed diabetic animals treated with GA-AUNPs 0.25mg/kg in normal saline. Group 5 (n=6) served as high fat diet fed diabetic animals treated with GA-AUNPs 0.5mg/kg in normal saline.

2.10 Biochemical estimation:

2.10.1 Estimation of blood glucose, insulin and lipid profiles:

Blood glucose level was determined by one touch horizon blood glucose meter (One Touch, Johnson & Johnson Ltd, Mumbai) using one drop of blood collected from tail vein. At the end of 30th day animals (n=3) were sacrificed by carbon dioxide euthanasia and blood was collected. Lipid profiles, total cholesterol and lactate dehydrogenase (LDH) are determined by using standard bio chemical Kit (Auto analyser). Insulin was measured by Radio immuno assay method.

2.10.2 Estimation of oxidative stress markers and antioxidants in vital organs:

At the end of 30th day animals (n=3) were sacrificed by carbon dioxide euthanasia and organ like heart, and liver were isolated, weighed and homogenized with ice cold phosphate buffer (pH 7.2) in Teflon glass homogenizer. The homogenate was centrifuged at 1000 rpm 4◦C for 15 min. Protein was estimated by the method Lowry *et al.,* (1951).[15] The supernatant was used for estimation of oxidative stress markers, and antioxidants.

2.10.3 Estimation of super oxide dismutase (SOD):

Super oxide dismutase measurement was done based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome. 2.78ml of sodium carbonate (0.05mM) buffer (pH 10.2), 100µl of EDTA (1.0mM) and 20µl of tissue homogenate or sucrose (blank) at 30°C for 45 min. After 45 min absorbance was adjusted to zero to sample. Thereafter, reaction was initiated by adding 100 µl of adrenaline solution (9.0mM). The change in the absorbance was recorded at 480 nm for 8-12 min. Throughout the assay procedure temperature was maintained at 30°C. Similarly, SOD calibration curve was prepared by taking 10 units/ml of standard solution. One unit of SOD produced approximately 50% inhibition of auto oxidation of adrenaline. Percent inhibition of the sample was calculated by calculating dy/dx of the straight line portion of both blank and sample. The results are expressed as units (U) of SOD activity/mg protein (Saggu *et al.,* 1989)[16].

2.10.4 Estimation of Catalase (CAT):

Catalase measurement was done based on the ability of CAT to oxidize hydrogen peroxide (H_2O_2). 2.25 ml potassium phosphate buffer (65mM, pH 7.8) and 100 µl of the tissue homogenate or sucrose (0.32M) were incubated at 25°C for 30 min H_2O_2 (7.5mM) 650 µl was added to initiate the reaction. The change in absorption at 240 nm was measured for 2 to 3 min. dy/dx for every minute for each assay was calculate and the result are expressed as CAT µM/min/g of protein (Beers and Sizer, 1952)[17].

2.10.5 Thiobarbituric acid reactive substance (TBAR):
LPO end product malondialdehyde (MDA) was measured by the method Okhawa *et al.* (1979). 0.1 ml of tissue homogenate was treated with 20% of 1.5 ml of acetic acid (pH 3.5), 1.5 ml thio barbituric acid and 0.2 ml sodium dodecyl sulphate (8.1 %). The mixture was then heated at 100°C for 60 min. The mixture was cooled with tap water and 5 ml of n- butanol-pyridine mixture (15:1 % v/v) followed by 1 ml of distilled water was added. The mixture was shaken vigorously. After centrifugation of the mixture at 4000 rpm for 10 min, the organic layer was withdrawn and absorbance was measure at 532 nm. The concentration of MDA formed is expressed as n mole/mg of protein.[18]

2.11 Histopathological examination:
After 30 days of STZ and STZ+ GA-AUNPs treated animals were euthanized. The pancreas was dissected out quickly, fixed in 10% formalin and 10-µm thick sections were taken. The sections were processed and stained in 0.1% Hematoxylin and Eosin. The stained sections were observed under a binocular light microscope and photographed. Quantitative scoring of histopathological examination was performed according to (Block and Schwarz (1996)) method with slight modifications. Scoring of damaged pancreas induced by STZ was done by assessing the histological picture as follows: 0–10% = 1 (no morphological signs of damage and few dark stained cells); 11–30% = 2 (edema or inflammation); 31–50% = 3 (Per vascular infiltrate) and 51–100% = 4 (Acinar necrosis). A total histological score of the pancreatic area was calculated by adding all the regional scores and then expressed based on their respective percentage of damage.

2.12 Statistical Analysis:
For *in-vivo* experiments values are represented by mean ± SEM. The mean values are analyzed by one way ANOVA followed by Dunnets test. The $p<0.05$ was considered as statistically significant.

3. RESULTS AND DISCUSSION:

3.1 Synthesis and Characterization of AuNPS:
The physicochemical properties like particle size, charge and shape highly influence the biodistribution, clearance and metabolism of nanoparticles *in vivo*.20 Hence the synthesis and characterization of nanoparticles is the crucial step to determine its potential as a therapeutic drug carrier at *in vivo* level.[16] In this method chitosan monolayer protected gymnemic acid coated gold nanoparticles were successfully synthesized using previously described wet-chemical reduction method using chitosan biopolymer with required modification. During synthesis, a chitosan concentration appears to be very crucial for controlling the particle size of the AuNPs. Hence tetrachloroauric acid concentration was kept constant (1 mM) and varied concentrations of chitosan were used (0.2–1.0%) to get the desired monodispersed nanoparticles (Table 5.1 and Table 5.2). Finally, the optimal concentration for the synthesis was determined. Chitosan monolayer protected GA-AuNPs were synthesized rapidly and confirmed by UV-visible spectrophotometer with defined characteristic narrow plasmon resonance peaks in the visible region at 520 nm shown in Figure 6.1.The FTIR and HR-TEM analysis of the synthesized GA-AuNPs shows monodispersive spherical nanoparticles shown in Figure 6.2(a&b), Figure 6.3(a&b), ranging average particle size of 64nm±1.99 nm in diameter with uniform distribution. Shows SAED pattern of GA-AuNPs that shows planar atom arrangement corresponding to the lattice parameters (FCC) from inner to outer layer at 111, 200, 220 and 311 and EDX spectrum confirms the presence of GA-AuNPs shown in Figure 2(d). Fig 6.4 The DLS analysis of synthesized GA-AuNPs shows hydrodynamic size of 29.62± 0.32 d.nm in diameters and zeta potential of about (+) 50.77 mV with low polydispersive index 0.382±0.00. This positive surface charge of GA-AuNPs shows the higher colloidal stability with no agglomeration for several months. Interestingly, synthesized nanoparticles aid the tissue uptake and no capping agent requires for maintaining its stability. The concentration of synthesized AuNPs was determined by ICP-OES analysis and found to be 77mg/l.

