International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(3): 2652-2657

Received: 10-03-2019
Accepted: 12-04-2019

Amarnath Chaudhary
Instructor, Nepal Rastriya Technical, Sec. School, Mahespur, Bara, Napal

Ajeet kumar Jha
Scientist, Nepal Agricultural Research Council, Nepal

Rakesh Yadav
Asst. Professor, Department of pharmacology, IAAS, Paklihawa, TU, Nepal

Kalyani Ray
Professor and Head, Department of Veterinary Gynaecology and Obstetrics, WBUAFS, Kolkata, West Bengal, India

Rakibul Islam
Professor, Dept. of physiology and pharmacology, HSTU, Dinajpur, Bangladesh

Correspondence
Kalyani Ray
Professor and Head, Department of Veterinary Gynaecology and Obstetrics, WBUAFS, Kolkata, West Bengal, India

Effect of cinnamon cassia extracts on hyperglycemia and renal function in Streptozotocin induced diabetic mice

Amarnath Chaudhary, Ajeet kumar Jha, Rakesh Yadav, Kalyani Ray and Rakibul Islam

Abstract
Diabetes mellitus (DM) is defined as a group of metabolic diseases manifested by hyperglycemia which results from defects in insulin production and/or insulin action. The present study was conducted to investigate the effect of Cinnamon cassia extracts on hyperglycemia, and renal profile in Streptozotocin induced diabetic mice. Thirty six male Swiss albino mice were kept in six different groups with six mice in each group for 21days. Group T_0 served as normal controls; Group T_1 Streptozotocin induced 100 mg/ kg of body weight served as positive control mice,; Groups T_2, T_3 and T_4 mice were also Streptozotocin induced 100 mg/ kg of body weight and mice treated with 300 mg/kg, 400 mg/kg and 500mg/kg of *Cinnamon* extracts (70% ethanol), respectively; and Group T_5 were induced mice Streptozotocin 100 mg/ kg and treated with 5mg/kg Glibenclamide drug. The effect of extracts on hyperglycemia, and renal function were tested by chemistry analyzer. Results were analyzed using one way ANOVA at a 5% level of significance. The fasting blood glucose level was significantly (p<0.05) reduced at 400mg/kg and 500mg/kg of *Cinnamon* extract concentration as compared to the diabetic group. It also reduces urea and creatinine in induced diabetic mice. Reduction in the fasting blood glucose, urea and creatinine by *Cinnamon* extract indicates that it has anti-hyperglycemic, and renal failure restoration effect in Streptozotocin induced diabetic mice.

Keywords: Diabetes mellitus, cinnamon cassia extracts, hyperglycemia, renal function

Introduction
Diabetes mellitus is the commonest endocrine disorder, which arise from many environmental and genetic factors often acting together causing an absolute or relative insulin deficiency, leading to hyperglycemia in the blood and urine, sometimes accompanied by thirst and graduate loss of weight (Jahadar, 1993., Murrag *et al.*, 1996) [6, 8]. In diabetes mellitus, the major contribution was made in the field of hypoglycemic action of various plant products and drug interaction to hypoglycemic agents. Thus, in some countries the use of medicinal plants as anti-diabetic remedies is a common practice. These plants were found to possess active constituents called hypoglycemia agents. Cinnamon is widely used in Ayurveda medicine to treat diabetes in India, Cinnamon extract has strong antioxidant activity due to the presence (cinnamaldehyde, eugenol, weitherthin, cinnamic acid and pinene, terpenoids, Mucilage, Diterpenes, Proanthocyanidins, Mannitol, Gum and coumarins and is beneficial in preventing and controlling the glucose intolerance and diabetes by activating insulin, glucose transport and improving glucose metabolism. It also lowered blood level fat and bad cholesterol which are, also, partly controlled by insulin (Preuss *et al.*, 2006) [12].
It was hoped that this study has been important in giving direction for finding new application on hyperglycemia, and renal dysfunction of *cinnamon extract* that could be used for future treatment of T2DM. The general objective of this study was to see that the effect of cinnamon on blood glucose level in Streptozotocin induced mice with the following specific objectives:
1. To determine the effect of cinnamon on blood glucose level in Streptozotocin induced mice.
2. To know the effect of cinnamon on serum urea, creatinine in Streptozotocin induced mice.

