Pakistan J. Zool., vol. 48(3), pp. 645-650, 2016.

Amelioration of Streptozotocin Induced Diabetes in Rats by Eco-friendly Composite Nano-Cinnamon Extract

Mai A Elobeid*

Department of Zoology, Faculty of Science, King Saud University, Riyadh, Saudi Arabia

A B S T R A C T

The study includes eco-friendly synthesis and characterization of silver/gold composite nano-cinammon particles and antidiabetic assessment of orally administered aqueous extracts of cinnamon (JF) and silver/gold nano-cinnamon (SG JF) in streptozotocin induced diabetic rats. The experimental groups (36 rats) were equally divided: a diabetic control group treated with metformin (standard drug), diabetic group treated with aqueous cinnamon extract, and diabetic group treated with silver/gold nanoparticles. The nano silver/gold cinnamon particles were characterized. The body weight and blood glucose levels were estimated. Nanoparticles characterization employed zetasizer, scanning electron microscopy, and transmission electron microscopy. The size and shape of the SG JF were 45.34 nm, stable, homogenous and showed mono dispersity distribution. The morphology of SG JF was highly variable with variety of shapes, following various arrangements. Body weights of rats treated with cinnamon vs nano-cinnamon showed no significant change. However, JF and SG JF showed a significant decrease in body weights as compared to metformin. SG JF significantly decreased glucose levels among diabetic rats in comparison with the rats treated with metformin and control JF. Similarly, metformin lowered glucose levels when compared to JF. Thus,eco-friendly synthesized nano-cinnamon possesses a potent antihyperglycemic that ameliorated and improved insulin sensitivity and body weight.

Article information
Received 27 May 2015
Revised 8 October 2015
Accepted 25 October 2015
Available online 14 March 2016

Key words:
Diabetes, nanotechnology, cinnamon extract, drug delivery.

INTRODUCTION

Diabetes mellitus II (DMT2) is a disease of altered glucose homeostasis and persistent hyperglycemia leading to many complications in both humans and animals (Salman *et al.*, 2015). Around 230 million people worldwide have been affected by diabetes and around 366 million people are expected to get affected by 2030 (Singh *et al.*, 2011). The pattern of prevalence has been the same in the Gulf including Saudi Arabia. Saudi Arabia has experienced an exponential socioeconomic growth over the past few decades which led to a sedentary and affluent lifestyle of the people in the urban society. An epidemiological study showed an alarming increase in the prevalence of DMT2 during the past few years (Al-Daghri *et al.*, 2011). This has stimulated public awareness of this endocrine disorder, the identification of risk factors associated with it and ways of prevention and treatment.

The fact that DMT2 can be delayed and prevented by a glycemic control and modifications in lifestyle (Midhet *et al.*, 2010) had led to a quest for better antidiabetic medications, triggering a search for novel phytochemicals to be used as alternative drugs in the therapeutic management of diabetes, believed to be less toxic and free of side effects than the synthetic drugs commonly used in therapy. However, discovery of new molecules and manipulating those available naturally in nanosize could be appealing for their greater potential to improve health care (Shrivastava and Dash, 2009).

Nanotechnology provides a broad knowledge of applied science and technology to control the matter on the atomic and molecular scale. It is an important and emerging technical tool for development of eco-friendly and reliable methodology for synthesis of nanoscale materials using biological sources (Gilaki, 2010). In modern nano science and technology, the interaction between inorganic nano particle and biological structures are one of the most exciting areas of research. To the best of our knowledge, this is the first work that evaluates the effect of biologically eco-friendly synthesized silver/gold composite nanoparticles coated with cinnamon powder extract (SG JF) to control the body weight and the hyperglycemic conditions in streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHODS

Chemicals

All chemicals and drugs were obtained commercially and were of analytical grade. Streptozotocin was used to induce diabetes (Sigma, U.S.A.). Metformin was used to control diabetes as a

* Corresponding author: maielobeid@gmail.com
0030-9923/2016/0003-0645 $ 8.00/0

commercial drug (Merck Serono, Middle East). Commercial kits for the estimation of serum glucose were used (United Diagnostic Company, Saudi Arabia). For the green synthesis of silver/gold nanoparticles, silver nitrate ($AgNO_3$) and chloroauric acid ($HAuCl_4$) (both were used as received without further purification) were obtained from Techno Pharmchem and Loba Chemie, India, respectively and powdered *Cinnamon cassia* was purchased from a local market, in Riyadh, Saudi Arabia.

