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BIOCOMPATIBLE SILVER NANOPARTICLES SYNTHESIZED USING *RUMEX HYMENOSEPALUS* EXTRACT DECREASES FASTING GLUCOSE LEVELS IN DIABETIC RATS

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The objective of this study was to evaluate the antihyperglycemic activity of silver nanoparticles (SNPs) produced using green chemistry in a model of stretozotocin (STZ)-induced experimental diabetes in rats. Green SNPs (GSNPs) were synthesized using *Rumex hymenosepalus* extracts as reducing agent. In control and hyperglycemic rats was performed the measurement of blood glucose levels and glucose tolerance tests. Posteriorly, low dose of GSNPs were administrated in diabetic rats and was evaluated fasting blood glucose and glucose tolerance tests, pre and post-treatment. The basal level of fasting blood glucose of all rats (103.5 ± 4.4 mg/dL) increased after diabetes induction with STZ (315.2 ± 36.1 mg/dL). The treatment with GSNPs during 9 days decreased 50% the blood glucose in diabetic rats. The glucose tolerance test showed that in diabetic rats treated with GSNPs induces a minimal increase in blood glucose. In conclusion, a low dose of GSNPs showed antihyperglycemic activity.

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Keywords: Nanomedicine, green silver nanoparticles, diabetes

1. Introduction

The diabetes is a metabolic disorder characterized by complete or relative lack of insulin, and insulin resistance, which results in hyperglycemia, which continues growing to be major worldwide epidemic; it is estimated that in the 2025 the prevalence can be of 380 million [1]. Type 2 diabetes mellitus is the most common form of diabetes, with more 90% of diabetes cases, its varying prevalence among different racial and ethnic groups. The diabetes has been described as a new epidemic in the pediatric population in America because the overall 33% increase in the incidence and prevalence of diabetes observed during the last decade [2]. Similarly, Europe the incidence of diabetes in childhood has been rising by 3.9% each year during recent years [3]. In the case of Mexico, the proportion of adults diagnosed with diabetes in 2012 was 9.2%, while in 2000 and 2006 was 4.6% and 7.3% respectively. The increases in incidence and prevalence of diabetes in children and adults justify the study of new techniques and therapeutic procedures that can help to mitigate the effects of this serious disease.

The nanotechnology is commonly defined as the manipulation of mater about the nanometers order (1-100 nm) to create materials with novel properties and functions with a possible wide range to applications [4, 5]. The nanomedicine is the application of the nanotechnology to medicine. Because of the similarity in the domain of size of nanotechnology with the biological structures and certain functional properties it is expected to make significant

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advances in the areas of gene therapy, imaging and novel drug discovery and drug delivery in the treatment of diseases like diabetes and cancer [6, 7]. The cancer is a studied disease in nanomedicine, with nanostructures development with powerful therapeutic functions. Liposomes, polymer micelles and dendrimers are nanostructures responsible to carry drugs and small interfering RNA into target cells resulting in a powerful therapeutic modality [8-11].

Due to increase in morbidity and mortality associated to diabetes and cardiac diseases has aroused interest in the nanomedicine field. Recent studies with nanostructures show a promising progress in the diagnosis and treatment of cardiovascular diseases [12]. Today, the diabetes investigations are focused in improve the clinical diagnosis, in the development of blood glucose biosensors [13] and in the control and treatment with recent progress in the oral therapy trying to improve the bioavailability bioactivity and drug delivery [14]. Recently, it has been reported that insulin-chitosan nanostructured complexes improve the efficiency of oral insulin delivery [15]. Another interesting study reported that carbon nanotubes functionalized with nicotinamide increase the production of insulin in pancreatic beta cell generating a new expectative in the antidiabetic therapy [16].