3.2 INVITRO STUDIES:
The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. It depends both on the number of viable cells and on the mitochondrial activity of cells. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay is based on the assumption that dead cells or their products do not reduce tetrazolium. Tetrazolium salts are reduced only by metabolically active cells. Thus MTT can be reduced to a blue coloured formazan by mitochondrial enzyme succinate dehydrogenase. The amount of formazan produced is directly proportional to the number of active cells. GA-AUNPs toxicity was tested in vitro in L6-GLUT4 myc cell following MTT as described in the materials and methods. Test concentrations that kept at least 90% cell viability were considered as safe. Gymnemic acid fraction (Fig. 6.) was found to be safe up to 50µl concentration. Accordingly, all the efficacy studies were performed at safe concentrations.

Hence, in this study L6 cell lines are used to determine the glucose uptake activity of GA-AUNPs and the results are presented in Table 1. The glucose utilization in L6 cell lines showed that the GA-AUNPs were found to be prominent over control. The L6 cell lines enhance caspase and the glucose uptake by 70.19±**1.72**at 500

μg/ml concentration (Fig. 6.7 and Fig 6.8). These results were compared with insulin and metformin, which were used as the standard antidiabetic drugs. Insulin at a concentration of 1IU/ml and metformin at a concentration of 100 μg/ml were found to enhance the glucose uptake over control.

3.3 Anti- diabetic effect of GA-AUNPs in high fat meal treated diabetic rats:

The anti-diabetic effect of GA-AUNPs has shown in table 5.3. HFM and STZ treated diabetic rats treated with two different GA-AUNPs had significant ($P<0.05$) decrease in reducing blood glucose levels at 7th Day as compared with saline treated HFM + STZ rats. The similar significant anti-hyperglycemic effect was noted at 14th day (as well as at the end of the experiment at 30th day as compared with HFM + STZ treated diabetic control. The anti diabetic effect of GA-AUNPs in reducing the blood glucose level at 30th day was comparable to that of metformin (111.0 ± 4.41).(Table 1)

3.4 Anti-hyperlipedimic effect GA-AUNPs in high fat meal treated diabetic rats:

Fig 6.9 depicts the effect of GA-AUNPs on total cholesterol and lipid profiles of the high fat meal fed diabetic rats. Administration of HFM significantly ($P<0.01$) increase the serum TC, LDL, Triglycerides (TG) with significant decrease ($P < 0.01$) in HDL level compared with normal rats. Administration GA-AUNPs significantly ($P<0.05$) protected the rats against HFM induced hyperlipidemia as observed by decrease in the TC and LDL level. In addition GA-AUNPs significantly increased the HDL level respectively. However, the effects of tannins on TG levels were insignificant.

3.5 Effect of GA-AUNPs on serum CPK LDH and Uric acid:

Fig 6.10 Depicts the effect of GA-AUNPs on CPK and LDH the high fat diet treated diabetic rats. There is a significant increase in CPK and LDH level was observed in HFM treated diabetic rats compared with normal rats. Attenuation of CPK and LDH level was observed in HFM treated diabetic rats fed GA-AUNPs.

3.6 Effect of GA-AUNPs on SOD level of heart, liver and kidney of HFM fed diabetic rats:

It is observed from the Fig 6.11 that significant ($P<0.01$) depletion in superoxide dismutase level (SOD) in heart, Kidney and liver of GA-AUNPs fed diabetic rats as compared with non diabetic control rats. Per oral administration of GA-AUNPs significantly ($P <0.05$) increased the SOD levels in heart, liver and Kidney of the HFD treated diabetic rats as compared with vehicle treated hyperlipedemic diabetic rats. The effect was dose dependent.

3.7 Effect of GA-AUNPs in catalase (CAT) in Heart, liver and kidney of HFM fed diabetic rats:

Fig 6.12 shows the significant ($P< 0.01$) decrease in the catalase (CAT) level was observed in heart, liver and kidney of HFM fed diabetic rats as compared with non – diabetic control rats. GA-AUNPs at two different doses significantly ($P<0.05$) increase the catalase level of the insulin dependent liver tissue and non-Insulin dependent tissue kidney and heart.

3.8 Effect of GA-AUNPs in TBARS level of heart, liver and kidney of HFM fed diabetic rats:

Fig 6.13 depicts the effect of TF on TBARS levels in vital organs of the rats fed with HFM and streptozotocin. Significant ($P<0.05$) increase in the TBARS was observed. High fat diet treated diabetic rats heart, liver and kidney has compared with non-diabetic control a animal group. Administration of insulin, different doses of GA-AUNPs are significantly ($P<0.05$) decrease the elevated TBARS level in insulin dependant liver and non insulin dependant kidney of the high fat diet treated diabetic rats compared with saline treated high fat treated diabetic animals.

3.9 Histopathology:

The effect of GA-AUNPs at high dose on histopathlogical findings on the pancreas shown in Fig 6.14(A-E). It is observed that diabetogenic agent Streptozotocin produced lesion in the pancreatic islets as viewed by very scanty islets with acinar tissue. Treatment with insulin has decreased the degree of lesions as indicated by partial intact pancreatic cells with acini. However attenuation of pancreatic degeneration was observed in high fat diet treated diabetic animals treated with GA-AUNPs 0.5mg/kg.