Experimental animals

Adult male Sprague Dawley rats weighing 200-250 g were used. The animals were fed with standard laboratory chow and had free access to water under well ventilated conditions of 12 h day and 12 h dark cycles. The animals were acclimatized for two weeks to laboratory conditions prior to the experiment. The animals were handled according to standard protocols for the use of laboratory animals (NIH, 2002). The experimental groups contained thirty six rats, equally divided as follows: 1) Diabetic control group treated with metformin, 2) Diabetic group treated with cinnamon, and 3) Diabetic group treated with silver/gold cinnamon.

Induction of diabetes

The rats were made to fast 12 hrs before the induction of diabetes. Thereafter, they were injected with streptozotocin (70 mg/kg, i.p.). Five days after injection the rats with fasting blood glucose higher than 150 nmole/L were considered diabetic and used for the experiment. Feeding was stopped 12 hrs before blood sampling (Elobeid *et al.*, 2013).

Preparation of aqueous cinnamon powder extracts

Cinnamon cassia (3 g) powder was soaked in 90 mL of boiled distilled water overnight. The extract was filtered and immediately used for preparation of the nanoparticles. The resultant aqueous filtrate was treated individually with 50 mL aqueous solution of $AgNO_3$ and $HAuCl_4$.

Synthesis of silver and gold nanoparticles and characterization of green silver/gold nanoparticles

One mmole/ml silver nitrate and 1 mmole/ml chloroauric acid were dissolved in 50 ml of distilled water individually under vigorous stirring at 80°C for 5 minutes. Five ml of *Cinnamon cassia* extract was added to the solutions of both silver nitrate and chloroauric acid, separately. A change in color of the colloidal solutions occurred, which confirmed reduction of Ag ions, Au ions and the formation of green silver and green gold particles. This change in color of the reaction solutions was noted visually. Then the two green nanoparticles solutions were mixed together to produce a green Ag/Au composite nanoparticles which were incubated at room temperature until further use. Three methods were employed to characterize the composite nanoparticles. Firstly, the synthesized green nanoparticles (Ag/Au) were characterized using dynamic light scattering (DLS), zetasizer. The average size of green Ag/Au nanoparticles collectively was analyzed through zetasizer, Nano series, HT Laser; ZEN3600 from Molvern Instrument, UK. Secondly, transmission electron microscopy (TEM) [JEM-1011, JEOL, Japan] was employed to characterize the shape and morphologies of formed green synthesized nanoparticles. A drop of nanoparticles suspension was deposited on carbon coated copper grid and the film was then dried. The TEM was operated and the measurements were performed at accelerating voltage of 100 KV.

Elemental analysis of silver and gold on single composite nanoparticles was carried out using energy dispersive spectrometer (EDS), Oxford Instrument, Incax-act, equipped with scanning electron microscopy (SEM) using JEOL-FE SEM. Thirdly; scanning electron microscopy was employed to characterize the shape and morphologies of formed biogenic synthesized silver/gold composite nanoparticles. The samples were dried at room temperature and then analyzed for samples composition of the synthesized composite nanoparticles.

Statistical analysis

A one-way analysis variance was performed using Graph pad Prism 5 statistical software. Tukey's test was used for group comaparisons. The values were considered significantly different when the *p*-value was lower than 0.05.

RESULTS

Particle size determination using zetasizer

Figure 1 (DLS, zetasizer) showed that the average size of green Ag/Au nanoparticles was 45.34 nm with mono dispersity, which can be also observed clearly from the appearance of one peak with intensity 100 % and width 23.18 nm which gives very high stability and homogeneity of nanoparticles for a long period of time. Transmission electron microscopy technique was employed to visualize in depth the shape and morphology of green nanoparticles produced. TEM images (Fig. 2) confirmed that the data obtained from the DLS and TEM of green silver/gold composite nanoparticles has mono dispersity distribution. Figure 2 indicated that the morphology of nanoparticles are highly variable with a variety of shapes being rod, spherical and irregular (Fig. 2A), spherical (Fig. 2B) and follow a certain arrangement such as pyramidal (Fig. 2C-D), triangular, and diamond shaped.

