J Bangladesh Agril Univ 23(2): 166-174, 2025 https://doi.org/10.3329/jbau.v23i2.82584

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau

Research Article

Mollification of Diabetic Nephropathy in Streptozotocin-Induced Diabetic Mice Using Bitter Gourd Extract and ZnO Nanoparticles

Mst Misrat Masuma Parvez[1]✉*, Banosree Saha Bandhan[2*], Md. Fazlur Ahsan[3] and Md. Juman Miah[3]

[1]Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200 Bangladesh

[2]Department of Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200 Bangladesh

[3]Department of Medicine, Surgery and Obstetrics, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200 Bangladesh

ARTICLE INFO

Article history
Received: 08 March 2025
Accepted: 22 June 2025
Published: 30 June 2025

Keywords
Diabetes mellitus, *Momordica charantia,* Phytomedicine, Nanoparticles, Nephroprotection

Correspondence
Mst Misrat Masuma Parvez
✉: parvez.misrat@gmail.com

*These authors contributed equally to this work

ABSTRACT

The study was carried out to evaluate the efficacy of bitter gourd extract (BGE) and zinc oxide nanoparticles (ZnONPs) against streptozotocin (STZ) induced diabetes nephropathy (DN) in male Swiss Albino mice. Multiple intraperitoneal (IP) injection of STZ was used to induce diabetes in experimental mice and were divided into five groups *viz.*, T_0 = control (no diabetes and no treatment), T_1 = STZ-induced DN without treatment, T_2 = STZ-induced DN treated with BGE, T_3 = STZ-induced DN treated with ZnONPs and T_4 = STZ-induced DN treated with both BGE and ZnONPs. The respective groups received single oral daily dose of BGE @ 5g kg^{-1} body weight and ZnONPs @ 8 mg kg^{-1} body weight, where control and T_1 groups were given normal distilled water orally. Data were recorded on blood glucose level, blood urea nitrogen levels and serum creatinine at 5 days after STZ administration and thereafter 7, 14 and 21 days after administration of bitter gourd extract and ZnO nanoparticles. Bitter gourd extract and ZnONPs were found operative to reduce the blood glucose level, creatine level and blood urea nitrogen level in nephropathic mice where combined effect was more effective than the individual effects against diabetic nephropathy. The findings provide considerable evidence about the efficacy of BGE and ZnONPs which could help to design the clinical application of BGE and ZnONPs on a standardized formulation to protest the development of diabetic nephropathy. However, BGE and ZnONPs possess a potential anti-diabetic as well as anti-nephropathic activity, which could be validated by further mechanistic investigations.

Introduction

Diabetic nephropathy is one of the vital microvascular hitches of diabetes mellitus has become a worldwide epidemic, accounting for approximately one third of all cases of end-stage renal disease. According to the World Health Organization, diabetes causes 1.5 million deaths each year worldwide (Souto et al., 2019) and the number of diabetic patients is increasing at an alarming rate all over the world (Souto et al., 2019; Vieira et al., 2019; Lin and Sun, 2010). The development of several medications with multiple modes of actions having glucose-lowering activity is needed to manage diabetes. There is currently a great interest of medical and food industry in the detection of anti-diabetic compounds with pharmacological potential from natural source without any side effects or at least with minimal side effects. Due to the high cost and side effects of chemical drugs today, it has become a top priority to study on plants used in traditional medicine to achieve further progress in medical sciences. Herbal remedies are natural substances with lower risk of side effects. Many of these herbs provide a rich source of natural anti-diabetic compounds that can curtail the side effects of chronic diabetics.

Bitter gourd *(Momordica charantia*) a popular vegetable was found to possess antiviral, antibacterial, anti-HIV, anticancer, and immunomodulatory properties with great attention on its blood glucose-lowering effect (Grover and Yadav 2004). Bitter gourd fruit contains

Cite This Article
Parvez, M.M.M., Bandhan, B.S., Ahsan, M.F. and Miah, M.J. 2025. Mollification of Diabetic Nephropathy in Streptozotocin-Induced Diabetic Mice Using Bitter Gourd Extract and ZnO Nanoparticles. *Journal of Bangladesh Agricultural University,* 23(2): 166-174. https://doi.org/10.3329/jbau.v23i2.82584

charantin, polypeptide-P insulin, and lectins which are useful substances to decrease blood glucose level (Meles et al., 2019). Saponins, flavonoids, polyphenols, and vitamin C from the bitter gourd acts as antioxidants that prevent the free radicals which can interfere with the presence of leydig cells due to diabetes mellitus (Meles et al., 2017). In addition, the vegetable has shown promising effects in preventing as well as delaying in progression of diabetic complications (nephropathy, neuropathy, gastroapresis, cataract and insulin resistance) in animals (Grover et al., 2001; Rathi et al., 2002; Sathishsekar and Subramanian, 2005) mediated by insulin-like activity of a polypeptide-p (phyto-insulin) and enhancement of pancreatic beta cell.