Materials and Methods
Experimental Animals and Study Protocol: Laboratory male Swiss albino mice (36-40) gm, 8th week age, were obtained from the department of Pharmacology, Institute of Agriculture and Animal science (IAAS), Tribhuban University, Nepal.

All experimental animal procedures were in accordance with the standards set forth in guidelines for the care and use of experimental animals by research Committee of department. The animals were allowed to acclimatize in the laboratory environment for a week before the commencement of the experiment. The mice were housed in a standard plastic cage measuring 30×13×15 cm at temperature (25±2) ^{0}C and 12/12 light/dark cycle under controlled environment and sawdust substrate was changed weekly. The mice were fed a standard commercial pellet diet at a dose of (120-150) gm/kg recommended or advised by Nimbus feed ltd. and water *ad libitum* throughout the experimental period.

Experimental Layout: Group T_0 (Normal control group): Feeding only 0.5 ml normal saline daily for each up to 21 days
Group T_1 (Positive control group): Induced diabetics by STZ 100mg/kg body wt. intraperitoneally and given only 0.5ml normal saline for each up to 21 days
Group T_2 (Positive control): Induced diabetic mice by STZ 100mg/kg body wt. intraperitoneally and treated with 300mg/kg body wt. of cinnamon extract solution orally for each up to 21 days
Group T_3 (Positive control) : Induced diabetic mice by STZ 100mg/kg body wt. intraperitoneally and treated with 400mg/kg body wt. of cinnamon extract solution orally for each up to 21 days
Group T_4 (Positive control): Induced diabetic mice by STZ 100mg/kg body wt. intraperitoneally and treated with 500mg/kg body wt. of cinnamon extract solution orally for each up to 21 days
Group T_5 (Positive control): Negative control induced diabetic mice by STZ 100mg/kg body wt. intraperitoneally and treated with 5mg/kg of body wt. of Glibenclamide (GCB) orally for each up to 21 days

Instruments, Reagents and Drugs: The following instruments, reagents and drugs were used for this study.

Instruments: Whatman filter paper No.1, test tube, gel tube, volumetric flask 5 L, beakers 400 mL, funnels, measuring cylinder 1000 mL, glass rod, spatula, magnetic stirrer, semi-automatic pipettes of 10, 200 and 1000 µL, gavage (oral feeding syringe), Syringe 1 mL, 3 mL, desiccator, heater, refrigerator, digital electronic balance, pH meter, one touch basic glucometer, strip,(Johnson & Johnson company, India).

Reagents: Ethanol, citric acid, sodium hydroxide, tri-sodium citrate, 5% glucose solution, diethyl ether.

Drugs: Streptozotocin was also purchased from Sisco Research Laboratories Pvt. Ltd. (SRL) - India.
Glibenclamide (Maan Medex private limited, India) was purchased from a local drug store in Patna of India.

Preparation and Alcoholic Extract of *Cinnamon* bark: *Cinnamon bark* was purchased from the local market, Bhairwaha, Rupendehi Nepal. It was identified and authenticated by taxonomist in Tribhuban University, Nepal, The bark were washed carefully with distilled water to remove any extraneous materials and then grounded to coarse powder using electric grinder. Three hundred gram of dried and grounded bark was extracted with ethanol 70% in a soxhlet apparatus for 48 hr. at 60 °C. After extraction, the solvent was evaporated to dryness at (40° - 45 °C) by using a rotary evaporator and the extract left behind was stored at 4 °C

Yield of Cinnamon Extracts: The percentage yield of ethanolic 70% extract of the dried *Cinnamon extract* was found to be 5.7% (w/w).The extract was dark-brown jelly and solidified when stored in a deep freezer and turn to semisolid state on re-exposure to room temperature.

$$\% \text{ Yield} = \frac{\text{Actual mass obtained from experment (gm)}}{\text{Theoretical Mass (gm)}} \text{x } 100\%$$

$$\% \text{ Yield} = \frac{17.2\text{gm}}{300\text{gm}} \text{x } 100\% = 5.7\%$$

Acute Toxicity Test of *Cinnamon bark* extracts: Acute oral toxicity study was conducted according to Organization for Economic Cooperation and Development guideline 423, and six male mice were orally administered a single concentration of 2000mg/kg body weight of *cinnamon bark* extracts. Mortality and toxicity signs such as coma, anxiety, polyuria and other behavioral changes were observed and recorded after 1, 3 and 6 hours of administration of the extract for three days.