The development of metal NPs stabilized and synthesized using green [17] or chemical reduction methods [18] have attracted interest in the clinical field for its possible beneficial effects or toxicological that can have in the biological systems. In experimental diabetes, it has reported that ZnO and silver NPs decrease the glucose levels and increase the production of insulin [19, 20]. Moreover, the ingestion of SNPs can cause high blood pressure, liver injury and endocrine glands, with doses and time dependent manner [21]. However, the metal NPs synthesized by chemical methods may have the presence of some toxic chemical species adsorbed on the surface, causing detrimental effects on cell proliferation and protein functions [22, 23]. The bio-compatible NPs synthesized from plant extracts can contribute to toxic compounds elimination. Studies have reported that the gold NPs synthesized through green methods improve the renal function and decreased the blood glucose in diabetic rats [24, 25], while, another study demonstrated that the selenium NPs have not effect on the glucose level. However, it exerts protective effect on the development of diabetic nephropathy [26]. Few studies are focused to investigate effects of green metal NPs on the diabetes. To our knowledge, only one study considers the antidiabetic effect evaluation of GSNPs using a reducing agent *Costus pictus* [27], commonly known as insulin plant, which has an antidiabetic effect [28]. These results show that SNPs produce high inhibition glycosylation and inhibit α-amylase and β-glucosidase enzymes. In the present work, we evaluated the antihyperglycemic activity of GSNPs reduced with *Rumex hymenosepalus*, in rats with STZ-induced experimental diabetes.

2. Experimental

2.1 Animals care

Male rats of the Wistar strain (n=16) with a weight 150 ± 15 g were used for the experiments. The rats were housed in individual cages with water and food ad libitum (18% proteins, 5% lipids, 5% fiber, enriched with vitamins and minerals). It were maintained in room with light-dark cycles (12 h/12 h) and room temperature controlled (25 °C). All animal studies were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH) and approved by the Institutional Animal Care and Use Committee of the University of Colima.

2.2 Synthesis of green silver nanoparticles

The synthesis of NPs was carried out following the methodology described by Rodríguez-León et. al. in 2013 with slight modifications [29]. The *Rumex hymenosepalus* extract was prepared with 15 g of dried root in 100 ml of ethanol: water (70:30 v/v), this extract was filtered and mixed with a 0.1 M $AgNO_3$ solution in a 1:1 ratio. We observed the synthesis of SNPs by visual inspection. Green silver nanoparticles were isolated by centrifugation and resuspended in ultrapure deionized water. The reaction products were analyzed using Transmission Electron Microscopy (TEM) and UV-Vis spectroscopy to determine the size of NPs obtained. Finally, with

this methodology are obtained SNPs stabilized by polyphenols mixture with the average diameter of 9 nm which were used in this study. The concentration of SNPs in the *Rumex hymenosepalus* extract is 1.07 mg/ml or 1 070 μg/ml.

2.3 Experimental diabetes induction

Fasting blood glucose levels and glucose tolerance tests were evaluated in all animals as control conditions or baseline prior to induction of diabetes. Hyperglycemia was induced by intraperitoneal administration of STZ dissolved in citrate buffer pH = 4.5 in a single dose of 55 mg / kg body weight; 3 and 7 days after was performed the measurement of blood glucose levels and glucose tolerance tests to ensure that the rats were diabetic; the blood samples were collected from rat tail. Glycemia was assessed with glucose oxidase reaction using a Cobas Plus Accutrend® system.

2.4 Experimental design

The rats were divided into 2 groups: diabetic rats (n = 8) and treated diabetic rats (n = 8). Treated diabetic rats group received an administration of GSNPs in a single dose of 150 μg / kg body weight i.p during 9 days. After treatment, the fasting blood glucose and glucose tolerance tests were measured to assess their antihyperglycemic activity.

2.5 Statistical analysis

Data were expressed as means ± standard error. Experimental groups were compared using an analysis of variance with post hoc tests of Bonferroni or Mann Whitney to compare means between pairs of groups. The significance level used in the study was 95% ($p \leq 0.05$). All analyzes were conducted with the Sigmaplot software (version 10.0).

3. Results and Discussion

3.1 Structural characterization of SNPs obtained with *Rumex hymenosepalus*

The UV-Vis spectrum of SNPs synthesized by *Rumex hymenosepalus* extracts shows two absorption bands: 1) the first in 278 nm is a feature of polyphenols presents in extract and 2) the absorption in 450 nm correspond to SNPs (Fig. 1). The decreasing of absorbance in 278 nm band after NPs synthesis can be attributed to the oxidation of polyphenols when their electrons (obtained from deprotonation) are transferred to silver ions to generate metal silver [30]. This curve is asymmetric and finds and the range between 260 and 350 nm this suggest the presence of different types of molecules, this was confirmed using nuclear magnetic resonance [29].

