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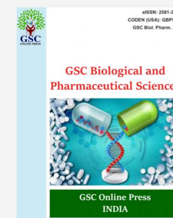

(Research Article)

Antidiabetic and wound healing potential of biologically fabricated chitosan nanoparticles of aqueous seed extract of *Hunteria umbellata*

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Abstract

Background: *Hunteria umbellata* (HU) is a tropical rainforest shrub in the Apocynaceae family, with various medicinal properties. Nanostructured systems improve pharmacokinetic and pharmacodynamic properties of bioactive compounds. The current study evaluates the antidiabetic and wound healing efficacy of *Hunteria umbellata*-chitosan nanoparticles (HUCNPs).

Methods: Aqueous seed extract was prepared from mature HU pods. Chitosan nanoparticles of *H. umbellata* were created by ionic gelation of chitosan with tripolyphosphate anions (TPP) and characterized using Dynamic light scattering (DLS) and Fourier Transform Infrared Spectroscopy (FT-IR). The streptozotocin-induced diabetic rat model and wound excision model were used to investigate the antidiabetic activity and wound healing efficacy of HUCNPs. Serum biochemical marker enzymes and antioxidants from kidney and liver tissues were quantified.

Result: FT-IR confirmed the successful cross linkage of bioactive compounds to the chitosan nanoparticles. HUCNPs had particle size of 408.6 ± 1.5 nm, zeta potential of 32.4 ± 1.6, and polydispersity index of 0.47. Rats treated with HUCNPs had a significantly reduced mean fasting blood glucose level. Serum levels of Glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), and alkaline phosphatase were closer to normal than diabetic control. Catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) levels were higher in HUCNPs-treated liver and kidney tissues, while lipid peroxidase (LPO) levels were lower. The topical application of the HUCNPs ointment significantly accelerated wound healing (81.6 %) and prevented the microbial invasion of the wound surface.

Conclusion: This study suggests HUCNPs as an excellent strategy for targeted drug delivery for diabetes management and accelerated wound healing.

Keywords: Antioxidant enzymes; Bioactive compounds; Diabetes; *Hunteria umbellata*; Nanoparticles; Wound healing

1. Introduction

Diabetes Mellitus is a chronic metabolic condition defined by sustained hyperglycemia and caused by either a low systemic insulin level or insulin resistance, both of which can have long-term health effects [1]. Diabetes Mellitus is

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expected to affect approximately 500 million adults by 2030 [2], with African and Asian populations expected to bear the brunt [3]. It has been linked to impaired wound healing, which frequently leads to the development of foot ulcers. According to research, diabetic patients are up to 25% more likely to develop foot ulcers [4]. Diabetic wounds are difficult to heal due to prolonged hyperglycemia, which is thought to encourage the production of biofilms [1]. Diabetes is characterized by impaired angiogenesis, increased oxidative stress, neuropathy, a suboptimal chronic inflammatory response, barrier disruption, and subsequent polymicrobial infections [1]. All of these factors contribute to the prolonged wound healing in diabetes.

Despite advancements in diabetes treatment development, current medicines appear insufficient in preventing diabetic complications, particularly in type 2 diabetes [5]. As a result, novel therapies for the holistic treatment of diabetes are required. In searching for an alternative therapy for managing diabetes, there has recently been a shift toward indigenous plants and herbal formulations [5]. Plant extracts are generally thought to be less toxic than synthetic drugs.

Hunteria umbellata is a tropical rainforest shrub belonging to the Apocynaceae family. It has glabrous, smooth fruits with disc-shaped seeds in a gelatinous jelly [6]. It is common in Nigeria and other parts of western and central Africa [7]. It has been widely used in treating infectious diseases, pain management, and metabolic disorders such as diabetes and obesity [8], as well as treating abdominal discomfort, piles, and infertility [9]. The antidiabetic, anti-obesity and anti-hyperlipidemic effects of aqueous seed extract of *H. umbellata* have been studied in various diabetic experimental models [10, 11]. Oral toxicity studies on experimental animals have validated its safety [12]. However, there has been a report of frequent vomiting caused by an allergic reaction to the extreme bitterness of its seed extract [13]. More so, the bioactive molecules abundant in plants are usually unstable in the gastro intestinal tract due to elevated pH and harsh conditions that render them less effective.

Plant extracts can be improved for disease treatment by encapsulating them in a biocompatible polymeric material. This novel strategy can potentially improve the controlled delivery of plant bioactive molecules and thus increase bioavailability [14]. Polymeric nanoparticles are a popular and promising drug delivery system widely used for this purpose. By employing biodegradable and biocompatible polymer materials like chitosan, nanotechnology provides drug molecules with several advantages, including improved solubility, bioavailability, and pharmacological activity, protection from toxicity, sustained delivery, and resistance to physical and chemical degradation [15, 16].

Chitosan is a polycationic linear polysaccharide derived from the deacetylation of chitin [17]. Its versatile use as a polymer for biomedical applications is due to its biocompatibility, biodegradability, and low toxicity, as well as its ability to promote the absorption of poorly absorbed macromolecules across epithelial barriers [18, 19, 20]. It can also participate in various chemical reactions via its amino group [21]. Its numerous pharmaceutical and biomedical applications are well documented [22]. Furthermore, high molecular weight chitosan has been shown to reduce hyperglycemia and hypercholesterolemia in streptozotocin-induced diabetic rats [23]. As a result, chitosan-based nanoparticles could be very important carriers for antidiabetic drug delivery.

The current study aimed to formulate and assess the potential of *Hunteria umbellata*-CNPs as an effective alternative for targeted drug delivery and diabetes treatment, as well as their efficacy in promoting diabetic wound healing in streptozocin-induced diabetic rats.

2. Material and methods

2.1. Material

Chitosan nanoparticles were prepared using medium molecular weight chitosan (190-310 kDa, deacetylation degree: 75-85%; Sigma-Aldrich). Tripolyphosphate, TPP (Fischer Scientific), and glacial acetic acid (Fischer Scientific) were purchased commercially. All other reagents were all of the analytical grades.