Table 5. 1. Effect of chitosan concentration on the physicochemical characteristics of synthesized AuNPs.

S.No	Physicochemical characteristics of AuNPs	Chitosan Concentration 1 (%) (w/v)
1	Hydrodynamic Size (d.nm)	29.62± 0.32
2	PDI	0.382± 0.00
3	Zeta Potential (mV)	50.88 ±0.16
4	Zeta Deviation	4.43
5	Conductivity (mS/cm)	1.73

Ratio (3:1, $HAuCl_3$: Chitosan)

Table5. 2. Physicochemical characteristics of GA- AuNPs

S.No	Physicochemical characteristics of AuNPs	GA-Chitosan Concentration 1 (%) (w/v)
1	Hydrodynamic Size (d.nm)	71.78±0.99
2	PDI	0.27±0.01
3	Zeta Potential (mV)	+46.4±0.78
4	AuNPS	+50.6± 1.15

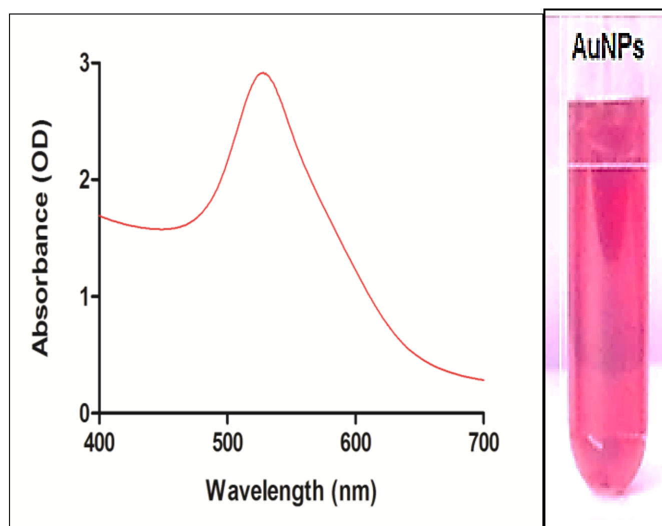


Figure:6.1 UV-visible absorption Spectrum of Synthesized Gold Nanoparticles and GA coated Gold nanoparticles

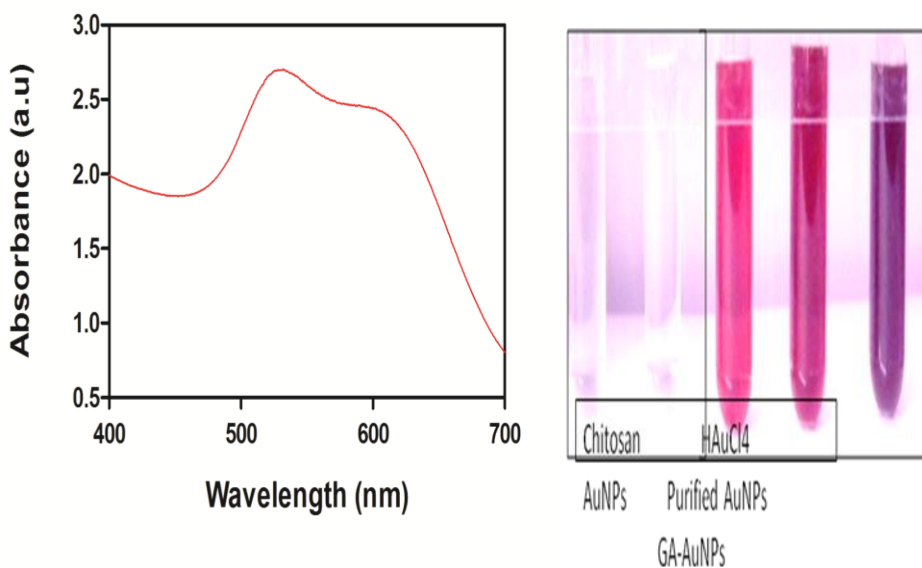
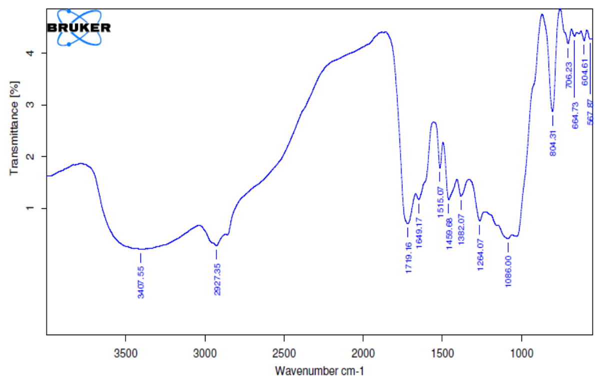


Figure 6.2 a&b FTIR Spectrum of Synthesized gymnemic acid and GA coated chitosan reduced Gold nanoparticles