In recent decade, the use of nanomedicine has increased exponentially, being utilized for diagnosis, prevention and treatment of different diseases (Rizzo et al., 2011). As an important agent of nanomedicine, zinc oxide nanoparticles (ZnONPs), a novel source of zinc, have great implications in many disease therapies including diabetes mellitus (Tang, 2019). The loading of insulin and other sugar-lowering drugs and nutraceuticals into nanoparticles has been proposed as a more convenient, non-invasive and safer approach through alternative administration routes (Souto et al., 2019). Zinc (Zn) has been reported to play pivotal role in blood sugar maintenance and used in diabetes therapy (Arthur and Chausmer, 1998). Studies showed that zinc oxide nanoparticles (ZnONPs) had anti-hyperglycemic, anti-oxidative stress and anti-inflammatory effects in a diabetic animal model (Jiang et al., 2018). Haase et al., (2008) reported that, over 300 enzymes in the body are activated by zinc and it plays a key role in different metabolic pathways, including glucose metabolism. Zinc is also known to keep the structure of insulin (Sun et al., 2009) and plays a vital role in insulin biosynthesis, storage and secretion (Edmin et al., 1980). It has been proved by researches that several zinc transporters in pancreatic β-cells like zinc transporter-8 have a potent role in insulin secretion (Rungby, 2010; Smidt et al., 2009). Human system acquires Zn in traces and vital for many biological functions in the human body (Auld, 2001; Kim and Ahn., 2014). Generally, compounds having Zn are considered toxic for mammals and plants (Husen, 2020) but ZnONPs are generalized more biocompatible and safer to take over time when used in amounts not greater than 40 mg daily. Excess zinc is readily removed from the body via excretion in feces or removed from the blood by the pancreas or liver (Wafaey et al., 2024; McClung and Cai, 2014). It has been well established and highly publicized in research literature and clinical use that antioxidants, like zinc, are not only good for human health, but also good for diabetes (Kim and Ahn., 2014). Zinc happens to be a strong inducer of metallothionien making zinc a great candidate for helping to prevent the onset or progression of diabetes and diabetes complications (Jayawardena et al., 2012). Moreover, ZnONPs coated with some polymeric material is considered effective against wound healing, ulcers and a strong antimicrobial along with cancer treatment (Siddiqi and Husen, 2017). Synthesizing ZnONPs has anti-oxidant, hypoglycemic and anti-inflammatory properties, and may be used in a variety of biomedical sectors (Rajakumar et al., 2018). Hence, the development of zinc-based agent would be promising in the treatment of diabetes and its associated complications as zinc supplement have shown ameliorating effect in preclinical studies (Tang, 2019; Ukperoro et al., 2010). A new and exciting research field has set out to unveil the mystery of zinc-based nanoparticles for curing the diabetes mellitus and its allied complications.

Considering the fact, the investigation was carried out to evaluate the efficacy of bitter gourd extract and ZnO nanoparticles as therapeutic agents on the biochemical alterations of diabetic mice in order to develop a treatment model against diabetic nephropathy using herbal and nanoparticles.

Materials and Methods

Site and duration

This study was performed at Department of Physiology and Pharmacology, Faculty of Veterinary and Animal Sciences, Hajee Mohammad Danesh Science and Technology University, Dinajpur during March 2022.

Collection and management of experimental animals

Laboratory Swiss albino mice (male, 10-15 g weight and 3 weeks old) were collected from International Centre for Diarrhoeal Disease Research (ICDDR), Dhaka, Bangladesh. The mice were allowed to acclimatize in the laboratory environment for a week before the commencement of the experiment. The mice were housed in a wire cage ($30\times13\times15cm^3$) and exposed to 12 hours of darkness and 12 hours of light at a condition of 25±2°C temperature and 48±5% relative humidity. The animals were fed with a standard commercial pellet diet @100-150 g kg^{-1} and *ad-libitum* water through the specific nipple of the water bottle throughout the experimental period as recommended by ICDDR. The cages of the animals were cleaned regularly, and water and food were supplied twice daily.

Induction and confirmation of diabetes in mice

Multiple intraperitoneal (IP) injection (45 mg kg^{-1} body weight) of streptozotocin (STZ) (Sigma, USA) was used to induce diabetes in experimental mice. STZ was dissolved in freshly prepared 0.01M sodium citrate

buffer (pH = 4.5) and administered on mice for 5 consecutive days. After STZ injection, 5% (w/v) sucrose was supplemented for 24 hours to prevent the animals from fatal hypoglycemia which might be induced by STZ. Control mice received an equivalent amount of citrate buffer only. The blood glucose levels were measured in all experimental animals before the beginning of the experimental procedures. When the dosing was completed, STZ-treated mice were kept in normal conditions for 5 days. After this period, The STZ-treated mice were fastened for 12h and blood samples were collected from the tail vein for determining the blood glucose levels using a glucometer. Mice with fasting blood glucose levels higher than 11.10 $mmolL^{-1}$ were considered as diabetic and hence they were selected for further investigation. Blood urea nitrogen and serum creatine of experimental mice were also measured for confirming the STZ-induced diabetic nephropathy.

Grouping of animals

For the antidiabetic activity test, the experimental procedure of Alkaladi et al., (2014) was followed for random grouping of the diabetic mice into four groups (T_1 = STZ-induced diabetic nephropathy without treatment, T_2 = STZ-induced diabetic nephropathy treated with bitter gourd extract, T_3 = STZ-induced diabetic nephropathy treated with ZnO nanoparticles and T_4 = STZ-induced diabetic nephropathy treated with both bitter gourd extract and zinc oxide nanoparticles) along with a control group (T_0 = control; no diabetes and no treatment) having six animals in each group. Mice were housed in separate metal cages while maintaining constant environment and nutritional conditions.

Preparation and administration of bitter gourd extract and ZnO nanoparticles

The extract of bitter gourd was prepared following the protocol as described by Yoon et al., (2017). Dried unripe fruit of bitter gourd was shattered and extracted by heating twice at 70^0C (4 h and 2 h) using 70% ethanol. The extract was then filtered and concentrated to 15-20 degrees Brix at 65^0C. The T_2 and T_3 groups received single oral daily dose of BGE @ 5 g kg^{-1} body weight and ZnONPs (a white powder with an amount of purity ≥ 99%, Sigma-Aldrich, Germany) @ 8 mg kg^{-1} body weight, respectively. Combined oral dose of BGE and ZnONPs was applied in T_4 group following similar rate of T_2 and T_3. Control group (T_0) and group T_1 were given normal distilled water orally. The treatments were given daily on single dose basis and continued up to 20 days.

Biochemical determination

Twenty-four hours after the last administration, blood tests were gathered. Blood samples (2.0-3.0 µl blood) were collected from the tail vein of all experimental groups after overnight fasting and the blood glucose levels were determined following the glucose oxidase method using the 'ACCU-CHEK active' kit at 7, 14 and 21 days after administration of bitter gourd extract and ZnO nanoparticles treatment. Blood urea nitrogen levels (BUN) and serum creatinine were determined spectrophotometrically using commercially available kits (ARCOMEX, Jordan) at 7, 14 and 21 days after administration of bitter gourd extract and ZnO nanoparticles treatment.