2.2. Methods

2.2.1. Collection, Identification and preparation of aqueous seed extract of Hunteria umbellata (HU)

Fresh *Hunteria umbellata* leaves, inflorescence and mature pods were collected in March 2020 from the coniferous forest of Agu-amanyi Orba in Udenu Local Government of Enugu State, Nigeria. Botanical identification was performed by Mr AO Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), and a voucher specimen was deposited in the institution's herbarium. The plant was authenticated by Mr. Onoja JO of the Department of Pharmacognosy, University of Nigeria, Nsukka. The *Hunteria umbellata* seeds were harvested from the fresh fruit

pods and gently washed in running water. It was then dried at room temperature (25–27 °C) for one month. The seeds' light brown coatings were removed when they were dried.

2.2.2. Preparation of the aqueous seed extract of Hunteria umbellata

The aqueous seed extract of HU was prepared using the method of Adeneye and Adeyemi [24], with minor modifications. Using a hammer mill (500# grinder/Fuyu metal, Linyi Fuyu metals products Co. Ltd., China), 200g of HU dry seeds were pulverized to a fine powder. 50g of the finely powdered sample was boiled in 500 ml of distilled water in a 1L Pyrex beaker for 1 hour at 100 °C while stirring continuously. After cooling for 8 hours, the mixture was rapidly filtered through a piece of white cotton muslin cloth. The filtrate was dried completely in an oven set at 40 °C, yielding a deep brown, aromatic solid residue. The residue was then placed in an airtight container and stored in a refrigerator at -4 °C. This was used as the "fresh stock" for subsequent experiments.

2.2.3. Bio-synthesis of Chitosan Nanoparticles (CNPs)

The HU-loaded chitosan nanoparticles were created using the nanoprecipitation method described by Venkatachalam *et al* [25] with slight modifications. In brief, an aqueous seed extract of HU was prepared at a concentration of 100 mg/ml. Then, at a rate of one drop per second, 150 ml of chitosan (1% w/v in 1% v/v acetic acid) was added drop wise to 150 ml of HU aqueous seed extract containing 0.5% v/v tripolyphosphate (TPP) anion. For 30 minutes, the nanoparticle suspension was continuously stirred at 120 rpm. The above procedure was used to prepare blank chitosan nanoparticles. The nanoparticles were collected by centrifugation at 1200 rpm for 15 minutes and then washed three times with distilled water. The nanoparticles were then characterized using various techniques.

2.3. Nanoparticles Characterization

2.3.1. Zeta potential (ZP) and size distribution of the nanoparticles

The zeta potential, particle size, and mean particle size distribution of nanoparticles (Chitosan nanoparticles (CNPs) and *Hunteria umbellata* chitosan nanoparticles (HUCNPs)) were determined using a zetasizer nano-Zs90 (Malvern Instruments) at 25 °C at an angle of 90°. The nanoparticles were dispersed in an aqueous solution, and 3 mL of the solution was placed in a quartz cuvette and analyzed.

2.3.2. Fourier Transform Infrared Spectroscopy (FT-IR)

The nanoparticles were examined for chemical structure integrity and potential interactions between their constituents. The chitosan, CNPs, and HUCNPs infrared spectra were obtained using a Perkin-Elmer-1600 Fourier infrared spectrophotometer (Shimadzu-8300 FT-IR). The samples were scanned at wavelengths ranging from 500 to 4000 cm^{-1}, and spectra were recorded on KBr tablets in transmittance mode (1 mg of powder sample per 400 mg of KBr).

2.4. Antidiabetic Study

2.4.1. Experimental animals

The study used Wister albino rats (150–200 g) that were in-house bred in the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka. Rats were housed in metallic cages with free access to food and water in standard environmental conditions (temperature: 27 ± 2 °C, relative humidity: 60-65 %, and 12:12-light: night cycles). The University of Nigeria, Nsukka's animal ethics committee reviewed and approved the experimental protocols prior to the start of the experiments. The rats were handled following the guidelines for the care and use of laboratory animals [26].

2.4.2. Induction of diabetes

Streptozotocin, STZ (Sigma-Aldrich, San Louis, MO, USA) induced diabetes in experimental animals. Freshly prepared STZ (50mg/kg b.w) dissolved in 0.1M citrate buffer (pH 4.5) was administered intraperitoneally to overnight fasted rats. Blood glucose levels were measured 48 hours after STZ administration [27]. Animals with blood glucose levels greater than 200 mg/dL were considered diabetic and used for the study.

2.4.3. Acute toxicity and dose calculation

The acute oral toxicity of HUCNPs in rats was investigated using the method previously described by Lorke [28]. The rats were unaffected by HUCNPs up to a dose of 2 g/kg b.w. In most cases, one-fifth to one-tenth of the lethal dose is used to calculate the effective dose. In this study, HUCNPs was given at a dose of 400 mg/kg b.w.

2.4.4. Animal grouping

Rats were divided into six groups, each with five rats.

- Group I: Normal control
- Group II: Diabetic control rats
- Group III: Diabetic rats treated with Glibenclamide (0.5 mg/kg b.w)
- Group IV: Diabetic rats treated with aqueous seed extract of HU (400 mg/kg b.w)
- Group V: Diabetic rats treated with chitosan nanoparticles, CNPs (400 mg/kg b.w)
- Group VI: Diabetic rats treated with HU-chitosan nanoparticles, HU-CNPs (400mg/kg b.w)

Each formulation was reconstituted in an appropriate amount of distilled water, and 1 ml of solution was given orally using a 2 ml syringe for all administrations. The respective formulations were given for 28 days.

2.4.5. Estimation of blood glucose

Blood glucose level was measured using the glucose-oxidase-peroxidase reactive strips in a glucometer (Accuchek®, Roche, USA). Blood was collected from the tail tip and used for estimation on study days 1, 7, 14, 21, and 28.

2.4.6. Biochemical estimations

Biochemical marker enzymes such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) were measured in the serum of experimental animals [29, 30]. The glycosylated haemoglobin kit (A1CNow®, USA) was used to determine HbA1c levels. Protein concentrates in tissue homogenates were measured by the method of Lowry *et al* [31]. Liver homogenates prepared in an ice-cold 10% w/v KCl solution were used to measure the levels and activities of lipid peroxidation, LPO [32]; superoxide dismutase, SOD [33]; catalase, CAT [34]; and reduced glutathione, GSH [35].