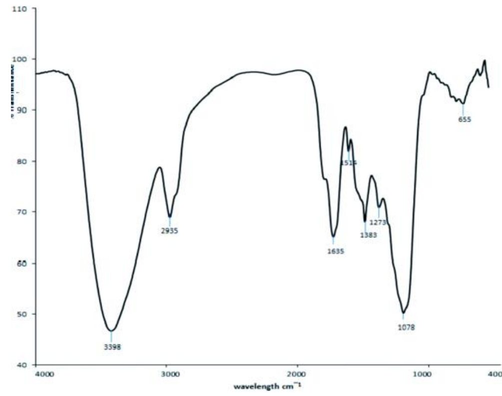
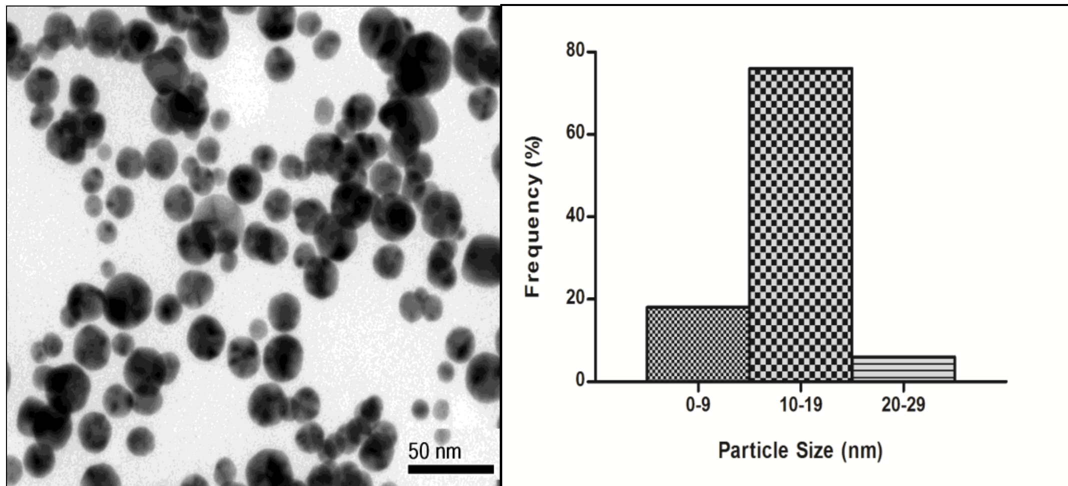
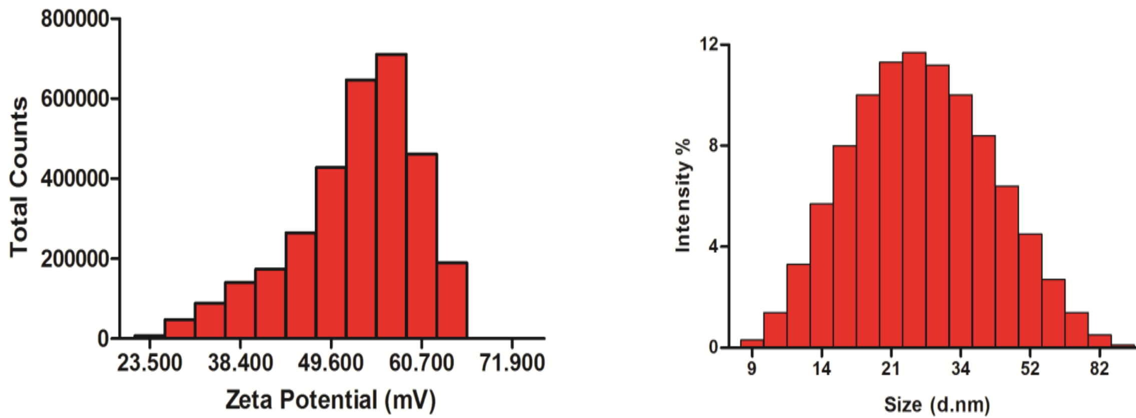


Fig.6.3. a&b. HR-TEM images of Synthesized Gold Nanoparticles .



a) Size distribution of AuNPs mean particle size was found to be around 64 nm in dm. (Image J Software)b. Size distribution of AuNPs mean particle size was found to be around 64nm in dm

Figure 6.4 Hydrodynamic Size distribution of GA- AuNPs



Mean particle size & PDI was found to be 29.62± 0.32 d.nm. & PDI=0.382±0.00 (b) Zeta potential of synthesized AuNPs was 50.77 mV.

Fig.6.5. AFM images of Synthesized gymnemic acid coated chitosan reduced Gold Nanoparticles

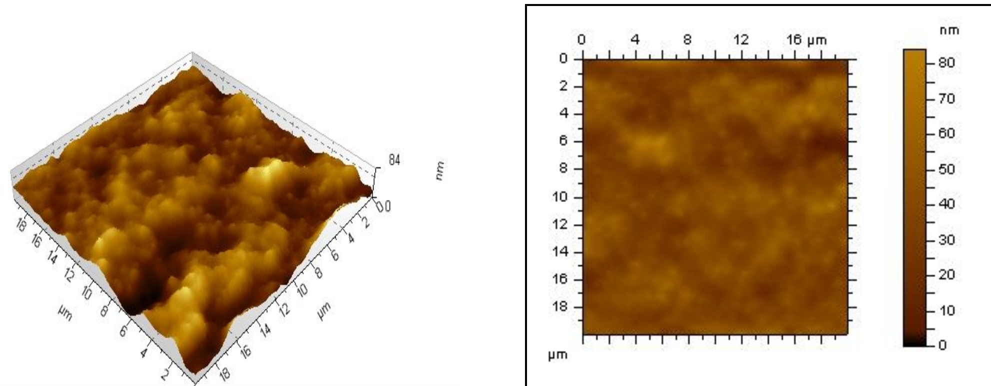
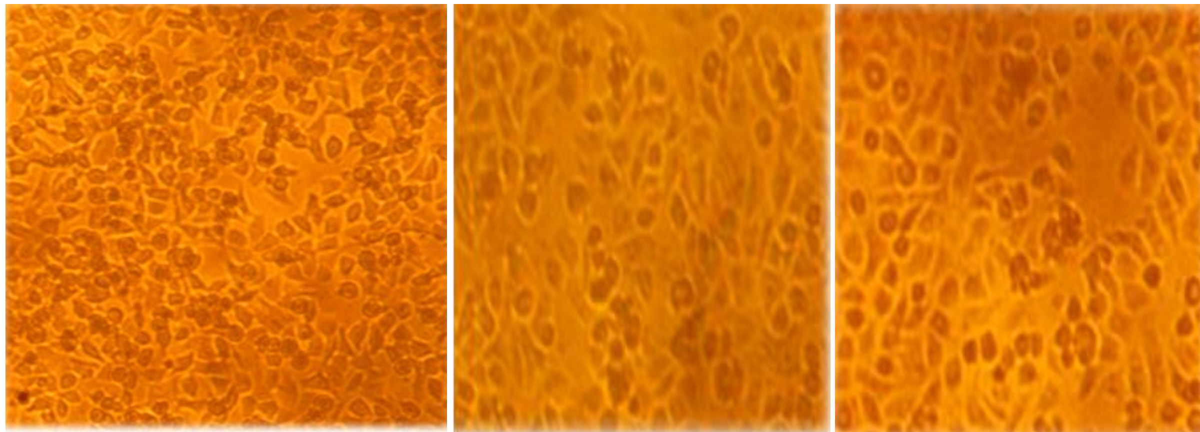