Statistical analyses

The collected data were analyzed with Statistix 10 software program for analysis of total variance and the means were separated by Tukey test at 5% level of probability.

Results

Effect of STZ administration in inducing diabetes resulted in nephropathy

Figure 1 indicates the blood glucose level, creatinine level and blood urea nitrogen (BUN) level of different treatment groups before starting the treatment. The results showed that after 5 days of STZ administration, diabetic mice showed significant ($P \leq 0.05$) increment in blood glucose (17.83±0.6432, 17.12±0.7024, 18.00±0.4905 and 17.54±0.5015 mmol L^{-1} in T_1, T_2, T_3 and T_4 groups, respectively), creatinine (0.71±0.0326, 0.73±0.0468, 0.69±0.0461 and 0.77±0.0712 mg dL^{-1} in T_1, T_2, T_3 and T_4 groups, respectively) and BUN (75.34±3.7533, 76.35±3.3316, 74.56±2.4432 and 77.10±2.2523 mg dL^{-1} in T_1, T_2, T_3 and T_4 groups, respectively) compared with the non-diabetic control mice group (6.27±0.3895 mmol L^{-1} glucose, 0.16±0.0061 mg dL^{-1} creatinine and 31.56±0.8434 mg dL^{-1} BUN).

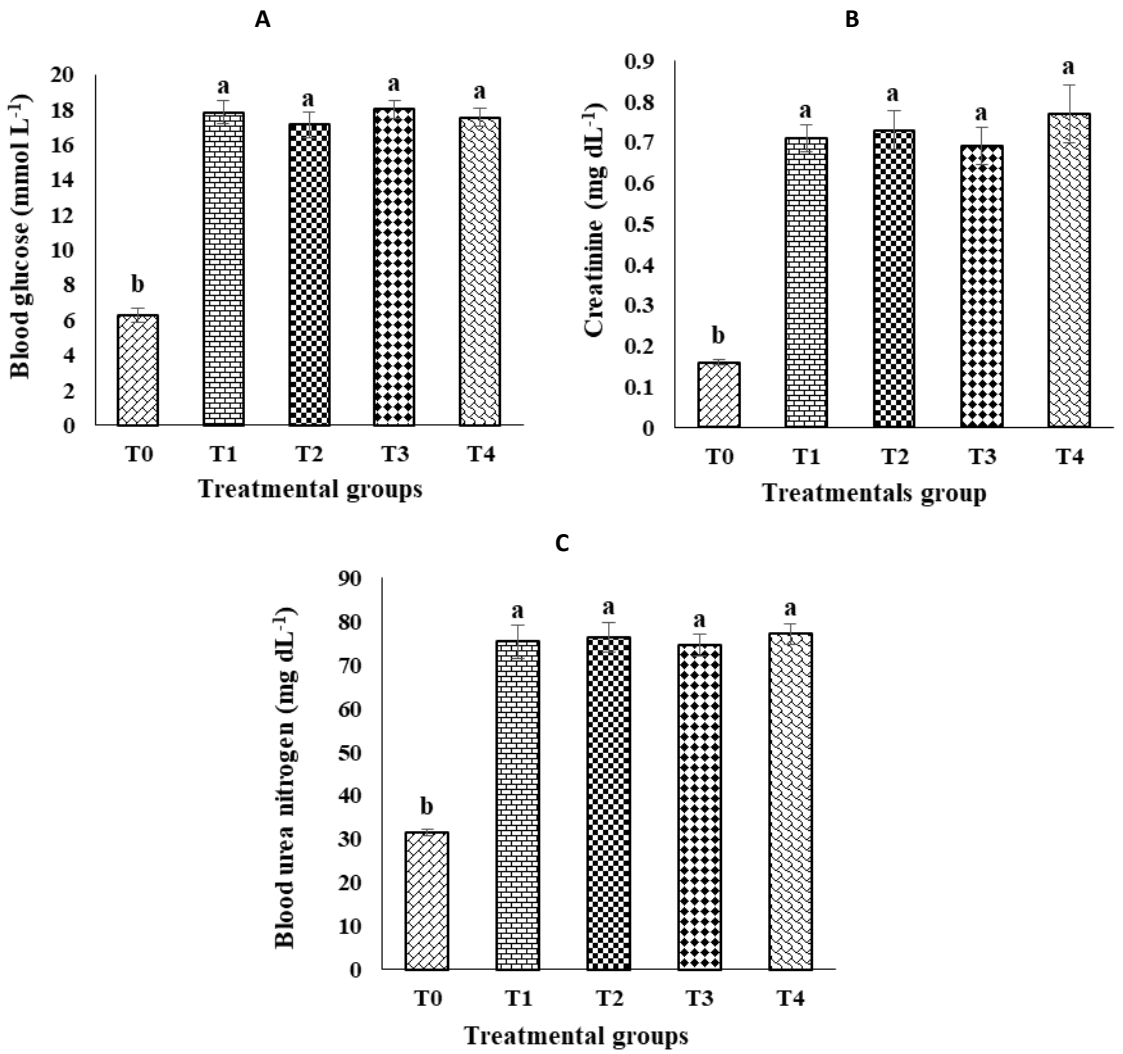

Figure 1. A) Blood glucose, B) creatinine and C) blood urea nitrogen (mean ± standard error) of different treatment groups recorded before treated with bitter gourd extract and zinc oxide nanoparticles.

T_0 = Control, T_1 = STZ-induced diabetic nephropathy (DN) without treatment, T_2 = STZ-induced DN treated with bitter gourd extract, T_3 = STZ-induced DN treated with zinc oxide nanoparticles and T_4 = STZ-induced DN treated with both bitter gourd extract and zinc oxide nanoparticles.