2.5. Wound healing study

The wound healing study used diabetic rats from the procedure described in 2.4.2 above.

2.5.1. Excision of wound

The excision of the wound model reported by Isenberg [36] was used in excising wounds in diabetic animals. The animals were first anaesthetized with 10 mg/kg b.w ketamine hydrochloride, and the furs at the desired injury site shaved off. The shaved-off portion was disinfected with 70% ethanol, and the wound was excised. To mimic a typically exposed wound and ensure a regular size before treatment, the wound was left undressed and exposed to the environment for 24 hours with no systemic or topical antimicrobial agent applied.

2.5.2. Treatment of wound

A simple ointment base (white petroleum jelly) was used as control and to impregnate all other formulations. 10g of petroleum jelly was weighed into a beaker and melted in a water bath. Appropriate amounts of each formulation were triturated with the molten ointment base. The rats were then divided into four groups of five rats each. Group 1 was treated with a simple ointment base, group 2, with reference gentamicin ointment, group 3, with HU-CNPs ointment and group 4, with HU seed extract ointment. The same amount of ointment base and gentamicin were used as the control and standard, respectively. The various treatment formulations were applied once daily for 20 days.

2.5.3. Evaluation of wound infection and healing

Exudates from wound sites were extracted and cultured on days 5, 10, and 15 to identify the prevalent organism capable of causing wound infections. The wound diameter was measured and compared to the baseline on treatment days 4, 8, 12, 16, and 20. Equation 1 below was used to calculate the degree of wound healing:

$$\frac{D_0 - D_y}{D_0} \times 100 \qquad \text{equation 1}$$

Where D_0 = Wound diameter on day 1 and D_y = wound diameter on corresponding days.

2.6. Statistical analysis

All grouped data were statistically evaluated using IBM SPSS 22 software. The hypothesis testing method included a one-way analysis of variance (ANOVA) followed by Duncan's multiple range comparison tests. *P*-values less than 0.05 were considered to indicate statistical significance. All results are expressed as means ± SEM of the five animals in each group.

3. Results and discussion

3.1. Synthesis of chitosan nanoparticles

Chitosan nanoparticles were prepared through ionic crosslinking of positively charged chitosan with negatively charged STPP and biomolecules of HU (in the case of HUCNPs). When STPP was added to the chitosan solution, the previously clear solution became an opalescent emulsion, indicating that CNPs had formed.

3.2. Characterization of the nanoparticles

Table 1 shows the particle size, particle size distribution, and zeta potential of nanoparticles determined by dynamic light scattering and a particle size analyzer. CNPs had a particle size distribution (polydispersity index) of 0.36, a zeta potential of 39.1 ± 0.8, and a mean particle size of 356.1 ± 0.5 nm. HUCNPs, on the other hand, had a particle size distribution (polydispersity index) of 0.47, a zeta potential of 32.4 ± 1.6, and a mean particle size of 408.6 ± 1.5 nm. Figure 1 shows the Fourier Transform Infrared (FT-IR) spectrum of chitosan, CNPs, and HUCNPs.

In the characterization of nanoparticles, particle size and zeta potential are crucial. The nanoparticle size and distribution affect their saturation solubility, dissolution rate, physical stability, and *in vivo* performance [37]. The zeta potential reflects the particle's overall surface charge density. It indicates the overall stability of the nanoparticles. A high zeta potential value indicates a more stable suspension free of aggregation caused by elastic repulsion. A low zeta potential value indicates colloidal instability, which leads to nanoparticle instability [38]. The particle size distribution, on the other hand, indicates the homogeneity of the nanoparticles [39]. In our study, CNPs and HUCNPs had particle size distributions less than 0.5, indicating a unimodal particle size distribution and high homogeneity of the nanoparticles. However, HUCNPs had a wider size distribution than CNPs. This could be attributed to bioactive molecules enriched with chitosan nanoparticles. Venkatachalam *et al.* [25] obtained a similar result. The high zeta potential of the nanoparticles indicates good stability.

A nanoparticle formulation is considered stable if its ZP value is less than -30 mv or greater than +30 mv [40]. The Zp values of these formulations are expected. Chitosan is a polycationic electrolyte in aqueous solutions with amino groups having a rigid intramolecular and intermolecular bond. Due to electrostatic repulsion between the chains, it adopts an extended conformation with more flexible chains. STPP, on the other hand, is a phosphate group-containing polyanionic electrolyte. When chitosan and STPP are mixed in dilute acetic acid, they spontaneously form complex nanoparticles with a net positive surface charge. The amino and hydroxyl groups (of chitosan) form hydrogen bonds with the hydroxyl group or water or oxygen atoms, resulting in a positive zeta potential [41]. The presence of negatively charged groups in HU seed extracts, such as [OH^-] and [COO^-], could explain why HUCNPs have a lower zeta potential than CNPs. The dissociation of the hydroxyl and carbonyl groups of their phytochemical constituents is thought to produce these anions. This trend is similar to what Servat-Medina *et al* [14] reported, but it contradicts the reports of Venkatachalam *et al* [25].

Table 1 Sizes and zeta potential values of the chitosan and *Hunteria umbellata*-loaded chitosan nanoparticles

Samples		
Parameters	**Chitosan nanoparticles (CNPs)**	***Hunteria umbellata* chitosan loaded nanoparticles (HUCNPs)**
Particle size (nm) at pH 6.0 (mean ± SEM)	356.1 ± 0.5	408.6 ± 1.5
Polydispersity index (mean ± SEM)	0.36	0.47
Zeta potential (mV) at pH 6.0 (mean ± SEM)	39.1 ± 0.8	32.4 ± 1.6

FT-IR offers an approach for qualitative verification of functional groups and possible interactions in a formulation. The result of the FT-IR spectra is presented in figure 1. The FT-IR spectra for chitosan showed a broad peak of 3263.53 cm^{-1}, thought to be due to a combination of stretching modes of hydroxyl and amino groups [42]. For the nanoparticles, a wider band was observed, possibly due to the enhancement of hydrogen bonds resulting from the hydrostatic interactions between the amino group of chitosan and the phosphate group of TPP ions[43]. More so, there was a shift in the peak of NH_2 bonding vibrations from 1650 cm^{-1} (in chitosan) to 1628 cm^{-1} for the nanoparticles and then from 1153.67 cm^{-1} to 1112.84 cm^{-1} for the nanoparticles. This could be attributed to interactions between the amino group and the phosphate anion[44]. A similar result has been observed for chitosan-TPP nanoparticles [45]. There was a new absorption band at 1220.35 cm^{-1} in the samples of CNPs due to PO stretching [17].The FT-IR of HU extract revealed bands at 1380.78 cm^{-1}, 1600.86 cm^{-1}, and 1705.55 cm^{-1} that were not present in CNPs but in HUCNPs. This indicates that the CNPs were successfully functionalized and incorporated [44].