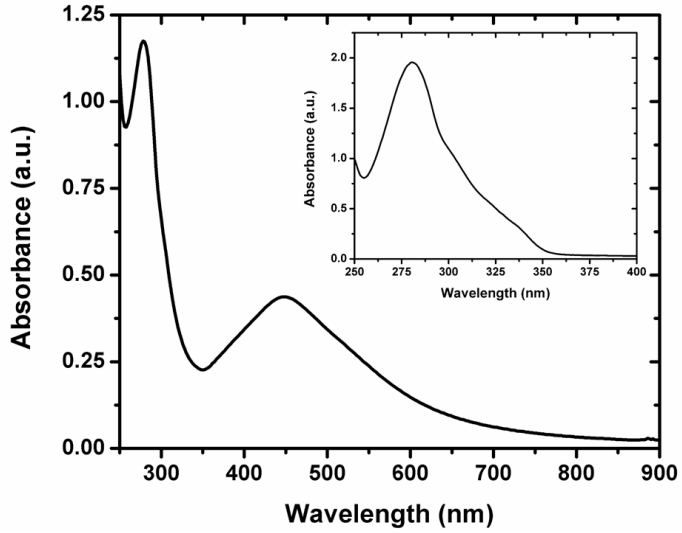

Fig. 1. Absorption spectrum of SNPs synthesized by Rumex hymenosepalus extract. Figure Inset corresponds to the absorption spectrum of Rumex hymenosepalus extract.

We use equipment Jeol 2010F operating to 200 keV to obtain images (Fig. 2a) by TEM that show the size and shape characteristics of the system. The histogram was obtained for the statistics with 300 particles where the histogram (Fig. 2b) shows a size mean of 9 ± 3 nm and the shape is cuasi spherical.

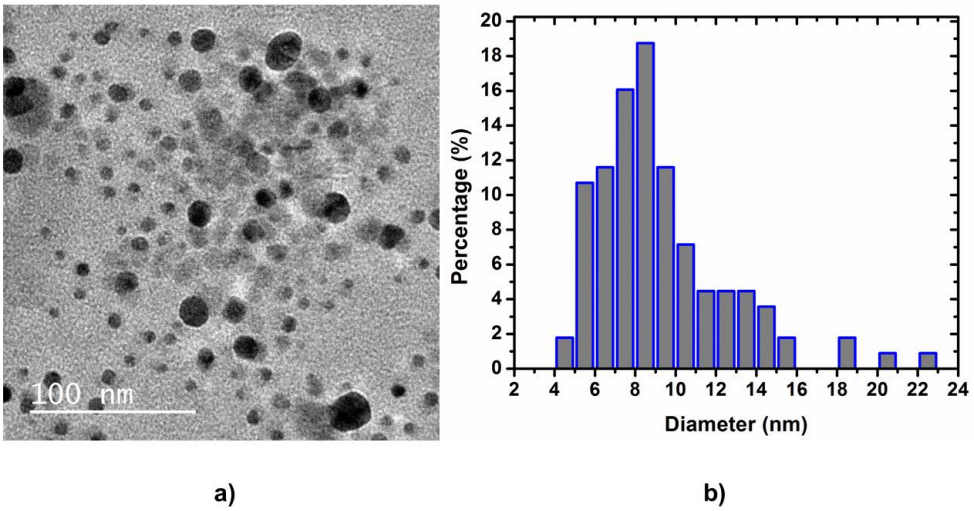

Fig. 2. a) SNPs microphotography was obtained with a TEM that operating to 200 kV. b) Histogram for sizes distribution, mean size is 9 nm

The crystalline structure was analyzed with images of High resolution of TEM (HRTEM), where we observed the interplanar distances characteristics of a hexagonal structure (Fig. 3a) for Ag. With the Fast Fourier Transformation of electron diffraction pattern (Fig. 3a inset) the Micrograph digital was generated the structure of the Fig. 3b where the interplanar distances are 2.50 y 2.41 Å correspond with the indexes Miller (100), (101).