Fig 6.6 ***Invitro*** **Cytotoxicity effect of gymnemic acid coated chitosan reduced AUNPs sample on skeletal muscle cell on (L6 myotubes).**



Normal L-6 cell lines GAF-AUNPS 12.5µg/mL GAF-AUNPS 25.00µg/mL

Fig 6.7 Caspase studies of gymnemic acid fraction and gymnemic acid coated chitosan reduced AUNPs sample on skeletal muscle cell on

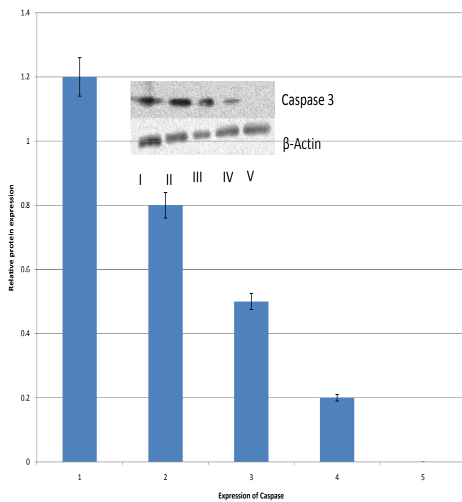


Fig 6.8 Glucose uptake assay of gymnemic acid coated chitosan reduced AUNPs sample on skeletal muscle cell on

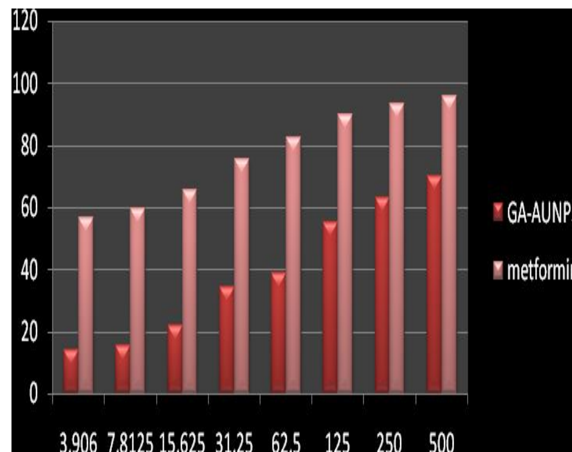
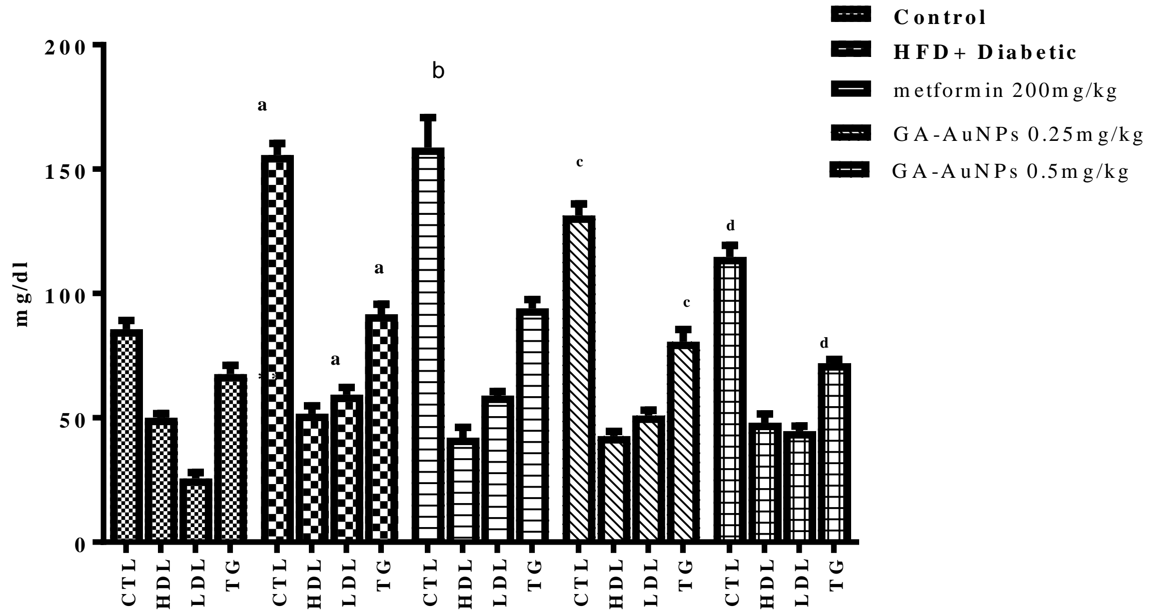


Figure 6.9 Effect of GA-AuNPs in lipid profile levels of the high fat treated Diabetic rats