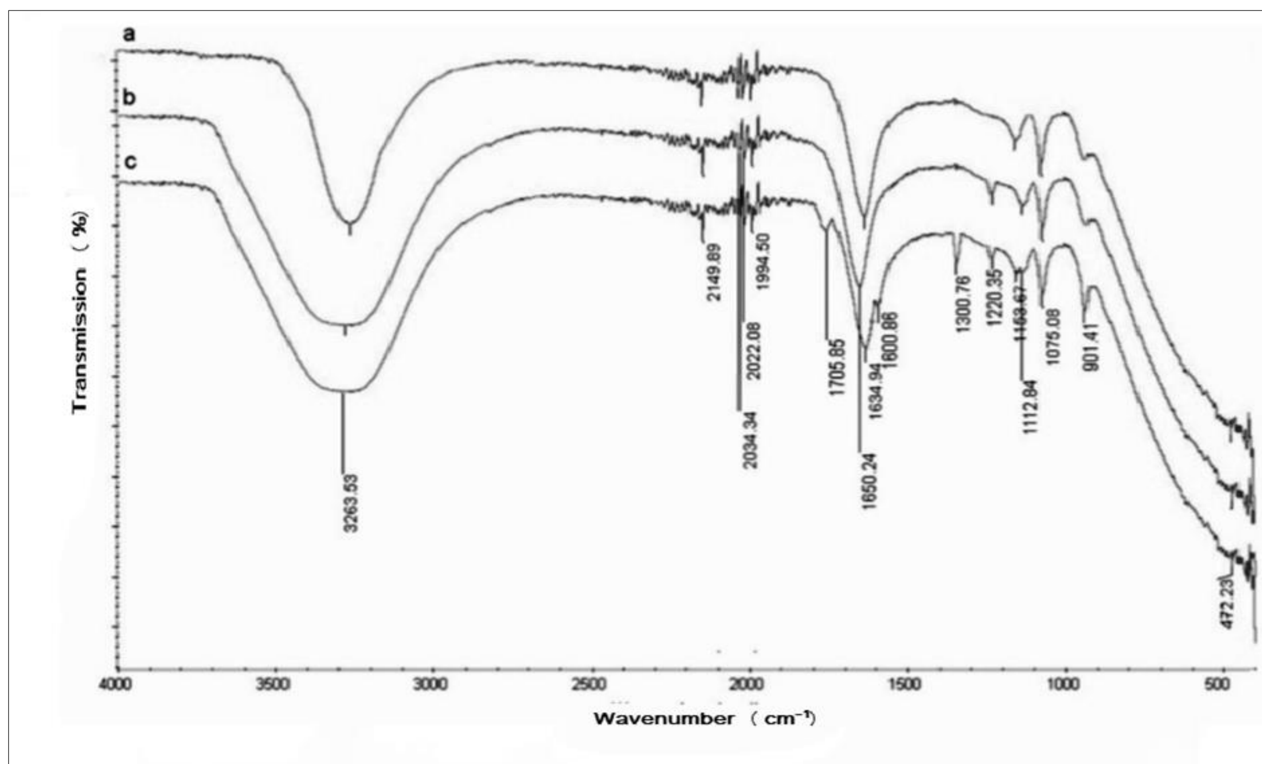


Figure 1FT-IR spectra of chitosan (a), chitosan nanoparticles (b) and *Hunteria umbellata* chitosan nanoparticles, HUCNPs (c)

3.3. Effect of HUCNPs on fasting blood glucose and body weight in STZ-induced diabetic rats

The body weight of rats and fasting blood glucose levels were monitored during the experimental period. The result of the change in body weight is presented in figure 2. STZ caused a significant weight loss ($p<0.05$) in diabetic control rats (group 2) compared to normal control rats (group 1). Treatment with HUCNPs resulted in a significant weight gain on day 28 compared to day 1. The same trend was observed in rats treated with HU seed extract (group 3), but the effect of weight loss reversal was more significant in rats treated with HUCNPs. The result of the fasting blood glucose level is shown in figure 3. Compared to normal rats (group 1), STZ caused a significant increase in blood glucose levels. Fasting blood glucose levels were significantly ($p < 0.05$) reduced after 28 days of daily administration of 400mg/kg b.w HUCNPs. At the end of the experiment (day 28), HUCNPs had a 55.5% glucose reduction, whereas Glibenclamide had a 55.03% glucose reduction.