The mice groups with significant increment in blood glucose confirmed that, the mice were induced with diabetes, whereas significant increment in creatinine and BUN ensured that the mice were suffering with kidney disorder as well as nephropathy resulted due to STZ induced diabetes.

Effect of bitter gourd extract and ZnO nanoparticles in amelioration of blood glucose of diabetic mice

Extract of bitter gourd, ZnONPs and their combined application significantly ($P \leq 0.01$) decreased blood glucose level in STZ-induced diabetic mice at different days after treatment (Table 1). The therapeutic effect of bitter gourd extract and ZnONPs gradually decreased the blood glucose level of diabetic mice at 7, 14 and 21 days after treatment (DAT). At initial stage (7 DAT), the variation of glucose level of T_1 group with T_2, T_3 and T_4 groups was less but with the advancement of time (14 DAT and 21 DAT) the variation was found as more. The results recorded on blood glucose level depicts that, the T_0 group showed normal glucose level (6.80±0.2859, 6.55±0.2429 and 7.33±0.2864 mmol L^{-1} at 7, 14 and 21 DAT, respectively), whereas the T_1 group showed maximum glucose level in blood (18.97±0.5442, 20.77±1.5963, 18.15±0.4954 mmol L^{-1} at 7, 14 and 21 DAT, respectively) but other treatment groups treated with bitter gourd extract and ZnONPs showed less blood glucose level compared to T_1 group which indicates the alleviative influence of bitter gourd extract and ZnONPs on diabetes.

Table 1. Ameliorative effect of bitter gourd extract and zinc oxide nanoparticles on blood glucose level of STZ-induced diabetic nephropathic mice

Treatment groups	Blood glucose (mmol L^{-1}) ± Standard error		
	7 DAT	14 DAT	21 DAT
T_0	6.80±0.2859 d	6.55±0.2429 d	7.33±0.2864 d
T_1	18.97±0.5442 a	20.77±1.5963 a	18.15±0.4954 a
T_2	15.75 ±0.6094 b	14.59±0.3141 b	13.92± 0.5091 b
T_3	11.53±0.3926 c	10.77±0.4352 c	10.95±0.2834 c
T_4	11.57±0.4018 c	9.81±0.3873 c	7.15±1.0680 d
Level of significance	**	**	**
Critical value for comparison	1.6341	1.7369	1.1726
Co-efficient of variation (%)	4.69	5.17	3.79

T_0 = Control, T_1 = STZ-induced diabetic nephropathy (DN) without treatment, T_2 = STZ-induced DN treated with bitter gourd extract, T_3 = STZ-induced DN treated with zinc oxide nanoparticles and T_4 = STZ-induced DN treated with both bitter gourd extract and zinc oxide nanoparticles, DAT = Days after treatment, **Significant at P≤0.01 level of probability.

At 21 DAT, T_4 treatment group *i.e.* the combined administration of bitter gourd extract and ZnONPs was found as more effective in reducing blood glucose than the individual effect of bitter gourd extract and ZnONPs. But at 7 and 14 DAT, T_3 and T_4 groups were found as statistically similar and more effective in reducing blood glucose than the T_2 group.

Effect of bitter gourd extract and ZnO nanoparticles in amelioration of creatinine level of nephropathic mice

Extract of bitter gourd, ZnONPs and their combined administration meaningfully (P≤0.01) reduced blood creatinine level in STZ-induced diabetic mice at different days after treatment (Table 2). The alleviative effect of bitter gourd extract and ZnONPs gradually decreased the blood creatinine level of diabetic nephropathic mice at 7, 14 and 21 days after treatment.

At initial period after treatment (7 DAT), the variation of creatinine level of T_1 group with T_2, T_3 and T_4 groups was less but with the advancement of time (14 DAT and 21 DAT) the variation was found as more. The results on blood creatinine level indicates that, the T_0 group showed normal creatinine level (0.14±0.0031, 0.13±0.0059 and 0.13±0.0063 mg dL^{-1} at 7, 14 and 21 DAT, respectively), whereas the T_1 group showed maximum creatine level in blood (0.60±0.0126, 0.78±0.0533 and 0.75±0.0549 mg dL^{-1} at 7, 14 and 21 DAT, respectively) but other treatment groups treated with bitter gourd extract and ZnONPs showed less blood creatinine level compared to T_1 group which expresses the ameliorative effect of bitter gourd extract and ZnONPs on blood creatinine level of nephropathic mice.

Table 2. Ameliorative effect of bitter gourd extract and zinc oxide nanoparticles on creatinine level of STZ-induced diabetic nephropathic mice

Treatment groups	Creatinine (mg dL^{-1}) ± Standard error		
	7 DAT	14 DAT	21 DAT
T_0	0.14±0.0031 d	0.13±0.0059 d	0.13±0.0063 d
T_1	0.60±0.0126 a	0.78±0.0533 a	0.75±0.0549 a
T_2	0.57±0.0268 a	0.42±0.0385 b	0.32±0.0610 b
T_3	0.38±0.0361 b	0.34±0.0190 c	0.22±0.0509 c
T_4	0.22±0.0610 c	0.19±0.0061 d	0.19±0.0502 cd
Level of significance	**	**	**
Critical value for comparison	0.0714	0.0704	0.0747
Co-efficient of variation (%)	6.97	6.99	8.65

T_0 = Control, T_1 = STZ-induced diabetic nephropathy (DN) without treatment, T_2 = STZ-induced DN treated with bitter gourd extract, T_3 = STZ-induced DN treated with zinc oxide nanoparticles and T_4 = STZ-induced DN treated with both bitter gourd extract and zinc oxide nanoparticles, DAT = Days after treatment, **Significant at P≤0.01 level of probability.

Among the different treatment groups (T_2, T_3 and T_4), T_4 treatment group *i.e.* the combined administration of bitter gourd extract and ZnONPs was found as more operative in reducing creatine level in blood than the individual effect of bitter gourd extract and ZnONPs at 7, 14 and 21 DAT.