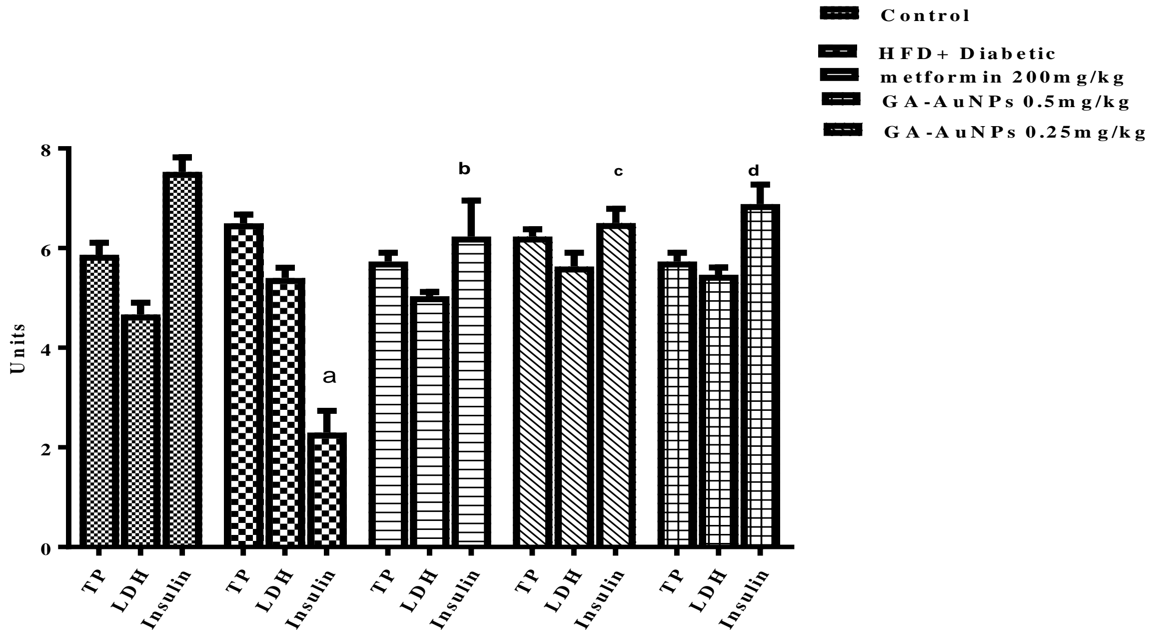
Table: 5.3 Effect of GA-AuNPs in lipid profile levels of the high fat treated Diabetic rats

Groups	Fasting Blood glucose levels
Control	76.03 ± 7.6
HFD+Diabetic	217 ± 6.1[a]
Metformin 100mg	72.98 ± 3.2
GA-AUNPs 0.25mg	85.98 ± 4.2
GA-AUNPs 0.5mg	81.65 ± 3.4



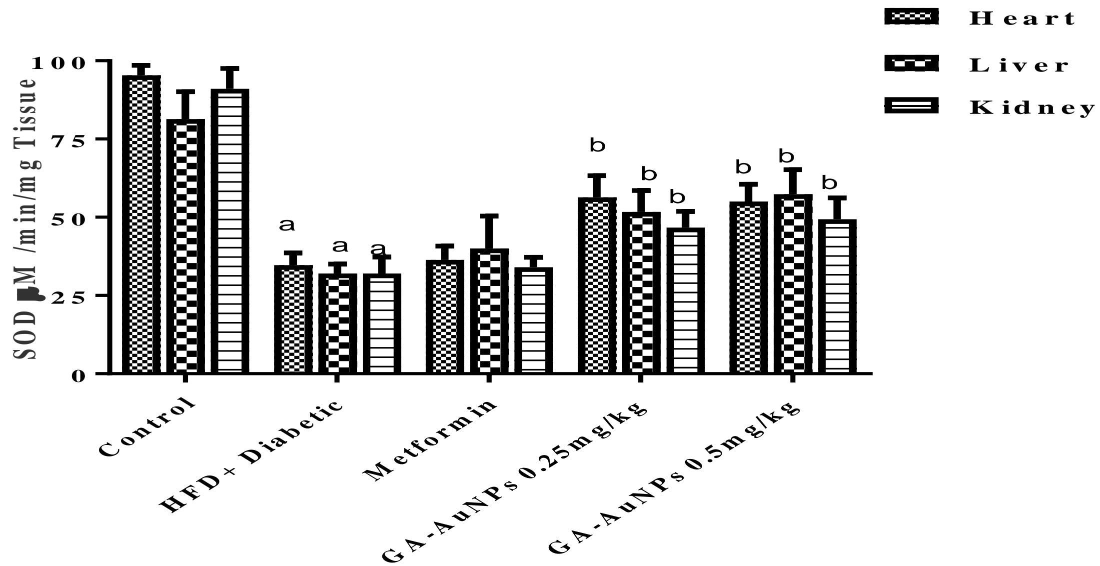
a indicates p<0.01 Control Vs diabetic; b, c &d indicates P<0.05 Treatment Vs diabetic; .05 Treatment Vs diabetic

Figure 6.10 Effect of GA-AuNPs on total protein insulin and LDH Levels of the High fat treated diabetic rats



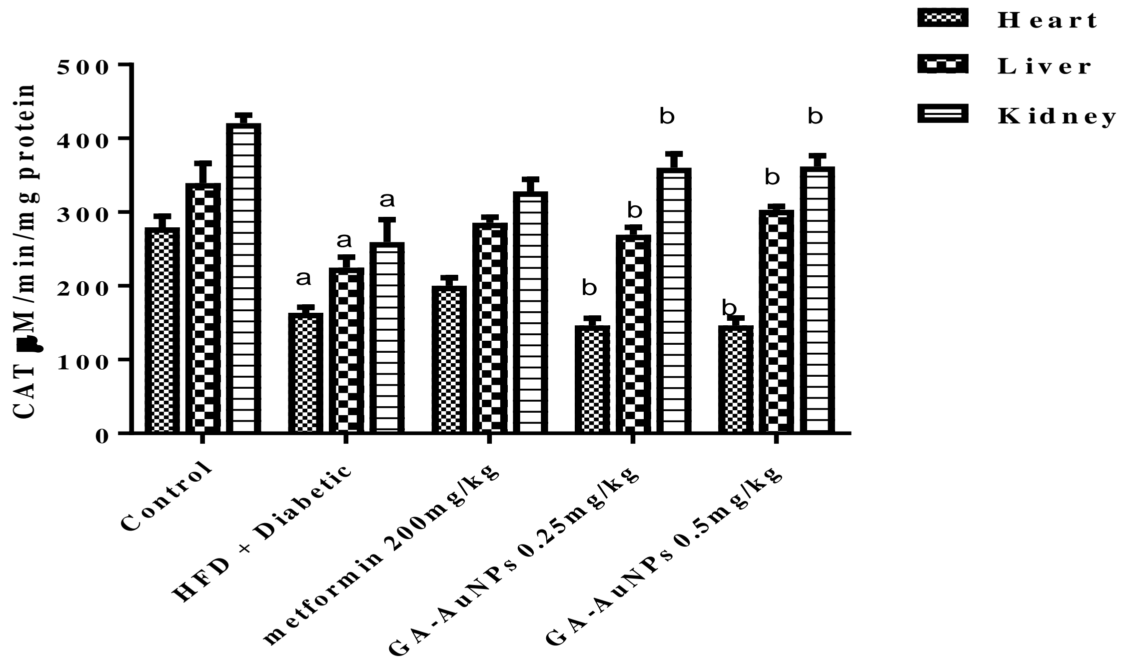
a indicates p<0.01 Control Vs diabetic; b, c indicates P<0.05 Treatment Vs diabetic ; .05 Treatment Vs diabetic