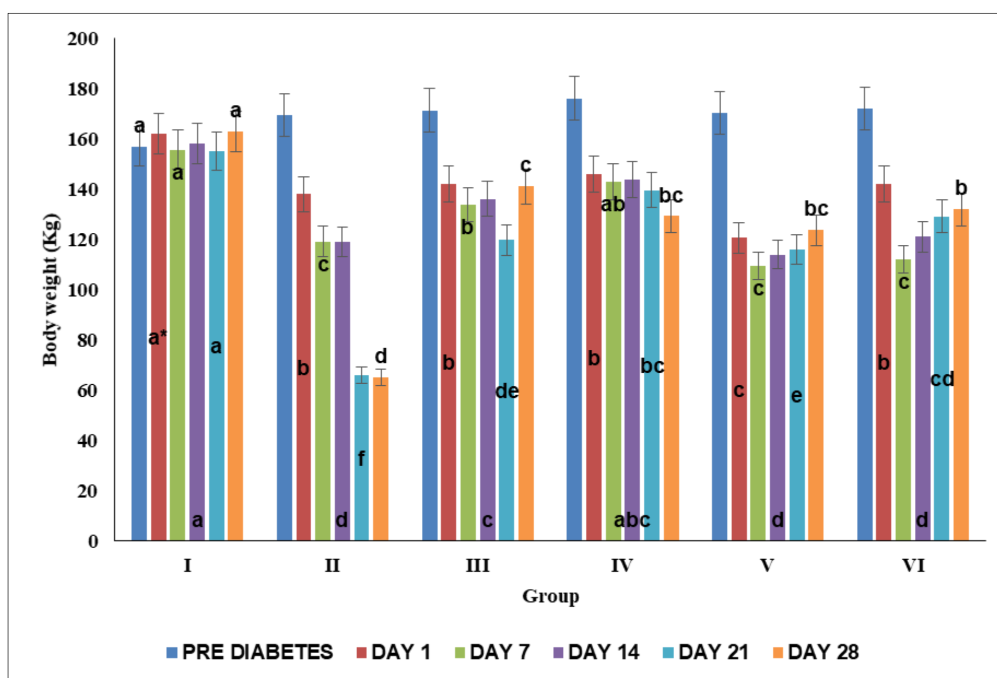
Experimental diabetes elucidates the physiological and biochemical abnormalities in the diabetic state. In our study, STZ was used to cause significant and sustained hyperglycemia. STZ causes diabetes by destroying beta cells in the islets of Langerhans [46]. In the diabetic state, beta-cell destruction and insulin secretion dysfunction cause physio-metabolic abnormalities such as weight loss, increased food and water intake, and urine volume. The severe weight loss observed in STZ-induced diabetes is most likely due to protein wasting caused by a lack of carbohydrates as an energy source, resulting in the excessive breakdown of tissue proteins [47]. The body weight of the diabetic rats decreased gradually,

as expected. However, treatment of diabetic rats with HUCNPs reversed the weight loss and significantly improved body weight gain. This indicates the prevention of hyperglycemia-induced muscle tissue damage.

Furthermore, HUCNPs significantly reduce blood glucose in diabetic rats. Pure HU seed extracts also reduced blood glucose significantly after 28 days, but not as much as HUCNPs. This finding implies that HU, like sulfonylureas, may stimulate insulin release from residual pancreatic beta cells [48]. Previous research has revealed the anti-hyperglycemic effect of HU aqueous seed extract on various experimental models of diabetes [49]. The hypoglycemic effect of the seed extract of HU has been documented in normal, high glucose, and nicotinic-induced hyperglycemic rats [50]. When compared to metformin, Ajibola *et al* [13] found that aqueous seed extract of HU reduced fasting blood glucose in type 2 diabetes patients in just a few weeks, with fewer side effects.

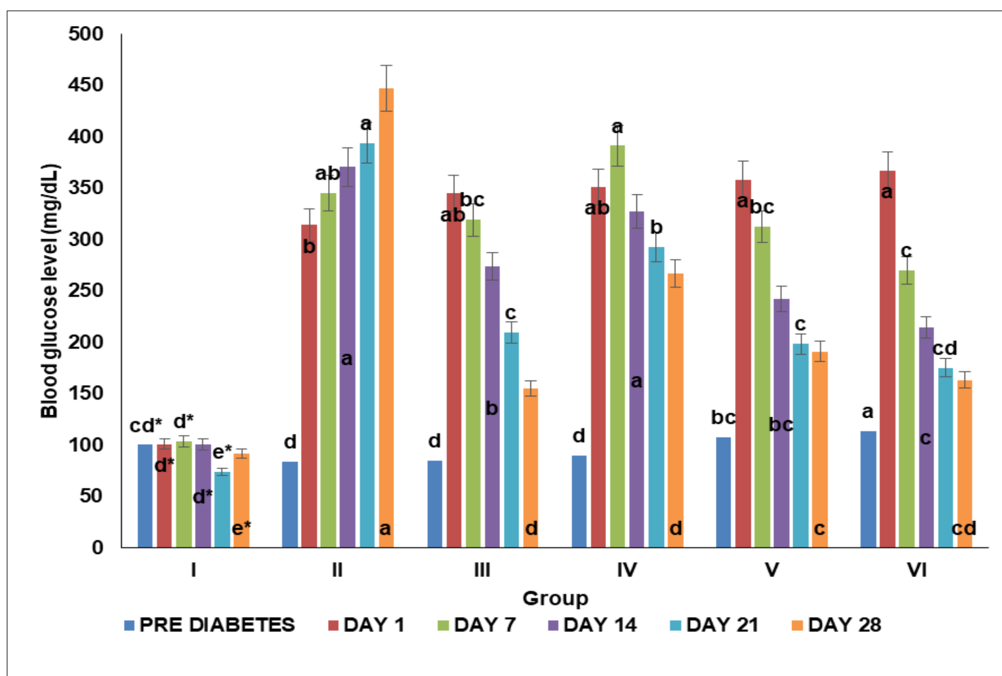
Similarly, in our study, daily HU-CNP administration for 28 days in STZ-induced rats abolished the blood glucose increase. This effect could be due to increased plasma insulin levels, which could influence pancreatic insulin stimulation from beta cells in the islets of Langerhans, or enhanced blood glucose transport to peripheral tissue [51]. Letitia and Timothy [52] suggested that flavonoids in the plant extract may contribute to its hypoglycemic effect because many flavonoids are antioxidants beneficial in the healing process of free-radical mediated diseases such as diabetes. The anti-hyperglycemic effect of HU has also been attributed to erinidine, a bisindole alkaloid [53]. Anti-hyperglycemic studies using erinidine *in vivo* and *in vitro* confirmed that HU mediated its anti-hyperglycemic action by inhibiting intestinal glucose uptake. The greater reduction in blood glucose observed in HUCNPs than in pure extract could indicate extract encapsulation in chitosan [39]. The HU encapsulation provides a method for furthering the application of HU, which could be a potential candidate for an antidiabetic pharmaceutical system. Nanostructured systems can enhance the actions of plant extracts, reducing the required dose and side effects while improving activity [16].