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (r.nm):	45.34	Peak 1:	58.73	100.0	23.18
PdI:	0.225	Peak 2:	0.000	0.0	0.000
Intercept:	0.909	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

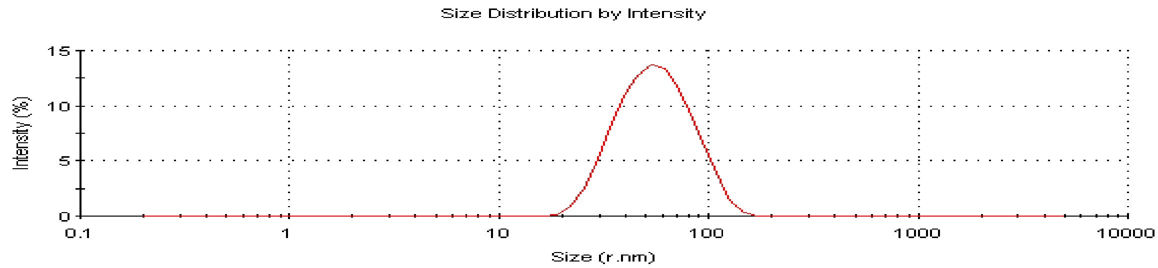

Fig. 1. Zetasizer measurement the average size of green silver / gold nanoparticles. TEM analysis of silver / gold nanoparticles.

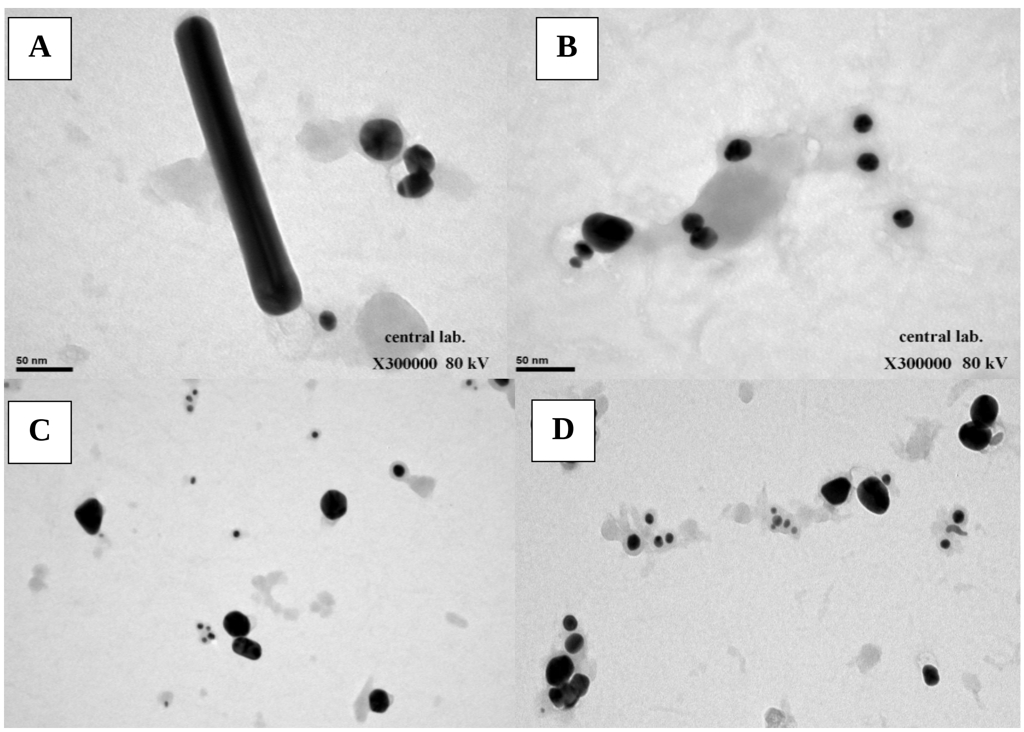

Fig. 2. TEM images of the green nanoparticles showing variety of shapes: rod, spherical and irregular (Fig. 2A), spherical (Fig. 2B), pyramidal, triangular, and diamond shaped (Fig. 2C-D).