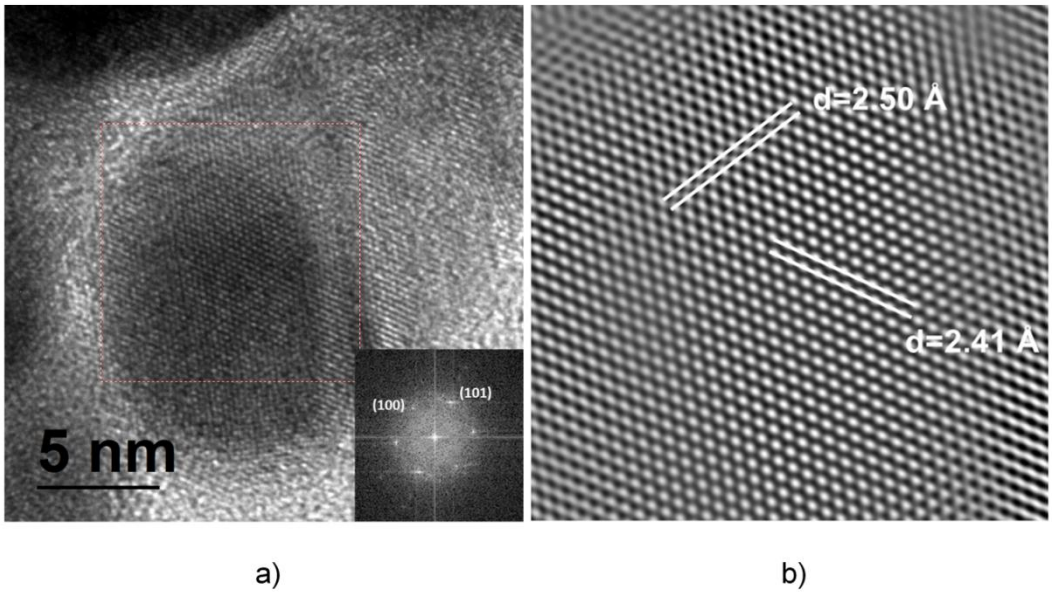

Fig. 3. a) HRTEM of silver nanoparticle that show interplanar distances consistent with a crystalline structure of type 4H hexagonal, in the inset we obtained the index Miller of the electron diffraction and b) reconstruction of the crystalline structure was realized using the digital micrograph.

3.2 Effect of SNPs obtained with *Rumex hymenosepalus* in experimental diabetes

The basal level of fasting blood glucose of all rats (103.5 ± 4.4 mg/dL) increased after diabetes induction with STZ (315.2 ± 36.1 mg/dL) (Fig. 4). In contrast, the treatment with GSNPs during 9 days decreased significantly the blood glucose level in diabetic rats (159 ± 15 mg/dL).

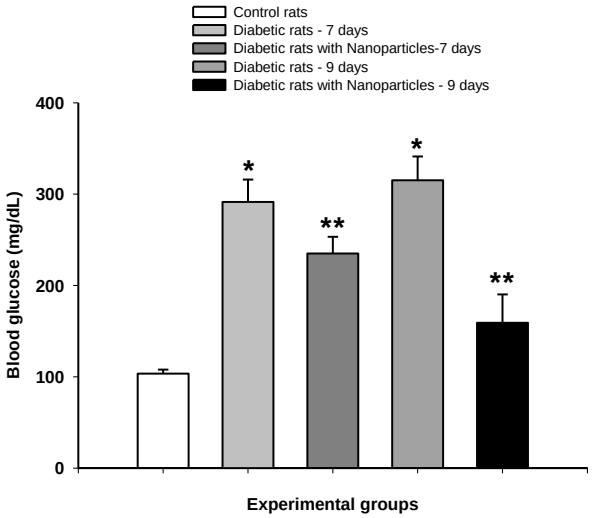

*Fig. 4. Effect of GSNPs on the fasting blood glucose level. The data correspond to means ± standard error. * p<0.05 respect to control group; ** p<0.05 respect to diabetic group. n=8 by group.*

On the other hand, the analysis of the glucose tolerance tests of the different experimental groups (Fig. 5) shows that in the case of healthy rats or control after the glucose load there is an increase in blood glucose during the first minutes and then decrease to return to baseline level. In contrast, diabetic rats with the glucose load administration significantly increased their glucose levels, but not returned to baseline level. Interestingly, diabetic rats treated with GSNPs induce a minimal increase in blood glucose levels after administrated glucose load, this increase is significantly less than the untreated diabetic group; and similar that the diabetic rats, the increase that occurs in the glycemia of treated rats with GSNPs do not return to baseline level within the time studied but also not increase.