Figure 6.11 Effect of GA-AuNPs in SOD level of the heart,liver and kidney of the high fat diet treated diabetic rats



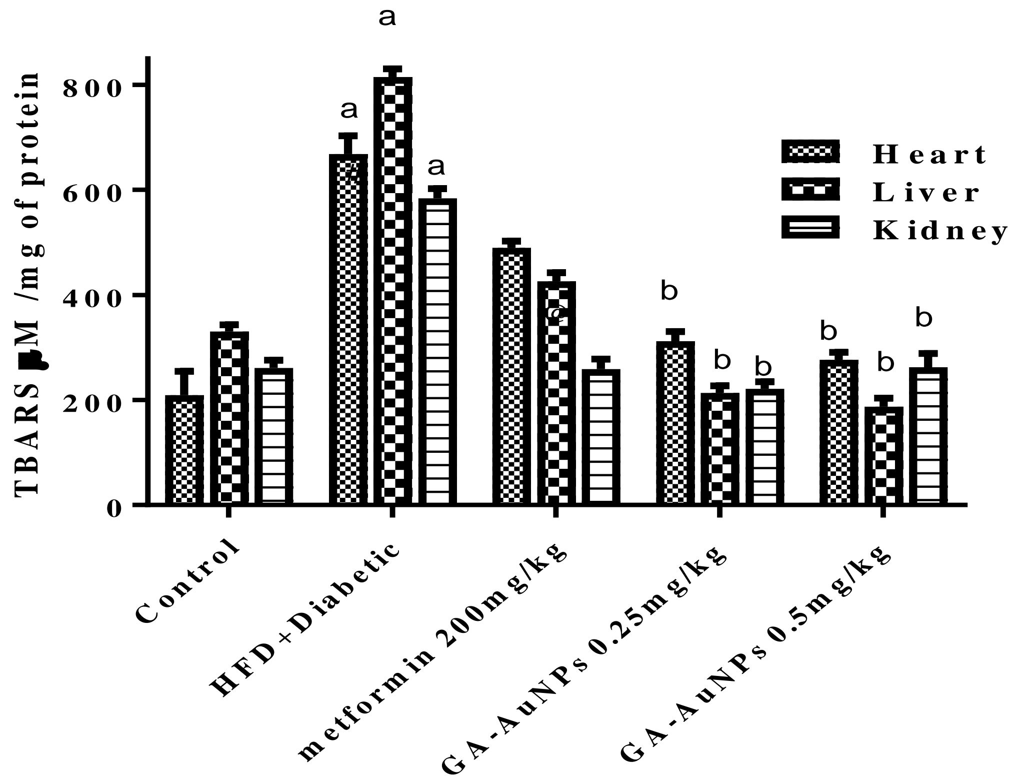
a indicates p<0.01 Control Vs diabetic; b indicates P<0.05 Treatment Vs diabetic ; .05 Treatment Vs diabetic

Figure 6.12Effect of GA-AuNPs in CAT level of the heart,liver,kidney of the High fat diet treated diabetic rats



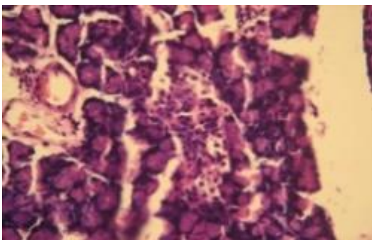
a indicates p<0.01 Control Vs diabetic; b indicates P<0.05 Treatment Vs diabetic ; .05 Treatment Vs diabetic

Figure 6.13 Effect of GA-AuNPs in TBARS level of the heart, liver,kidney of the High fat diet Treated diabetic rats

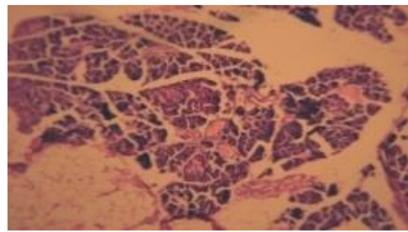


a indicates p<0.01 Control Vs diabetic; b indicates P<0.05 Treatment Vs diabetic ; .05 Treatment Vs diabetic

Figure 6.13



A)Histopathological Section of Control Pancreas (Magnification 400x)



B) Histopathological Section of high fat diet fed Streptozotocin treated Pancreas (Magnification 400x)

Figure C: Histopathological Section of high fat diet fed Streptozotocin + Metformin 100mg/kg treated Pancreas(Magnification 400x)

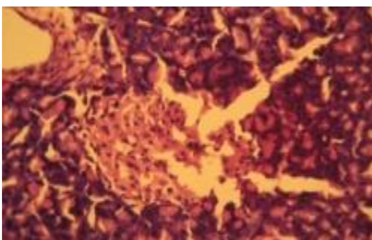


Figure D:
Histopathological Section of high fat diet fed Streptozotocin + GA-AUNPs 0.25 mg/kg treated Pancreas (Magnification 400x)

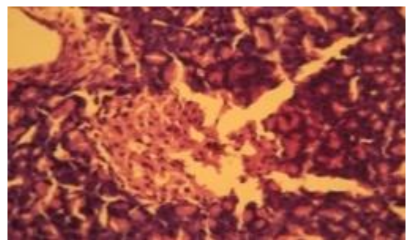


Figure E: Hstopathological Section of high fat diet fed Streptozotocin + GA-AUNPs 0.5 mg/kg treated Pancreas (Magnification 400x)