Effect of bitter gourd extract and ZnO nanoparticles in amelioration of blood urea nitrogen level of nephropathic mice

Extract of bitter gourd, ZnONPs and their combined application significantly (P≤0.001) decreased BUN level in STZ induced diabetic mice at different days after

treatment (Table 3). The therapeutic effect of bitter gourd extract and ZnONPs steadily decreased the BUN level of diabetic nephropathic mice at 7, 14 and 21 days after treatment. At early stage (7 DAT), the variation of BUN level of T_1 group with T_2, T_3 and T_4 groups was less but with the progression of time (14 DAT and 21 DAT) more variation was observed. The findings on BUN level specifies that, the T_0 group showed normal BUN (30.34±0.7354, 34.41±0.7285 and 32.00±0.7256 mg dL^{-1} at 7, 14 and 21 DAT, respectively), whereas the T_1 group showed the highest BUN (79.00±2.7832, 75.67±2.2498 and 80.40±2.6437 mg dL^{-1} at 7, 14 and 21 DAT, respectively) but other treatment groups treated with bitter gourd extract and ZnONPs showed less BUN compared to T_1 group which expresses the protective influence of bitter gourd extract and ZnONPs on BUN regarding diabetic nephropathy.

Table 3. Ameliorative effect of bitter gourd extract and zinc oxide nanoparticles on blood urea nitrogen of STZ-induced diabetic nephropathic mice

Treatment groups	Blood urea nitrogen (mg dL^{-1}) ± Standard error		
	7 DAT	14 DAT	21 DAT
T_0	30.34±0.7354 e	34.41±0.7285 d	32.00±0.7256 d
T_1	79.00±2.7832 a	75.67±2.2498 a	80.40±2.6437 a
T_2	68.15±2.3756 b	61.82±1.8756 b	59.00±1.5690 b
T_3	53.15±1.4682 c	51.55±1.5639 c	51.00±1.3615 bc
T_4	44.26±1.2420 d	39.00±1.2033 d	40.86 ±1.1570 cd
Level of significance	***	***	***
Critical value for comparison	6.4401	5.5941	11.079
Co-efficient of variation (%)	4.36	3.97	7.83

T_0 = Control, T_1 = STZ-induced diabetic nephropathy (DN) without treatment, T_2 = STZ-induced DN treated with bitter gourd extract, T_3 = STZ-induced DN treated with zinc oxide nanoparticles and T_4 = STZ-induced DN treated with both bitter gourd extract and zinc oxide nanoparticles, DAT = Days after treatment. ***Significant at P≤0.001 level of probability.

As like as the creatinine level, the different treatment groups followed the similar pattern regarding their efficacy in reducing BUN level in blood. Among the different treatment groups, T_4 *i.e.,* the combined application of bitter gourd extract and ZnONPs was found as more active in reducing BUN level in blood than the individual effect of bitter gourd extract and ZnONPs at 7, 14 and 21 DAT.

Discussion

Our findings have demonstrated that streptozotocin increased blood glucose, blood creatinine and blood urea nitrogen resulted in diabetes consequently nephropathy in albino mice. On the contrary, extract of bitter gourd and zinc oxide nanoparticles had dependently induced nephroprotective effect individually and combinedly in diabetes caused nephropathic albino mice. Both bitter gourd extract and ZnONPs reduced blood glucose, blood creatinine and blood urea nitrogen in nephropathic albino mice and reached near to the normal level. But the extent of reduction was different for different treatments. ZnONPs was found as more effective compared to bitter gourd extract against diabetes as well as nephropathy, whereas the combined application of both BGE and ZnONPs was found as more nephroprotective.

Bitter gourd is one of the plants that has been investigated thoroughly for the treatment of diabetes (Hasan and Khatoon, 2012). With the traditional use supported by modern scientific evidence of the beneficial function it is one of the most promising plants for diabetes today (Cefalu et al., 2008). Charantin is a typical cucurbitane type triterpenoid present in bitter gourd and is a potential substance with antidiabetic properties (Patel et al., 2010). Investigation of the traditional uses of bitter gourd revealed that, it is one of the most important plants for lowering blood glucose levels in patients with diabetes (Paul and Raychaudhuri, 2010). Bitter gourd and its various extracts and components are believed to exert their hypoglycemic effects via different physiological, pharmacological and biochemical modes (Taylor, 2002). Considerable number of studies worldwide have investigated anti-hyperglycemic and hypoglycemic effects of the different extracts and ingredients of bitter gourd in both human and animal models (Wehash et al., 2012; Fuangchana et al., 2011). Another author (Ahmed et al., 2004) investigated the effect of daily oral administration of *M. charantia* fruit juice and the distribution of α, β and δ cells in the pancreas of STZ-induced diabetic rats using immunohistochemical methods. These initiatives and their outputs are supportive to our findings of the present investigation.

Although, the exact mechanism of the role of ZnONPs treatment in diabetic nephropathy is not well understood in our study, we observed that ZnONPs treatment notably decreased the blood glucose, creatine and blood urea nitrogen in diabetic