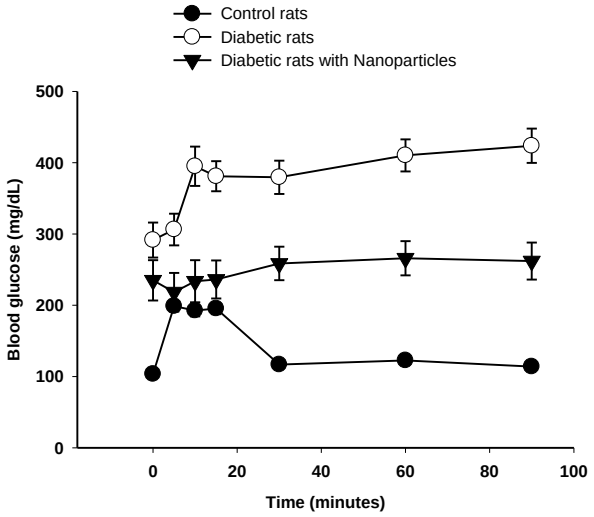

Fig. 5. Glucose tolerance tests. Load of 1 g/kg glucose was administrated via i.p. in all experimental groups. The data correspond to means ± standard error from glycemia level to different times. n=8 by group

The use of biocompatible metal nanoparticles in the treatment of the diabetes and its complications is a field promising in nanomedicine. In the present study, we are provided evidence of the antihyperglycemic activity in vivo of biocompatible GSNPs in experimental diabetes using NPs of 9 nm. Previous studies reported that higher doses (10 mg/kg) of SNPs decreases to 161 ± 10 mg/dL and ZnO NPs to 122 ± 7 mg/dL the glucose level respectively after of 30 days of treatment [20], others studies shows that ZnO NPs decreases 29% the glucose level [19], and the gold NPs 43% [24], interestingly in our study low dose (150 μg/kg) of GSNPs decreased hyperglycemia by 50% in short time.

In the literature, it is well documented that in healthy rats or control after the glucose load increases glucose rapidly and insulin action returns to basal levels whereas in diabetic rats increased blood glucose is much higher and does not return to its basal level due to low insulin production. In our study the diabetic rats that were treated with GSNPs attenuate the increasing in the blood glucose level in the glucose tolerance test, suggesting that the GSNPs exert a protective effect. Previous work studied the therapeutic effect of ZnO and SNPs on STZ-induced diabetic rats [20]. The ZnO NPs significantly increase of serum insulin level (358.6 ± 16.46 pg/mL) and the histopathological cuts of the pancreas showing some normal islets of Langerhans in between normal pancreatic acini and normal pancreatic ducts, in contrast, the SNPs treatment results in a low increase of serum insulin (206.2 ± 3.33 pg/mL) and pancreas showing disruption in the islets of Langerhans with damage in B-cells. In both treatments, blood glucose decreased in 75.8%, with ZnO NPs, and 68.2% with SNPs in compare with insulin treatment. They suggest that the effect of ZnO NPs is mediate by insulin and the effect of SNPs by an increase in the glucokinase activity and GLUT-2 expression in hepatic tissues. These authors applied a daily dose of ZnO NPs and SNPs of 10 mg/kg body weight for 30 days. This dose is around 66 times above the doses used in the present work: 150 μg/kg body weight for nine days. Therefore, to our knowledge is the first study using a low dose of GSNPs that induces an antihyperglycemic effect with less risk of toxic effects.

Based on the above findings, a plausible explanation for the effect of treatment with GSNPs is the enhancing effect on the activity of glucokinase and GLUT-2 expression in liver tissue which increase glucose uptake, however, futures experiments are required to elucidate the mechanism of activation that participates in the antihyperglycemic effect induced by GSNPs. Moreover the doses used in the order of micrograms per kg of body weight in the present study are very far from the LC50 and LD50 reported [31-33].

4. Conclusion

Low dose of biocompatible SNPs produced by green synthesis with *Rumex hymenosepalus* extract have significant antihyperglycemic effect in streptozotocin-induced experimental diabetes in rats. The glucose tolerance tests of the group treated with green SNPs suggest that the implicated mechanism have a rapid regulation of the circulating glucose; however, future biochemical and molecular studies are needed to elucidate the mechanism.

Acknowledgments

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References

[1] S. Golbidi, M. Badran, I. Laher, Exp. Diabetes Res. **2012**, 1 **(**2012).
[2] F.R. Kaufman, Clin. Diabetes. **20,** 217 (2002).
[3] S. Williamson, J. R. Coll. Physicians Edinb. **40,** 25 (2010).
[4] National Science and Technology Council. National nanotechnology iniciative strategic plan.