On the other hand, chitosan has been reported to have anti-hyperglycemic properties. Akande and Fasheun found that Chitosan had possible cardioprotective and beta cell regenerative effects in Wister albino rats [54]. Chitosan may exert its antidiabetic effect by inhibiting the expression of intestinal alpha-glucosidase activity, glucose transporters, and peroxisome proliferator-activated receptor-gamma (*PPARγ*) [55]. Incorporating HU extract into chitosan as nanoparticles may thus confer synergism on their anti-hyperglycemic control, resulting in the observed results.



Values are expressed as Mean ± SEM = Mean values ± Standard error of means for five rats in each group. *Mean values within a group followed by the same letter in superscript are not significantly different (P>0.05); Group I: Normal control; Group II: Diabetic control rats; Group III: Diabetic rats treated with Glibenclamide (0.5 mg/kg b.w); Group IV: Diabetic rats treated with aqueous seed extract of HU (400 mg/kg b.w); Group V: Diabetic rats treated with chitosan nanoparticles, CNPs (400 mg/kg b.w); Group VI: Diabetic rats treated with HU-chitosan nanoparticles, HUCNPs (400mg/kg b.w)

Figure 2 Effect of chitosan nanoparticle treatment on body weight in normal and strptozotocin-induced diabetic rats



Values are expressed as Mean ± SEM = Mean values ± Standard error of means for five rats in each group. *Mean values within a group followed by the same letter in superscript are not significantly different (P>0.05); Group I: Normal control; Group II: Diabetic control rats; Group III: Diabetic rats treated with Glibenclamide (0.5 mg/kg b.w); Group IV: Diabetic rats treated with aqueous seed extract of HU (400 mg/kg b.w); Group V: Diabetic rats treated with chitosan nanoparticles, CNPs (400 mg/kg b.w); Group VI: Diabetic rats treated with HU-chitosan nanoparticles, HUCNPs (400mg/kg b.w)

Figure 3 Effect of Chitosan nanoparticle treatment on fasting blood glucose level in normal and streptozotocin-induced diabetic rats

3.4. Biochemical Estimation

The impact of HUCNPs on biochemical enzymes was investigated. The result is shown in Table 2. According to the findings, STZ-induced diabetic rats had an elevated HbA1c level. Serum GOT, GPT, and ALP activity in these rats were significantly higher than in normal rats. The administration of HUCNPs, on the other hand, significantly ($p < 0.05$) reduced the elevation of HbA1c, SGOT, SGPT, and ALP levels to normal. These effects were comparable to those of the standard medication, Glibenclamide. Tables 3 show the antioxidant profiles (SOD, CAT, GSH, and LPO) of normal and diabetic rat liver and kidney tissues. When diabetic rats were compared to normal rats, there was a significant increase in LPO and a decrease in SOD, CAT, and GSH levels. LPO levels were significantly reduced after the oral administration of HUCNPs. In addition, oral administration of HUCNPs significantly increased SOD, GSH levels, and CAT activity like the standard antidiabetic drug, Glibenclamide.

HbA1c is the product of a non enzymatic reaction between glucose and free amino groups of haemoglobin [56]. In diabetic patients, it is typically used as an indicator of glycemic control. It forecasts the likelihood of diabetic complications developing or progressing [57]. A 10% stable reduction in HbA1c reduces the risk of retinopathy by 35%, the risk of nephropathy by 25%–44%, and the risk of neuropathy by 35% [58]. The ability of HUCNPs to effectively control glycemia and prevent the development of diabetes-related complications is demonstrated by the reduced glycosylated HbA1c level in treated animals after administration of HUCNPs. STZ is selectively accumulated in pancreatic beta cells and utilizes the low-affinity glucose transporter-2 in the plasma membrane. It damages the organs expressing glucose transporter-2, especially the liver and kidney [59]. Due to the hepatotoxic effect of the STZ, there may also be enzyme leakage from the cytosol into the bloodstream. As a result, serum GOT, GPT, and ALP levels are elevated, indicating that liver and kidney damage has occurred [60]. The observed decrease in these markers (Serum GOT, GPT, ALP) after administration of HUCNPs reflects its ability to prevent liver and kidney damage in diabetics. Another feature of STZ-induced hyperglycemia is the inactivation of antioxidant enzymes such as SOD, CAT, and GSH due to protein glycation. This, in turn, causes oxidative stress, which leads to lipid peroxidation [61]. As seen in STZ-induced diabetic rats, an increase in LPO may cause tissue damage and failure of the endogenous antioxidant mechanism that prevents excessive free radical production. Endogenous enzymatic antioxidants (CAT, SOD) and nonenzymatic antioxidants (GSH) function as reducing agents in the detoxification of highly reactive oxygen and nitrogen species [25]. The significant decrease in LPO levels and an increase in SOD, GSH, and CAT levels on administration of HUCNPs,

indicates its efficacy in attenuating oxidative stress in diabetic liver and thus its functionality in preventing diabetic complications.

Table 2 Effect of chitosan nanoparticles treatment on various serum biochemical parameters in streptozotocin-induced diabetic rats after 28 days of treatment

GROUPS						
Parameters	**I**	**II**	**III**	**IV**	**V**	**VI**
HbA1C (%)	3.95 ± 0.22^{e*}	9.75 ± 0.65^{a}	4.8 ± 0.86^{d}	6.20 ± 0.41^{b}	5.40 ± 0.26^{c}	5.0 ± 0.2^{c}
SGOT (U/dL)	17.4 ± 1.21^{c*}	24.5 ± 0.21^{a}	21.9 ± 1.1^{ab}	23.83 ± 2.7^{a}	22.27± 0.7^{ab}	18.57±3.2^{bc}
SGPT (U/dL)	43.56 ± 1.6^{e*}	61.66 ± 1.5^{a}	45.26± 2.1^{de}	54.62 ± 2.1^{b}	51.33 ± 3.7^{c}	47.33± 2.4^{d}
ALP (U/dL)	117.56± 3.1^{d*}	257.62±2.8^{a}	142.62±1.1^{bc}	203.01±1.5^{ab}	185.0±1.6^{b}	147.1±4.2^{bc}