nephropathic mice. It might be due to the decline in the expression and activity of MMP-9 contributes to mesangial matrix accumulation in the diabetic kidney (McLennan et al., 2002). Zinc could improve insulin signaling by several mechanisms, including increased insulin receptor phosphorylation, enhancing PI3K activity and inhibition of glycogen synthase kinase-3 (Jansen et al., 2009) which might be another reason behind the attenuative effect of ZnONPs against diabetes mellitus. In addition, increased degradation and decreased extracellular matrix (ECM) protein synthesis could subsidize to the anti-fibrotic effects of ZnONPs treatment in diabetic nephropathy. In many diseases, including DN, the antioxidant properties of Zn have been well documented by the researchers, Ranasinghe et al., (2015) and Barman et al., (2018). The beneficial role of zinc in diabetes has been previously implicated by studies of the zinc supplies in diabetic rats (Ukperoro et al., 2010). Zinc is also known to play role in biosynthesis, storing, secreting (Arthur and Chausmer, 1998) and keeping the structure of insulin (Sun et al., 2009) and promoting the hepatic glycogenesis through its actions on the insulin signaling pathways (Jansen et al., 2009). It has been reported that, the key mechanism related to hyperglycemia involves over-production (hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues (Lin and Accili, 2011). Zinc may provide insulin-like effects in the signal transduction mechanism of insulin and reduce the production of cytokines, which leads to β-cell death due to inflammation in the pancreas during diabetes (Jansen et al., 2009). Our finding is found to be consistent with the finding of Alkaladi et al., (2014) and Alomari et al., (2021) who also reported the alleviative effect of zinc oxide nanoparticles on diabetic nephropathy in rats induced by streptozotocin that support our present findings. The research finding of Gadoa et al., (2022) proved that ZnONPs had an ameliorative effect on blood glucose levels, antioxidant status, lipid profile, liver function enzymes, and mRNA expression of hepatic genes in Type II diabetic rats which is in a line with the findings of the current study. Virgen-Ortiz et al., (2020) provided compatible information about the acute response of ZnONPs on fasting glycemia in diabetic and healthy rat models that are helpful for possible forthcoming clinical approaches and supportive to our results on ZnONPs treatment against DN. From an in vitro and in vivo approach using biosynthesised zinc oxide nanoparticles on pancreatic beta cells, John et al., (2020) reported that, streptozotocin-fructose-induced type II diabetic rats treated with ZnONPs exhibited significant reduction in the blood glucose levels and increased number of beta cells (responsible for its increased insulin levels and reduced glucose levels) proclaiming its efficacious role as a potent hypoglycaemic (antidiabetic) drug. Other authors (Abd El-Baset., 2023; Umrani and Paknikar, 2014) also observed the alleviative result of ZnONPs on adverse effects of diabetic nephropathy in rats. These results are very much congruent with our results on ameliorative efficacy of ZnONPs in reducing the blood glucose, creatinine and BUN level in STZ-induced diabetic nephropathic mice.

Conclusion

The findings revealed that, the extract of bitter gourd and zinc oxide nanoparticles have signified nephroprotective effect. The combined effect of bitter gourd extract and zinc oxide nanoparticles was found more effective than the individual effect of those against diabetic nephropathy in mice. The present findings may help to design the clinical application of bitter gourd extract and zinc oxide nanoparticles for protection against the development of diabetic nephropathy. However, further studies should be focused to draw precise conclusion on the efficacy as well as standard dose of bitter gourd extract and zinc oxide nanoparticles against diabetic nephropathy.

Ethical Statement

The study was carried out following the University guidelines and all ethical practices for the use and care of experimental animals as recommended by Bangladesh Veterinary Council. All procedures of the current experiment have been approved by the Institutional Animal Experiment Ethics Committee of HSTU, Dinajpur, Bangladesh.

Acknowledgement

The authors gratefully acknowledge the Institute of Research and Training (IRT) and Department of Chemistry, Hajee Mohammad Danesh Science and Technology University Dinajpur, Bangladesh for providing research grant (Project number: 120; Fiscal year: 2021-2022) and laboratory support, respectively to conduct the research work successfully.

References

Abd El-Baset, S.A., Mazen, N.F., Abdul-Maksoud, R.S., and Kattaia, A.A.A., 2023. The therapeutic prospect of zinc oxide nanoparticles in experimentally induced diabetic nephropathy. *Tissue Barriers*, 11(1): e2069966. https://doi.org/10.1080/21688370.2022.2069966.

Ahmed, I., Cummings, E., Sharma, A.K., Adeghate, E., and Singh, J., 2004. Beneficial effects and mechanism of action of *Momordica charantia* fruit juice in the treatment of streptozotocin-induced diabetes mellitus in rats. *Molecular and Cellular Biochemistry*, 261: 63-70. https://doi.org/10.1023/B:MCBI.0000028738.95518.90

Alkaladi, A., Abdelazim, A., and Afifi, M., 2014. Antidiabetic activity of zinc oxide and silver nanoparticles on streptozotocin-induced diabetic rats. *International Journal of Molecular Science*, 15(2): 2015-2023. https://doi.org/10.3390/ijms15022015

Alomari, G., Al-Trad, B., Hamdan, S., Aljabali, A.A.A., Al Zoubi, M.S., Al-Batanyeh, K., Qar, J., Eaton, G.J., Alkaraki, A.K., Alshaer, W., Haifawi, S., Jemon, K., Chellappan, D.K., Dua, K., and Tambuwala, M.M., 2021. Alleviation of diabetic nephropathy by zinc oxide nanoparticles in streptozotocin-induced type 1 diabetes in rats. *ET Nanobiotechnology*, 15: 473-483. https://doi.org/10.1049/nbt2.12026

Arthur, B., and Chausmer, S., 1998. Zinc, insulin and diabetes. *Journal of American College of Nutrition*, 17(2): 109-115. https://doi.org/10.1080/07315724.1998.10718735

Auld D.S., 2001. Zinc coordination sphere in biochemical zinc sites. *Biometals*, 14(3-4): 271-313. https://doi:10.1023/a:1012976615056

Barman, S., Pradeep, S.R., and Srinivasan, K., 2018. Zinc supplementation alleviates the progression of diabetic nephropathy by inhibiting the overexpression of oxidative-stress-mediated molecular markers in streptozotocin-induced experimental rats. *Journal of Nutritional Biochemistry*, 54: 113-129. https://doi.org/10.1016/j.jnutbio.2017.11.008

Cefalu, W.T., Ye, J., and Wang, Z.Q., 2008. Efficacy of dietary supplementation with botanicals on carbohydrate metabolism in humans. *Endocr Metab Immune Disord Drug Targets*, 8: 78-81. https://doi.org/10.2174/187153008784534376

Edmin, S., Dodson, G., and Cutfield, S., 1980. Role of zinc in insulin biosynthesis: some possible zinc insulin interactions in the pancreatic beta cells. *Diabetologia*, 19: 174-182. https://doi.org/10.1007/BF00275265