Executive office of president of the United States. (2007).
[5] S. Logothetidis, Hippokratia. **10,** 7 (2006).
[6] K. Subramani, S. Pathak, H. Hosseinkhani, Dig. J. Nanomater. Bios. **7,** 85 (2012).
[7] G. Neil, S. Uri, Canadian NanoBussiness Alliance (2003).
[8] V.P. Torchilin, Nat. Rev. Drug Discov. **4,** 145 (2005).
[9] D. Peer, E.J. Park, Y. Morishita, C.V. Carman, M. Shimaoka, Science. **319,** 627 (2008).
[10] E. Blanco, A. Hsiao, A.P. Mann, M.G. Landry, F. Meric-Bernstam, M. Ferrari, Cancer Sci. **102**, 1247 (2011).
[11] E. Blanco, M.Ferrari, Breast. **23**, 10 (2014).
[12] M.P. Ferreira, V. Balasubramanian, J. Hirvonen, H. Ruskoaho, H. A. Santos, Curr. Drug Targets. (2014).
[13] M. Veerapandian , Y.T. Seo, H. Shin, K. Yun , M. H. Lee, Int. J. Nanomedicine. **7**, 6123 (2012).
[14] E. Gundogdu, A. Yurdasiper, Int. J. Endocrinol. Metb. **12,** :e8984 (2014).
[15] L. Song, Z.L. Zhi, J.C. Pickup, Int. J. Nanomedicine. **9,** 2127 (2014).
[16] I. Ilie, R. Ilie, T. Mocan, F. Tabaran, C. Iancu, L. Mocan, Int. J. Nanomedicine. **8**, 3345 (2013).
[17] G. Rajakumar, A.A. Rahuman, S.M. Roopan, I.M. Chung, K. Anbarasan, V. Karthikeyan, Parasitol. Res. **114**, 571 (2014).
[18] J.M. Ibarra-Hurtado, A. Virgen-Ortiz, A. Apolinar-Iribe, A. Luna-Velasco, Dig. J. Nanomater. Bios. **9,** 493 (2014).
[19] R. D. Umrani, K. M. Paknika, Nanomedicine (Lond). **9,** 89 (2014).
[20] A. Alkaladi, A. M. Abdelazim, M. Afifi, Int. J. Mol. Sci. **15,** 2015 (2014).
[21] M.H. Razavian, M. Masaimanesh, Nanomedicine J. **1,** 339 (2014).
[22] N. Lewinsky, V. Colvin, R. Drezek, Small. **4**, 26 (2008).
[23] E. K. Rushtona, J. Jiangb, S.S. Leonardc, S. Eberlyd, V. Castranovac, P. Biswasb, A. Eldera, X. Hana, R. Geleina, J. Finkelsteinae, G. Oberdörstera, J. Toxicol. Envirom. Health. A. **73,** 445 (2010).
[24] P Daisy, K. Saipriya, Int. J. Nanomedicine. **7,** 1189 (2012).
[25] V. Karthick, V. G. Kumar, T. S. Dhas, G. Singaravelu, A. M. Sadiq, K. Govindaraju, Colloids Surf. B. Biointerfaces. **122,** 505 (2014).
[26] G. S. Kumar, A. Kulkarni, A. Khurana, J. Kaur, K, Tikoo, Chem. Biol. Interact. **223,** 125 (2014).
[27] A. Aruna, S. R. Nandhini, V. Karthikeyan, P. Bose, K. Vijayalakshmi, Indo American J. Pharm. Res. **4**, 3217 (2014).
[28] M. A. Jyarsi, S. Gunasekaran, A. Radha, T. L. Mathew, Int. J. Diabetes Metabolism. **16**, 117 (2008).
[29] E. Rodríguez-León, R. Iñiguez-Palomares, R. E. Navarro, R. Herrera-Urbina, J. Tánori, C. Iñiguez-Palomares, A. Maldonado, Nanoscale Res. Lett. **8**, 318 (2013).
[30] K. S. N. Kamaldeep, S. Kaur, V. Bhalla, M. Kumar, A. Gupta, J. Mater. Chem. A. **2,** 8369 (2014).
[31] S. K. Singh, K. Goswami, R. D. Sharma, M. V. Reddy, D. Dash. Int. J. Nanomedicine. **7**, 1023 (2012).
[32] D. Shahbazzadeh, H. Ahar, R. N. Mohammad, F. Dastmalchi, M. Soltani, M. Fotovat, J. Rahmannya, N. Khorasani, Pak. J. Nutr. **8**, 1178 (2009).
[33] K. Bilberg, M. B. Hovgaard, F. Besenbacher, E. Baatrup, J. Toxicol. **2012**, 293784 (2012).