Values are expressed as Mean ± SEM = Mean values ± Standard error of means for five rats in each group. One-way ANOVA repeated measures with Duncan's multiple range test was used to calculate statistical significance. *Mean values within a row followed by the same letter in superscript are not significantly different (P>0.05); Group I: Normal control; Group II: Diabetic control rats; Group III: Diabetic rats treated with Glibenclamide (0.5 mg/kg b.w),; Group IV: Diabetic rats treated with aqueous seed extract of HU (400 mg/kg b.w); Group V: Diabetic rats treated with chitosan nanoparticles, CNPs (400 mg/kg b.w); Group VI: Diabetic rats treated with HU-chitosan nanoparticles, HUCNPs (400mg/kg b.w)

Table 3 Effect of chitosan nanoparticles treatment on antioxidant defense enzymes and total protein levels in liver and kidney of control and experimental groups of rats after 28 days of treatment

GROUPS						
Parameters	**I**	**II**	**III**	**IV**	**V**	**VI**
Liver						
SOD(Units/mg protein)	11.2±0.44^{a*}	5.62±0.38^{d}	10.19±0.56^{a}	6.58±0.6^{d}	7.75±0.50^{c}	9.04±0.7^{b}
CAT (mol/min/mg protein)	43.8±2.54^{a*}	26.8±1.53^{e}	34.8±2.35^{bcd}	32.64±2.25^{d}	36.69±2.63^{bc}	38.14±1.25^{bc}
GSH (mol/min/mg protein)	44.4±2.95^{a*}	12.83±3.37^{f}	30.3±2.97^{bcd}	28.66±2.7^{cd}	22.41±2.76^{e}	25.41±3.12^{cd}
TBARS (mol/min/mg protein)	1.0±0.07^{c*}	2.34±0.24^{a}	0.61±0.05^{d}	2.49±0.07^{b}	2.06±0.18^{c}	1.92±1.4^{c}
TOTAL PTN (mg/dL)	6.84±0.31^{a*}	4.2±0.11^{e}	7.38±0.16^{b}	5.58±0.5^{d}	6.81±0.36^{c}	7.39±0.57^{b}
Tissue						
SOD(Units/mg protein)	12.1±0.63^{a*}	5.63±0.78^{e}	9.83±0.71^{b}	6.54±1.8^{de}	7.84±0.58^{cd}	9.01±1.26^{bc}
CAT (mol/min/mg protein)	35.2±3.2^{a*}	24.56±0.8^{e}	33.26±1.86^{bc}	27.26±0.9^{d}	26.4±0.9^{de}	29.83±1.82^{c}
GSH (mol/min/mg protein)	42.2±4.47^{a*}	20.04±2.57^{e}	33.21±3.4^{bc}	24.3±3.2^{de}	28.43±4.32^{bc}	34.82±0.8^{b}
TBARS (mol/min/mg protein)	0.93±0.01^{e}	3.12±0.46^{a}	1.37±0.4^{de}	2.06±0.38^{ab}	1.89±0.15^{bc}	1.05±0.23^{cd}
TOTAL PTN (mg/dL)	5.96±0.2^{b}	4.01±0.11^{e}	6.31±0.11^{be}	4.58±0.2^{be}	4.92±0.5^{de}	4.97±0.42^{c}

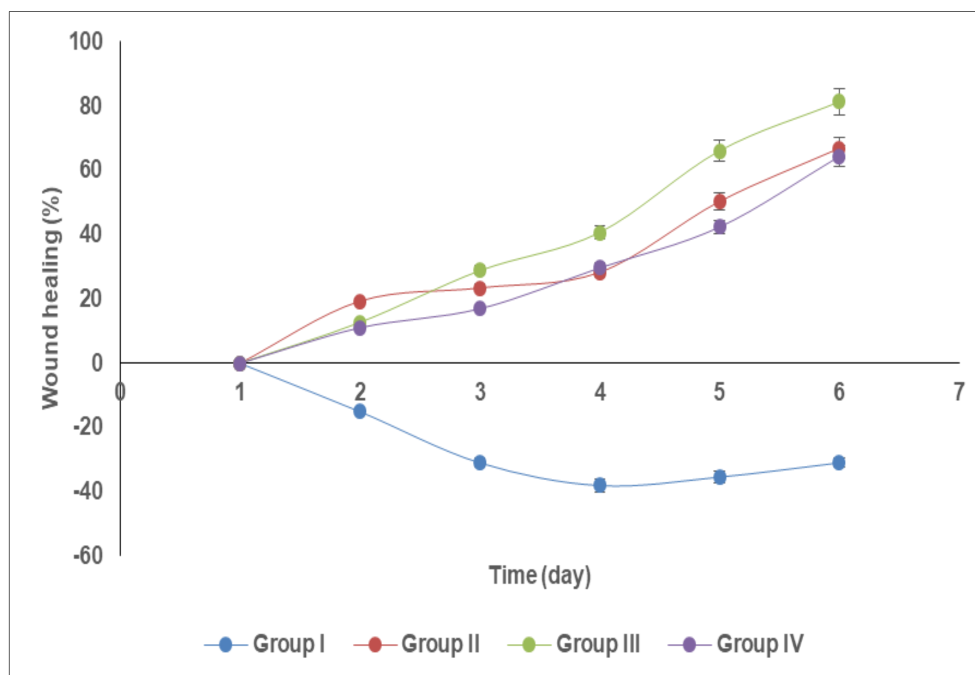
Values are expressed as Mean ± SEM = Mean values ± Standard error of means for five rats in each group. One-way ANOVA repeated measures with Duncan's multiple range test was used to calculate statistical significance. *Mean values within a row followed by the same letter in superscript are not significantly different (P>0.05); Group I: Normal control; Group II: Diabetic control rats; Group III: Diabetic rats treated with Glibenclamide (0.5 mg/kg b.w); Group IV: Diabetic rats treated with aqueous seed extract of HU (400 mg/kg b.w); Group V: Diabetic rats treated with chitosan nanoparticles, CNPs (400 mg/kg b.w); Group VI: Diabetic rats treated with HU-chitosan nanoparticles, HUCNPs (400mg/kg b.w)