Fuangchana, A., Sonthisombata, P., Seubnukarnb, T., Chanouanc, R., Chotchaisuwatd, P., Sirigulsatiene, V., Ingkaninan, K., Plianbangchang, P., and Haines, S.T., 2011. Hypoglycemic effect of bitter melon compared with metformin in newly diagnosed type 2 diabetes patients. *Journal of Ethnopharmacology*, 134(2): 422-428. https://doi.org/10.1016/j.jep.2010.12.045

Gadoa, Z.A, Moustafa, A.H., El-Rayes, S.M., Arisha A.A., and Mansour, M.F. 2022. Zinc oxide nanoparticles and synthesized pyrazolopyrimidine alleviate diabetic effects in rats induced by type II diabetes. *ACS Omega,* 7: 36865-36872. https://doi.org/10.1021/acsomega.2c05638

Grover, J.K., and Yadav, S.P., 2004. Pharmacological actions and potential uses of Momordica charantia: A review. *Journal of Ethnopharmacology*, 93(1): 123-132. https://doi.org/10.1016/j.jep.2004.03.035

Grover, J.K., Vats, V., Rathi, S.S., and Dawar, R., 2001. Traditional Indian anti-diabetic plants attenuate progression of renal damage in streptozotocin-induced diabetic mice. *Journal of Ethnopharmacology*, 76(3): 233-238. https://doi.org/10.1016/s0378-8741(01)00246-x

Haase, H., Overbeck, S., and Rink, L., 2008. Zinc supplementation for the treatment or prevention of disease: current status and future perspectives. *Experimental Gerontology*, 43(5): 394-408. https://doi.org/10.1016/j.exger.2007.12.002

Hasan, I., and Khatoon, S., 2012. Effect of *Momordica charantia* (bitter gourd) tablets in diabetes mellitus: Type 1 and Type 2. *Prime Research and Medicine*, 2(2): 72-74.

Husen, A., 2020. Carbon-based nanomaterials and their interactions with agricultural crops, in: Nanomaterials for agriculture and forestry applications. *Elsevier*, pp. 199-218. https://doi.org/10.1016/B978-0-12-817852-2.00008-1

Jansen, J., Karges, W., and Rink, L., 2009. Zinc and diabetes-clinical links and molecular mechanisms. *The Journal of Nutritional Biochemistry*, 20(6): 399-417. https://doi.org/10.1016/j.jnutbio.2009.01.009

Jayawardena, R., Ranasinghe, P., Galappatthy, P., Malkanthi, R.L.D.K., Constantine, G.R., and Katulanda, P., 2012. Effects of zinc supplementation on diabetes mellitus: a systematic review and meta-analysis. *Diabetology and Metabolic Syndrome*, 4: 13. https://doi.org/10.1186/1758-5996-4-13

Jiang, J., Pi, J., and Cai, J., 2018. The advancing of zinc oxide nanoparticles for biomedical applications. *Bioinorganic Chemistry and Applications*, Pp 1-18. https://doi.org/10.1155/2018/1062562

John, A.D., Ragavee A., and Selvaraj, A.D., 2020. Protective role of biosynthesised zinc oxide nanoparticles on pancreatic beta cells: an in vitro and in vivo approach. *IET Nanobiotechnology*, 14(9): 756-760. https://doi:10.1049/iet-nbt.2020.0084

Kim, J., and Ahn, J.,2014. Effect of zinc supplementation on inflammatory markers and adipokines in young obese women. *Biological Trace Element Research*, 157: 101-106. https://doi.org/10.1007/s12011-013-9885-3

Lin, H.V., and Accili, D., 2011. Hormonal regulation of hepatic glucose production in health and disease. *Cell Metabolism*, 14(1): 9-19.

Lin, Y., and Sun, Z., 2010. Current views on type 2 diabetes. *Journal of Endocrinology*, 204(1): 1-11. https://doi.org/10.1677/JOE-09-0260

McClung, K.A., and Cai, L. 2014. Fighting diabetes with the power of zinc. *Austin Journal of Endocrinology and Diabetes*, 1(3): 1011.

McLennan, S.V., Kelly, D.J., Cox, A.J., Cao, Z., Lyons, J.G., Yue, D.K., and Gilbert, RE., 2002. Decreased matrix degradation in diabetic nephropathy: effects of ace inhibition on the expression and activities of matrix metalloproteinases. *Diabetologia*, 45(2): 268-275. https://doi.org/10.1007/s00125-001-0730-4

Meles, D.K., Wurlina, A.D.P.A., Rinaldhi, C.P., Octaviani, R.R., and Cempaka, D.K.S., 2019. The antidiabetic effect of bitter melon (*Momordica charantia* L.) extracts towards glucose concentration, langerhans islets, and leydig cells of hyperglycemic mice (*Rattus norvegicus*). *EurAsian Journal of BioSciences*, 13(2): 757-762.

Meles, D.K., Wurlina, W., and Adnyana, D.P.A., 2017. Measurement of alkaloids achyranthes aspera linn level using thin layer chromatography method and high-performance liquid chromatography. *KnE Life Sciences*, 3(6): 378-385. https://doi.org/10.18502/kls.v3i6.1146

Patel, S., Patel, T., Parmar, K., Bhatt, Y., Patel, Y., and Patel, N.M.D., 2010. Isolation, characterization and antimicrobial activity of charantin from *Momordica charantia* Linn. Fruit. *International Journal of Drug Development and Research*, 2(3): 629-634.

Paul, A., and Raychaudhuri, S.S., 2010. Medicinal uses and molecular identification of two *Momordica charantia* varieties - a review. *Electronic Journal of Biology*, 6(2): 43-51.