Table I shows the percentage of silver and gold in the green nanoparticle suspension.

Scanning electron microscopy was further used to analyze the structure and morphology of the nanoparticles obtained from the described green synthesis method. The image showed relatively different shapes of the produced nanoparticles as shown in Figure 3A. The energy dispersive spectrometer known to provide information on the chemical analysis of the elements, has confirmed the presence of silver and gold nanoparticles. The spectrum analysis revealed signals in the silver and gold and confirmed the formation of silver/gold composite nanoparticles (Fig. 3B). Green silver/gold composite nanoparticles generally showed a typical optical absorption peak at approximately 3 keV for silver and gold showed four peaks in a wide range of 1-10 KeV.

Table I.- EDS results showing percentage of elements present in green silver/gold nanoparticle suspension.

Element	Weight%	Atomic%
Ag L	97.32	98.51
Au M	2.68	1.49
Totals	100.00	

A

B

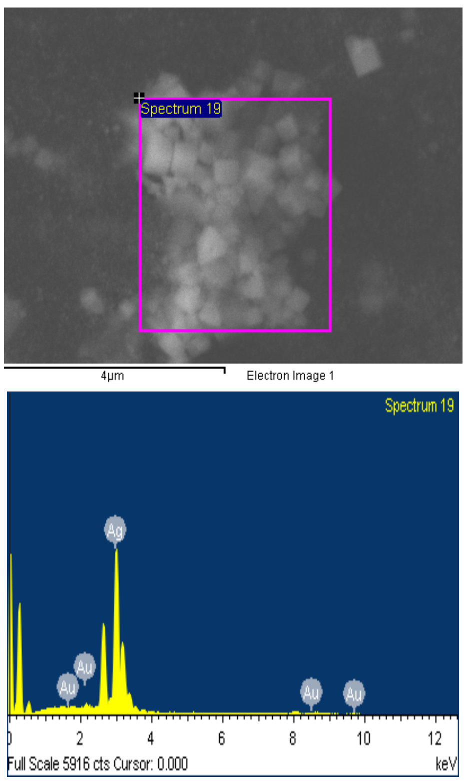

Fig. 3. (A) SEM image (B) EDS pattern of green silver / gold nanoparticles.

Body weights and glucose levels

During the experimental period, a non-significant decrease in body weight (Fig. 4A) was observed between the groups orally administered aqueous extracts of cinnamon and the group administered silver/gold nano-cinnamon. However, a significant decrease in body weight of rats treated with JF was observed compared to rats treated with control metformin (p<0.05) (231±37.1). Furthermore, a highly significant decrease in body weight of rats treated with SG JF was reported when compared to rats treated with metformin (p<0.05) (210±54.3). The