3.5. Wound healing studies

Wound healing is a series of complex and dynamic events to restore damaged cellular structures and layers of tissues to as close to their original state as possible [62].This process may be impaired in people with diabetes [63]. Figure 4 shows the effect of HUCNPs on wound healing in diabetic rats. The wounds treated with HUCNPs showed a significant ($p < 0.05$) wound contraction on the eighth day and a faster wound contraction than all other wound treatment groups. On day 20, the HUCNPs-treated rats had an 81.36% wound healing rate, compared to 66.9% in gentamicin-treated rats and 64.44% in pure HU seed extract-treated rats. This demonstrates the ability of HU to promote wound healing. The presence of phytochemicals in the plant may be responsible for the effect [64]. Previous research on the phytochemical

analysis of HU aqueous extract revealed the presence of tannins, phlobatannins, alkaloids, flavonoids, saponins, cardiac glycosides, and anthraquinones [50]. These metabolites are typically responsible for the pharmacological actions of medicinal plants [65]. Saponins and flavonoids have been shown to have wound healing properties [66]. Flavonoids reduce lipid peroxidation, which slows cell necrosis and prevents inflammatory reactions [67]. Tannins are known to be detoxifiers and to inhibit bacterial growth [68]. Phlobatannins have been shown to have wound healing activity [69]. Thus, it is safe to conclude that the wound healing ability of the pure extract is due to its phytoconstituents. The higher wound healing percentage observed with HUCNPs compared to pure HU extract can be attributed to its nanoparticle formulation. When formulated as nanoparticles, the extract has a higher surface area to volume ratio. Nanostructures give pharmacological agents a distinct advantage, contributing to improved pharmacological effects [70]. When HU extract is prepared as a nanoparticle, the chances of interactions with the wound surface and permeation into the wound site are increased. As a result, HU was released in a sustained and controlled manner, resulting in faster wound healing.

Microorganisms thrive in wounds because they provide an ideal environment for their growth. Among the organisms commonly implicated in wound infections are *Pseudomonas aeruginosa, Staphylococcus aeruginosa, Staphylococcus faecalis, Escherichia coli, Clostridium perfringens, Clostridium tetani, Proteus mirabilis, Corynebacterium spp, and Enterococcus spp* [71]. Pus exudates from wound sites were cultured on nutrient media plates to determine the presence of a wound infection-causing organism. Table 4 shows the results. On day 5, a few organisms most commonly associated with chronic and non-healing wounds were found in all the treatment groups. However, continued HUCNPs treatment up to day 10 was able to inhibit the activity of these organisms. Microbial infection in wounds slows healing and causes more pronounced acute inflammatory reactions, leading to further tissue injury and damage. They may also release free radicals and lytic enzymes at the wound site, causing a delay in the onset of the proliferative and re-modelling phases of wound healing [72]. After 10 days, the inhibition of microbial growth in the HUCNPs treated group suggests that the HU extract has *in vivo* antibacterial effects against these organisms. Previous research has demonstrated *H. umbellata's* antibacterial activity against *Eschericha coli, Staphylococcus aureus, and Streptococcus sp* pathogens [73]. The antimicrobial activity of HU-CNPs on wound isolates may contribute to faster wound healing by eliminating infections and allowing the initiation of natural tissue repair processes. HUCNPs may play an important role in accelerating the healing of old wounds in diabetic patients by eradicating already established infections.



Values are expressed as Mean ± SEM = Mean values ± Standard error of means for five rats in each group; Group 1(bland ointment); Group 2 (Gentamicin ointment); Group 3 (HU-CNPs ointment); Group 4 (HU extract ointment); On the vertical axis, 1 = day 0, 2 = day 4, 3 = day 8, 4 = day 12, 5 = day 16, 6 = day 20

Figure 4 Result of percentage wound healing of ointment preparations with time

Table 4 Result of wound infection evaluation

	GROUP I		GROUP II		GROUP III		GROUP IV	
DAYS	**Pus**	**Organism**	**Pus**	**Organism**	**Pus**	**Organism**	**Pus**	**Organism**
5	+	*P. aeruginosa* *S. aureus*	++	*P. aeruginosa* *S. aureus*	+	*P. aeruginosa* *S. aureus*	+	*P. aeruginosa* *S. aureus*
10	++	*P. aeruginosa* *S. aureus*	+	*P. aeruginosa*	-	-	+	*S. aureus*
15	+++	*P. aeruginosa* *S. aureus* *E. coli*	-	*P. aeruginosa*	-	-	-	-

Key: +++ (severely present); ++ (moderately present); + (mildly present); - (not present); Group 1(bland ointment); Group 2 (Gentamicin ointment); Group 3 (HU-CNPs ointment); Group 4 (HU extract ointment)

4. Conclusion

The *Hunteria umbellata*-loaded chitosan nanoparticles were fabricated using the nanoprecipitation method via dropping technique. Their antidiabetic and wound healing potentials were investigated using the streptozotocin-induced diabetic rat model and the excision wound model. HUCNPs significantly reduced fasting blood glucose levels in STZ-induced diabetic rats and enhanced antidiabetic activity. Compared to diabetic control groups, the levels of serum enzymes (SGOT, SGPT, ALP), antioxidant enzymes (CAT, GSH, SOD), and lipid peroxidase (LPO) in the HU-CNPs treated group were restored to normal. The findings suggest that HUCNPs have anti-diabetic properties and can activate multiple defence mechanisms in a coordinated fashion to alleviate STZ-induced oxidative stress in rats. There was significant wound healing in the HUCNPs ointment treated group, as evidenced by a higher percentage of wound closure compared to gentamicin ointment. This demonstrates the ability of HUCNPs to accelerate wound healing. The HU encapsulation provides a method for furthering the application of *H. umbellata* extract, which could be a potential candidate for antidiabetic and wound healing pharmaceutical systems. Our findings have clinical relevance and could be extremely useful in drug discovery and development. However, more research into the mechanism of *H. umbellata's* anti-hyperglycemic, wound healing, and antioxidant activity is recommended because it will provide significant progress toward drug design using the plant material.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

Animal experiments were carried out in accordance with guidelines of the Animal and Ethics Committee of the Faculty of Pharmaceutical Sciences at the University of Nigeria Nsukka, as well as the National Institute of Health (NIH) guide for the care and use of laboratory animals (Pub No: 85-23 Revised 1985)

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