Induction of Experimental Diabetes: Diabetic were induced to fasting mice by a single intraperitoneal injection of freshly prepared STZ at a concentration of 100 mg/kg body weight in 0.1 M citrate buffer (pH4.5) in a volume of 20 ml/kg body weight (Sachin *et al.*, 2009). After one week of Streptozotocin induction, fasting blood glucose levels were estimated and mice with blood glucose 200 mg/dl were considered as diabetic, and used for the experiments. Streptozotocin can selectively destroy the pancreatic β-cells with rapid and irreversible necrosis and can be used to generate a chronic model of hyperglycemia with diabetes complications.

Biochemical Test Assay: At the end of the experimental period, all groups of animals were euthanized by anesthetizing with diethyl ether and then blood was collected via lateral Tail vein. After the blood was coagulated at room temperature for 30 minutes, it was centrifuged for 10 minutes at 3000 rpm. Serum samples were stored in deep freezer at -20 °C until further analyses of various biochemical parameters were determined. Urea and creatinine were estimated with chemistry analyzer.

Fasting Blood Glucose Level: Blood sample was collected from the tail vein of the mice, and fasting blood glucose was estimated with One Touch Basic Glucometer after 6 hour fasting on 1st, 7th, 14th and 21stdays. The method involves two coupled reactions (Mukherjee, 1988). The increase in absorbance of NADPH at 340 nm was measured and directly proportional to concentration of glucose.

Serum Creatinine: Colorimetric estimation of serum creatinine is done by using the alkaline picrate method via Jaffe's Method (Peake and Whiting, 2006). Creatinine in an alkaline medium forms a colored complex with picric acid. The formation rate of the complex measured calorimetrically through the increase of the absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.

For this study; the sample was serum of the mice and, the reagents are standard and ready for use on automated analyzer. This enzymatic Assay was done at 400nm wavelength, 1cm optical path, room temperature and measurement done against air (increasing absorbance).

Serum Urea: Urea is split into ammonia and carbon dioxide in the presence of water and urease. Then, ammonia combines with 2-oxoluglutarete and NADH by the enzyme glutamate dehydrogenase (GLDH) to yield glutamate and NAD+. The reaction is monitored kinetically at 340 nm by the rate of the decrease in the absorbance resulting from the oxidation of NADH to NAD+, proportional to the concentration of urea present in the sample (Fawcett and Scott, 1960).
For this study, the sample was serum of mice and, the Reagents are standard and ready for use on automated analyzer. This enzymatic Assay was done at 340 nm wavelength, 1 cm optical path, room temperature and measurement done against air (decreasing absorbance).
Working reagent, samples and standards were pre-incubated at 37°C. The spectrophotometer was adjusted to zero absorbance with air. Samples (10µL) or standard (10µL) and working reagents (10 µL) were pipetted into cuvette and mixed gently by inversion. The cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance was measured at 340 nm exactly after 30 seconds (A_1) and exactly 90 seconds later (A_2) of the sample and standard addition. Finally, after 30 and 90 seconds absorbance, the difference was calculated.

Recording of body weight: Body weight was taken on day1 and 21st day of treatment. (During treatment). Body weight of all groups was recorded before treatment on Day 1 and21st day by the help of electric balance.

Recording of blood glucose: Blood sample were collected from tail vein at a day 1st, 7th , 14thand 21st (during treatment for estimation of blood glucose levels. estimation of blood glucose level was performed by OneTouch Ultra® 2 active monitor blood glucose system(Strip method)

Statistical Analysis: The results of various biochemical parameters were expressed as mean ± SEM. Data analysis of the Statistics were done using SPSS version 20 and Microsoft Excel. Statistical difference between means analysis was done using analysis of variance (ANOVA) followed at a 5% level of significance.

Results

Effects of Cinnamon extracts on Body Weight: The body weights were found to drop in diabetic mice as compared with normal control group. However, there were slight increases of the body weights in all concentrations of *Cinnamon extract* treated diabetic mice as shown below in figure 1.