A

B

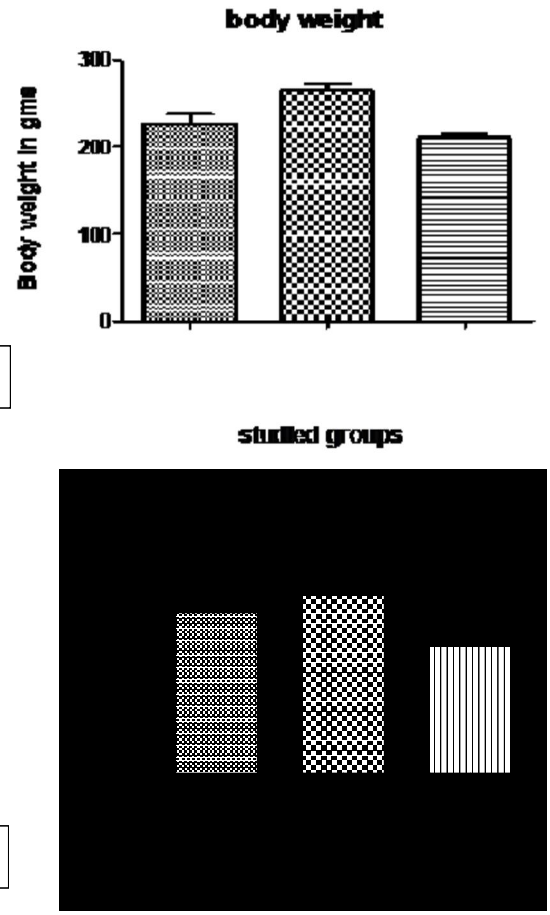

Fig. 4. Body weights (A) and glucose levels (B) in the studied groups.

administration of aqueous extract of cinnamon showed a significant decrease in the blood glucose level in STZ-induced diabetic rats (Fig. 4B). Metformin lowered blood glucose level more than JF. There was no statistical difference in the basal blood glucose levels of the metformin vs control JF (p<0.05) (134±14.3), however, blood glucose levels showed a highly significant decrease comparing SG JF vs metformin (p<0.05) (110±27.3), and significant decrease comparing control SG JF vs JF (p<0.05) (110±41.6).

DISCUSSION

Nanoparticles behavior is a function of their size, shape and surface reactivity with the surrounding tissue. In the present study, it was noticeable that the center of the particles was bigger than the edges, suggesting that biomolecules present in *Cinnamon cassia* extract have capped the silver/gold composite nanoparticles and were adhered to their surfaces, which is in agreement with a previous study (Ahmad *et al*., 2010). Also the difference in shapes and sizes could be due to aggregation and adsorption of compounds in *Cinnamon cassia* extract onto the surface of silver/gold nanoparticles. These results are in concord with another report (Awad *et al*., 2013), which reported the formation of silver/gold nanoparticles using *Balanitesa egyptiaca* extract with different sizes and shapes due to aggregation and adsorption of compounds in *Balanitesa egyptiaca* extract onto the surface of silver/gold nanoparticles, and that the silver/gold nanoparticles are surrounded by a faint thin layer of other materials, which they supposed was capping organic material from *Balanitesa egyptiaca* extract, in addition to few agglomerated particles. The silver and gold peaks could be due to the surface plasma on resonance (Palanive *et al*., 2013).

The promising potential of silver/gold nanoparticles in treating inflammatory and auto immune diseases (Mukherjee *et al*., 2005) have augmented greater interest to investigate the antihyperglycemic activity of the nanoparticles in the diabetic system. The gold nanoparticles are known for their tremendous applications in the field of therapeutics and diagnosis. Gold nanoparticles, an emerging nano medicine is renowned for its promising therapeutic possibilities, due to its significant properties such as biocompatibility, high surface reactivity, resistance to oxidation and plasmon resonance (Guo *et al*., 2005). The major drawback of ionic gold lies on the fact that they are easily inactivated by complexation and precipitation, thus, limiting their desired functions in human system. Here zerovalent gold nanoparticles can be a valuable alternative replacing the potential of metallic gold (Kalishwaralal *et al.*, 2009).

The biological synthesis process elucidates the importance of metal microbe interaction in several biotechnological applications including the field of bioremediation, biomineralization, bioleaching and microbial corrosion. Nanotechnology is undergoing explosive expansions in any areas serving mankind which may be considered an investment in future economic and social well-being. They effectively form a bridge between bulk materials and atomic or molecular structures, so they are of great scientific interest. Although nanopartcles size is very small but they are very effective when compared with the bulk material. Nanoparticles are of different types and exhibit enhanced properties. The present findings showed that the aqueous cinnamon modulated changes in body weight which is contrary to a previous study (Elobeid *et al*., 2013). While the present study showed a decrease in body weights among diabetic rats treated with JF and SG JF, another study using methanol extract of *Cinnamomum zeylanicum* increased the final body weights (Nyadjeu *et al*., 2013).