Figure 6.15(A-E)

4. DISCUSSION:

In the present study emphasized the protective effect of biocompatible nontoxic gymnemic acid coated- chitosan reduced gold nanoparticles (GA-AUNPs) synthesized from the gymnemic acid fraction isolated from *Gymnema sylvestre* leaves in high fat diet /streptozotocin treated type II diabetic rats. The present study is the first biochemical inspection to shows the anti diabetic effect of GA-AUNPs present in *Gymnema sylvestre* in animal model of type II diabetes associated with hyperlipidemia. Metallic nanostructures play a substantial role in nanotechnology. Tremendous efforts have been devoted toward developing new methods of preparing metal nanoparticles with different topologies, such as spheres, tetrapots and wires. Based on the interesting chemical and physical properties, gold nanoparticles used for catalysis, biological labeling and sensing. A number of methods have been used to synthesise the gold nano particles in both organic and aqueous media. Ever-growing epidemiological and recent clinical reports suggest that the high prevalence of cardiovascular diseases like coronary artery disease (CAD) is associated with hypercholesterolemia and diabetes[19,20,2122]. Treatment of rats with high fat diet and STZ is an established model for Type II. Diabetes is associated with profound alterations in the plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease [23, 24, 25].

Many medicinal herbs from Indian system of medicine have been shown to have hypoglycemic and hypolipidemic properties[26,27]. In this present study the well known anti diabetic plant Gymnema sylvestre is examined for its efficacy in type II diabetes. The main chemical constituent of of Gymnema sylvestre is gymnemic acid. The gymnemic acid chemically triterpenoidal saponin glycosides. The bioavailability of gymnemic acid is very low. So the present research designed to improve the bioavailability of gymnemic acid, by converting in to metallic nanoparticles and investigate its antidiabetic action.

While metal nanoparticles are being increasingly used in many sectors of the economy, there is growing interest in the biological and environmental safety of their production. The main methods for nanoparticle production are chemical and physical approaches that are often costly and potentially harmful to the environment. Type II diabetes are an increasingly serious threat to human health. It is necessary to overcome it with the help of nature. Therefore, there is an increase in the investigation of plants as a source of human illness management [28, 29] Medicinal plants have been a potential source of therapeutic agents for thousands of years. An impressive number of modern drugs have been derived from natural sources like plants which have been recognized as a part of the improvement of human healthcare for thousands of years [30].In addition, within the past decade, it has been demonstrated that many biological systems like plants, can transform metal ions into metal nanoparticles via the reductive capacities of metabolites present in these organisms [31]. Gold has been widely employed for many years in human history. Gold nanoparticles interaction with light is strongly dictated by their environment, size and physical dimensions. GA-AUNPs have been demonstrated to exhibit antidiabetic properties against high fat diet fed STZ induced type II diabetes with close attachment of the nanoparticles themselves to the anti diabetic activity being size dependent [32]. Fundamental studies showed that GA-AUNPs exhibit a rare combination of valuable properties, namely, unique optical properties associated with the surface plasmon resonance, catalytic activity, high electrical double layer capacitance, etc. In this regard an effective and versatile method was performed for the synthesis of gymnemic acid coated chitosan reduced gold nanoparticles. A monodisperse AuNPs were successfully synthesized by wet chemical reduction method. An optimal ratio were found for the synthesis of stable uniformed size AuNPs. Analytical results of UV-Visible spec, HR-TEM, AFM confirmed that formed AuNPs are spherical in shape and having size around 64nm. Zeta potential measurement of AuNPs shows high positive charge +50.77mV that confirms the high stability of AuNPs. The amount of Au in the nanoparticle solution was measured by ICP-OES through acid digestion method and it was found to be 77mg L-. FTIR spectra of Aurocholic acid shows the presence of the respective peaks and gymnemic acid shows the presence of triterpenoids, saponin and glycosides. The synthesized AuNPs were found to be stable for more than 6 months in Milli-Q water without any agglomeration.

We compared antidiabetic properties of standard drug metforminis compared with the biosynthesized GA-AUNPs. Antidiabetic effects of GA-AUNPs have been evaluated in several studies. These studies shows 90% of cell viability in MTT- cytoprotective assay in insulinoma cell lines(L6 cells). The glucose utilization in L6 cell lines showed that the GA-AUNPs were found to be prominent over control. The L6 cell lines enhance the glucose uptake by 70.19±1.72at 500 µg/ml concentration

Evidence is presented that the gymnemic acid coated chitosan reduced gold nanoparticles shows antidiabetic activity in addition lipid lowering action in diabetic animals. It is observed from the biochemical datas that elevated blood glucose level, increase in plasma cholesterol and lipid profile level was observed in high fat diet treated STZ induced diabetic rats. Treatment of GA-AUNPs normalized the blood glucose level,

triglyceride, LDL levels in plasma possibly by increasing glucose metabolism and controlling the hydrolysis of lipoproteins, their selective uptake and metabolism by different tissues. The effect of GA-AUNPs on controlled mobilization of serum triglycerides, total cholesterol and lipid profiles. The strong anti hyperlipidemic property of GA-AUNPs evidenced by control of hyperglycemia and control of total cholesterol and low density protein levels.

In this biological investigation shows protective effect of oxidative damage in high fat diet and STZ induced diabetic rats. Superoxide dismutase and catalase are the enzymes that protects our tissues from the effects of free radicals and lipid peroxides, both increased in diabetic animals because the animals affected free radical mediated injury and lipid peroxidation.

In conclusion, the results of this study show that 2 doses of GA-AUNPs had a favorable effect on plasma glucose and insulin concentrations. It also had an influence on attenuating oxidative stress in diabetic rats. In addition, larger amounts of GA-AUNPs supplementation may have beneficial effects on reducing plasma TC, triglyceride and LDL levels. Further characterization of active GA-AUNPs is warranted and studies are in progress to target the molecular levels.

5. CONFLICT OF INTEREST STATEMENT:

We declare that we have no conflict of interest.

6. ACKNOWLEDGMENTS:

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