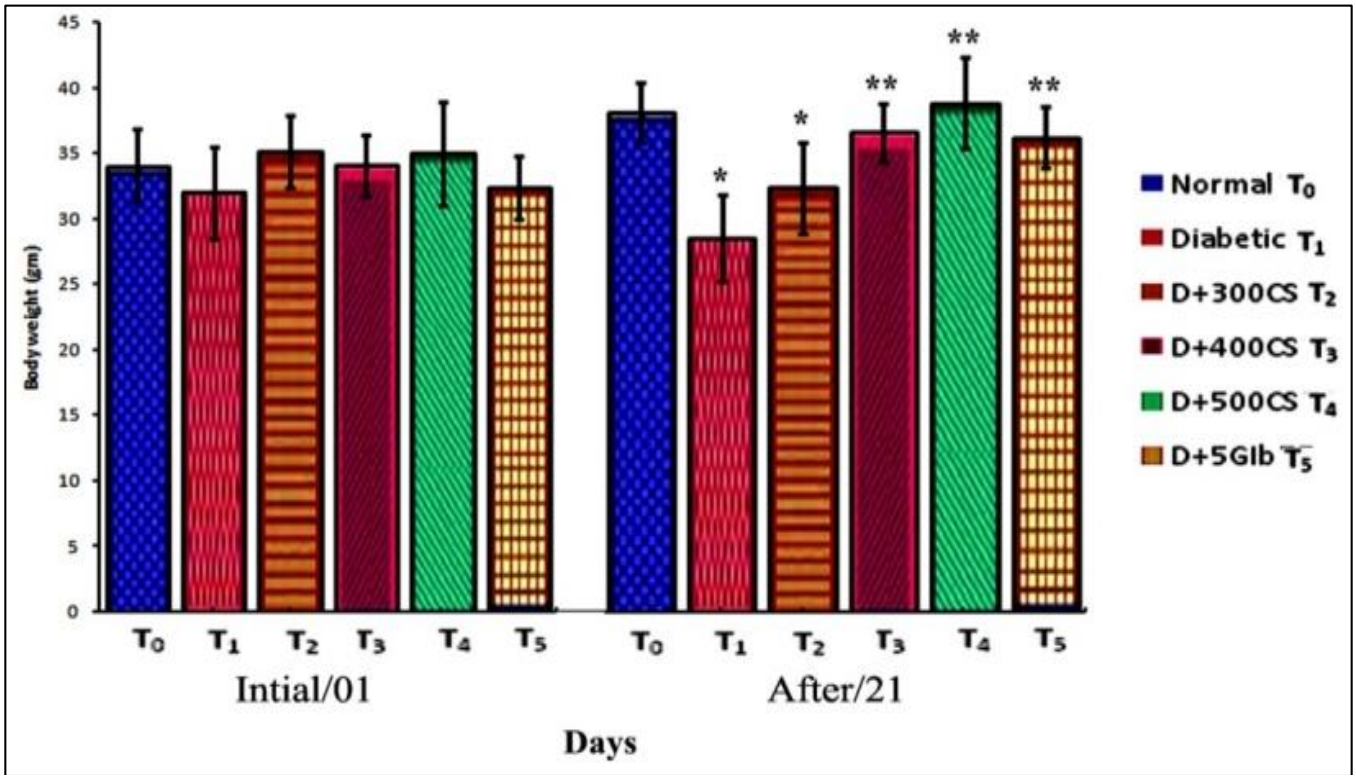

Fig 1: Effects of cinnamon extract on body weight in STZ inuced diabetic mice. *The each value is a mean ±SD for six mice is group. Values are statistically significant at *-p<0.05 diabetic control compared with normal control, **-p<0.001 treated group compared with diabetic control. D-Diabetic, CS - cinnamon extract solution, Glb -Glibenclamide*.