Blood glucose levels showed a decrease in both groups of rats administered cinnamon and nanocinnamon extracts. Thus, there is a growing trend of using plant products in therapy as they have anti-hyperglycemic effects. Beside medicines, the non pharmacological management of diabetes includes an appropriate diet management such as foods like cereals, vegetables and spices which had been assessed for their anti-hyperglycemic effect (Iyer and Mani, 1990; Vats *et al.*, 2002; Vinod *et al*., 2011) in experimental as well as clinical studies. There are several drugs of plant origin containing substantial amounts of alkaloids, glycosides and flavonoids bearing strong antioxidant properties, (for the treatment of diabetes), which are described in ancient literature. However, these drugs prove to be mostly effective in long-term treatment and so often lose their importance when compared to the faster onset of action of orthodox medicines. Therefore, efforts are needed to enhance their action and increase their bioavailability to targeted organs/organ systems. Considerable progress has been made in developing biodegradable nano particles as effective vehicles for the delivery of proteins and peptides (Kumaresh *et al*., 2001). The present study showed that the nano-formulation of cinnamon exhibited significantly enhanced anti-diabetic activity. The antidiabetic effect of cinnamon extract could be attributed to its insulin secretagogue effect which could be due to the stimulation of the beta cells or regeneration of beta cell functioning by alleviating the oxidative stress. This is in agreement with Ravichandran (2013), who reported that, gymnemic acids, the main phyto-constituents of *Gymnema sylvestre* possess potential natural pharmacological activities like suppression of taste sensitivity to sweetness, inhibition of intestinal glucose absorption and lowering the plasma glucose levels. Another study (Samadder *et al*., 2012), conducted *in vivo* (in mice) experiments, suggested the efficacy of encapsulated nano *Syzygium jambolanum* to have localized in the brain tissue of mice which suggested that it could efficiently cross the blood brain barrier, especially the hypothalamic region, proposed to be the glucose sensor region, which plays a critical role in initiating the counter regulatory response to glucose homeostasis. In conclusion, a good glycemic control is the cornerstone in diabetes management. The nano formulation of advanced organ/tissue/cell-specific drugs may enhance bioavailability of drugs to target organisms or organ systems with better efficacy at a minimum dosage. Nano encapsulated drugs appear to have greater advantages due to their small size, more rapid entry into target cells and biodegradable nature. The overall results suggested that nano-silver/gold cinnamon had a greater potential than that of cinnamon by itself, indicating its possible use in the future drug design and management of hyperglycemia. A long term study however, is imperative as plant products are slow in action than the synthetic drugs and at higher doses may also exhibit a plateau effect which would not help in diabetes management. Hence, further research is recommended in this field.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-033.

Conflict of interest

There is no conflict of interest.

REFERENCES

Ahmad, N., Sharma, S., Alam, M., Singh, N., Shamsi, S., Mehta, B. and Fatma, A., 2010. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. *Colloids Surf. B Biointerf.*, **81**: 81-86.

Al-Daghri, N., Al-Attas, O., Alokail, M., Alkharfy, K., Yousef, M., Sabico, S. and Chrousos, G., 2011. Diabetes mellitus type 2 and other chronic noncommunicable diseases in the central region, Saudi Arabia (Riyadh cohort 2): A decade of an epidemic. *BioMed. Cent. Med.*, **9**: 1-6.

Awad, M., El Dib, R., Almusayeib, N., Al-Massarani, S., Ortashi, K. and Hendi, A., 2013. Novel *Balanites aegyptica* mesocarp synthesized silver nanoparticles: formation, characterization, antimicrobial, cytotoxicity and antiviral effects. *Digest J. Nanomat. Biostruc.*, **8**: 1665-1677.

Elobeid, M., Virk, P., Siddiqui, M., Omer, S., ElAmin, M., Hassan, Z., Almarhoon, Z., Merghani, N., AlMahasna, A., Daghestani, M. and Al-Olayan, E., 2013. Antihyperglycemic activity and body weight effects of extracts of *Emblica officianalis*, *Tamarix nilotica* and cinnamon plant in diabetic male rats. *Wulfenia*, **20**: 19-31.