Rajakumar, G., Thiruvengadam, M., Mydhili, G., Gomathi, T., and Chung, I.M., 2018. Green approach for synthesis of zinc oxide nanoparticles from *Andrographis paniculata* leaf extract and evaluation of their antioxidant, anti-diabetic, and anti-inflammatory activities. *Bioprocess Biosystems Engineering*, 41(1): 21-30. https://doi:10.1007/s00449-017-1840-9

Ranasinghe, P., Pigera, S., Galappatthy, P., Katulanda, P., and Constantine, G.R., 2015. Zinc and diabetes mellitus: understanding molecular mechanisms and clinical implications. *DARU Journal of Pharmaceutical Sciences*, 23(1): 44. https://doi.org/10.1186/s40199-015-0127-4

Rathi, S.S., Grover, J.K., Vikran, V., and Biswas, N.R., 2002. Prevention of experimental diabetic cataract by Indian Ayurvedic plant extracts. *Phytotherapy* Research, 16(8): 774-777. https://doi.org/10.1002/ptr.1064

Rizzo, L.Y., Theek, B., Storm, G., Kiessling, F., and Lammers T., 2013. Recent progress in nanomedicine: therapeutic, diagnostic and theranostic applications. *Current Opinion in Biotechnology*, 24(6): 1159-1166. https://doi.org/10.1016/j.copbio.2013.02.020

Rungby, J., 2010. Zinc, zinc transporters and diabetes. *Diabetologia*, 53: 1549-1551.

Sathishsekar, D., and Subramanian, S., 2005. Medicinal uses and molecular identification of two *Momordica charantia*

varieties - a review. *Biological and Pharmaceutical Bulletin*, 28: 978-983.

Siddiqi K.S., and Husen A., 2017. Plant response to engineered metal oxide nanoparticles. *Nanoscale Research Letter*, 12: 92. https://doi.org/10.1186/s11671-017-1861-y

Smidt, K., Jessen, N., Petersen, A.B., Larsen, A., Magnusson, N., Jeppesen, J.B., Stoltenberg, M., Culvenor, J.G., Tsatsanis, A., Brock, B., Schmitz, O., Wogensen, L., Bush, A.I., and Rungby, J., 2009. SLC30A3 responds to glucose-and zinc variations in ß-cells and is critical for insulin production and in vivo glucose-metabolism during ß-cell stress. *PLoS ONE*, 4: e5684. https://doi.org/10.1371/journal.pone.0005684

Souto, E.B., Souto, S.B., Campos, J.R. Severino, P., Pashirova, T.N., Zakharova, L.Y., Silva, A.M., Durazzo, A., Lucarini, M., Alzzo, A., and Santini, A., 2019. Nanoparticle delivery systems in the treatment of diabetes complications. *Molecules*, 24: 4209. https://doi.org/10.3390/molecules24234209

Sun, Q., Van, D.R.M., Willett, W.C., and Hu, F.B., 2009. Prospective study of zinc intake and risk of type 2 diabetes in women. *Diabetes Care*, 32: 629-634. https://doi.org/10.2337/dc08-1913

Tang, K.S., 2019. The current and future perspectives of zinc oxide nanoparticles in the treatment of diabetes mellitus. *Life Science*, 239: 117011. https://doi.org/10.1016/j.lfs.2019.117011

Taylor, L., 2002. "Bitter melon (*Momordica charantia*)" herb. Secrets rainforest. 2nd Ed. Sage Press, Austin, Texas, USA, pp. 1-100.

Ukperoro, J.U., Offiah, N., Idris, T., and Awogoke, D., 2010. Antioxidant effect of zinc, selenium and their combination on the liver and kidney of alloxan-induced diabetes in rats. *The Mediterranean Journal of Nutrition and Metabolism,* 3: 25-30. https://doi.org/10.1007/s12349-009-0069-9

Umrani, R.D., and Paknikar, K.M., 2014. Zinc oxide nanoparticles show antidiabetic activity in streptozotocin-induced Type 1 and 2 diabetic rats. *Nanomedicine*, 9(1): 89-104. https://doi.org/10.2217/nnm.12.205

Vieira, R., Souto, S.B., Sanchez-Lopez, E., Machado, A.L., Severino, P., Jose, S., Santini, A., Silva, A.M., Fortuna, A., García, M.L., and Souto, E.B., 2019. Sugar-lowering drugs for type 2 diabetes mellitus and metabolic syndrome-strategies for in vivo administration: part-II. *Journal of Clinical Medicine*, 8(9): 1332. https://doi.org/10.3390/jcm8091332

Virgen-Ortiz, A., Apolinar-Iribe, A., Díaz-Reval, I., Parra-Delgado, H., Limón-Miranda, S., Sánchez-Pastor E.A., Castro-Sánchez, L., Castillo, S.J., Dagnino-Acosta, A., Bonales-Alatorre, E., and Rodríguez-Hernández, A., 2020. Zinc oxide nanoparticles induce an adverse effect on blood glucose levels depending on the dose and route of administration in healthy and diabetic rats. *Nanomaterials*, 10: 2005. https://doi:10.3390/nano10102005

Wafaey, A.A., El-Hawary, S.S., Abdelhameed, M.F., El Raey, M.A., Abdelrahman, S.S., Ali, A.M., and Kirollos, F.N., 2024. Green synthesis of zinc oxide nanoparticles using ethanolic extract of *Gliricidia sepium* (Jacq.) Kunth. ex. Walp., stem: Characterizations and their gastroprotective effect on ethanol-induced gastritis in rats. *Bioorganic Chemistry*, 145: 107225. https://doi.org/10.1016/j.bioorg.2024.107225

Wehash, F.E., Abpo-Ghanema, I.I., and Saleh, R.M., 2012. Some physiological effects of *Momordica charantia* and *Trigonella foenum-graecum* extracts in diabetic rats as compared with cidophage. *Journal of World Academy of Science, Engineering and Technology*, 64: 1206-1214.

Yoon, N.A., Park, J., Lee, J., Jeong, J.Y., Kim, H., Lee, H.S., Hwang I.G., Roh, G.S., Kim, H.J., Cho G.J., Choi, W.S., Lee D.H., and Kang S.S., 2017. Anti-diabetic effects of ethanol extract from bitter melon in mice fed a high-fat diet. *Development and Reproduction*, 21(3): 259-267. https://doi.org/10.12717/DR.2017.21.3.259