Gilaki, M., 2010. Biosynthesis of silver nanoparticles using plant extracts. *J. biol. Sci.*, **10:** 465-467.

Guo, R., Song, Y., Wang, G. and Murry, W., 2005. Does core size matter in the kinetics of ligand exchanges of monolayer-protected Au clusters? *J. Am. Chem. Soc.*, **127**: 2752-2757.

Iyer, M. and Mani, U., 1990. Studies on the effect of curry leaves supplementation (*Murraya koenigii*) on lipid profile, glycated proteins and amino acids in noninsulin dependent diabetic patients. *Pl. Fds. Hum. Nutri.*, **40**: 275-282.

Kalishwaralal, K., Deepak, V., Pandian, S. and Gurunathan, S., 2009. Biological synthesis of gold nano cubes using *Bacillus licheniformis. Bioresour. Technol.*, **100**: 5356-5358.

Kumaresh, S., Tejraj, M., Anandrao, K. and Rudzinski, W., 2001. Biodegradable polymeric nanoparticles as drug delivery devices. *J. Contr. Rel.*, **70**: 1-20.

Midhet, F., Al-Mohaimeed, A. and Sharaf, F., 2010. Lifestyle related risk factors of type 2 diabetes mellitus in Saudi Arabia. *Saudi med. J.*, **31**: 768-774.

Mukherjee, P., Bhattacharya, R., Wang, P., Wang, L., Basu, S., Nagy, J., Atala, A., Mukhopadhyay, D. and Shay, S., 2005. Antiangiogenic properties of gold nanoparticles. *Clin. Cancer Res.*, **11**: 3530-3534.

National Institute of Health, USA, 2002. *Public health service policy on human care and use of laboratory animals*.

Nyadjeu, P., Nguelefack-Mbuyo, E., Atsamo, A., Nguelefack, T., Dongmo, A. and Kamanyi, A., 2013. Acute and chronic antihypertensive effects of *Cinnamon zeylanicum* stem bark methanol extract in L-NAME-induced hypertensive rats. *BMC Complem. Altern. Med.*, **13**: 13-27.

Palanive, V., Sang-Myung, L., Mahudunan, I., Kui-Jae, L. and Byung-taek, O., 2013. Pine conemediated green synthesis of silver nanoparticles and their antibacterial activity against agricultural pathogens. *Appl. Microbiol. Biotechnol.*, **97**: 361-368.

Ravichandran, R., 2013. Pharmacokinetic and pharmacodynamic studies on nanoparticulate gymnemic acids. *Int. J. Biotechnol. Biochem.*, **2**: 282-288.Salman, K., Muhammad, I., Muhammad, A., Abdul Rehman, and Khalid, M., 2015. Prevalence and Chemotherapy of Canine Diabetes Mellitus in and Around Lahore, Pakistan. *Pakistan. J. Zool.*, **47**(3): 895-898**.**

Samadder, A., Das, S., Das, J., Paul, A. and Khuda-Bukhsh, R., 2012. Ameliorative effects of *Syzygium jambolanum* extract and its poly (lactic-co-glycolic) acid nano-encapsulated form on arsenic-induced hyperglycemic stress: a multi-parametric evaluation. *J. Acupunc. Merid. Stud.*, **5**: 310-318.

Singh, M., Manikandan, S. and Kumaraguru, A., 2011. Nanoparticles: A new technology with wide applications. *Res. J. Nanosci. Nanotech.*, **1**: 1-11.

Shrivastava, S. and Dash, D., 2009. Applying nanotechnology to human health: Revolution in biomedical sciences. *J. Nanotech.*, **2009**: 1-14,

Vats, V., Grover, J. and Rathi, S., 2002. Evaluation of antihyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum*, Linn, *Ocimum sanctum*, Linn and *Pterocarpus marsupium*, Linn in normal and alloxanized diabetic rats. *J. Ethnopharmacol.*, **79**: 95-100.

Vinod, K., Mishel, W. and Schlosser, J., 2011. Efficacy of a novel, biologically active food supplements in type 2 diabetes mellitus: A patient-blinded, prospective, clinical trial. *J. Nutri. Diet. Suppl.*, **3**: 59-66.