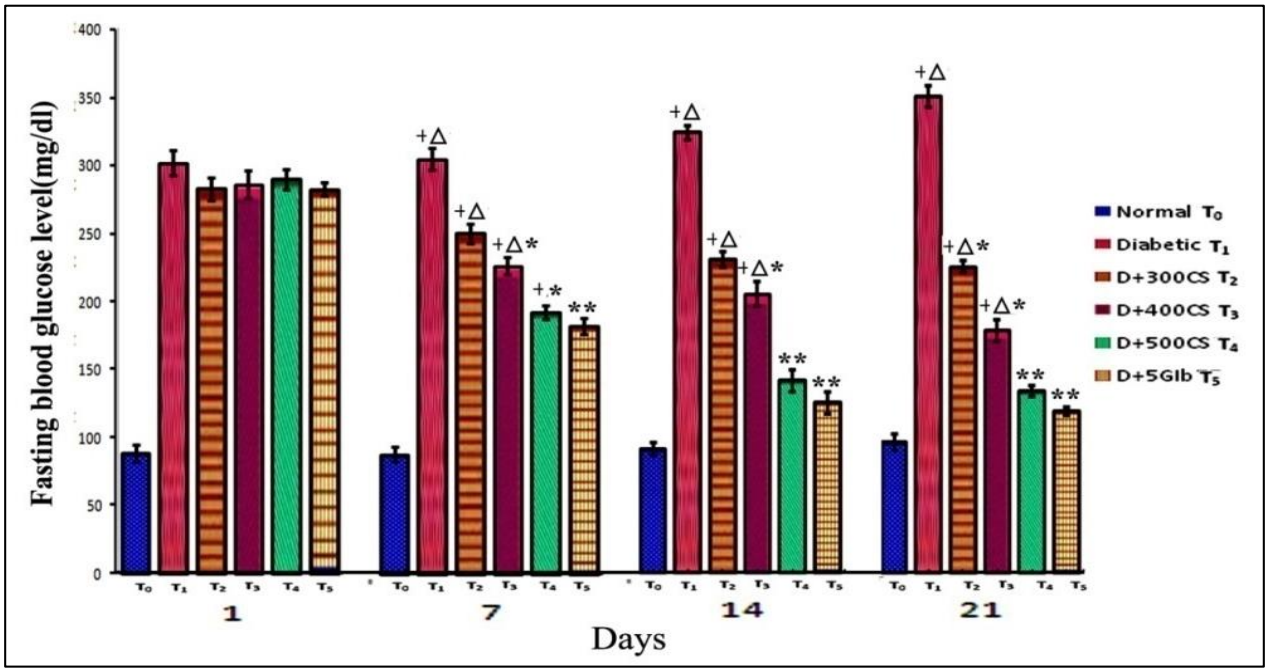

Fig 2: Effects of *Cinnamon extract* on fasting blood glucose in STZ induced diabetic mice on 1st, 7th, 14th and 21st days. *The results are expressed as mean ± SD (n =6).*
** – significant at p<0.05 compared with diabetic control,*
*** - significant at p<0.001 compared with diabetic control, + - significant at p<0.05 compared with normal control, Δ -significant at p<0.05 compared with (Glb) Glibenclamide treated group, D-Diabetic, CS- Cinnamon extract solution*

Effect of *Cinnamon extract* on Fasting Blood Glucose Level: The anti-hyperglycemic effects of graded concentration of *Cinnamon extract* on the FBG levels of STZ induced diabetic mice were presented in figure 2 as shown below. The FBG levels were significantly (p<0.001) increased as compared to normal control group throughout the study period. This increase of blood glucose was almost three-fold higher even after three weeks compared to normal control mice. However, treatments of diabetic mice with *Cinnamon* extracts, the FBG levels were significantly (p<0.01) decreased on 7th, 14th and 21st days. Similarly, treatment with Glibenclamide, which has been used as standard anti-diabetic reference drug to compare the beneficial effects of *Cinnamon extract*, also led to a significant (p<0.001) reduction in FBG levels on 7th, 14th and 21st days.

Effects of Cinnamon extract on Serum Creatinine: Figure 3. Describes the effect of *Cinnamon extract* on serum creatinine in normal and diabetic mice. There were significant (P<0.01) increases in serum creatinine in diabetic group as compared to normal control. However, serum creatinine was reduced after the administration of *Cinnamon extract* at all concentrations and 5mg/kg Glibenclamide in treated diabetic mice as compared to diabetic mice.

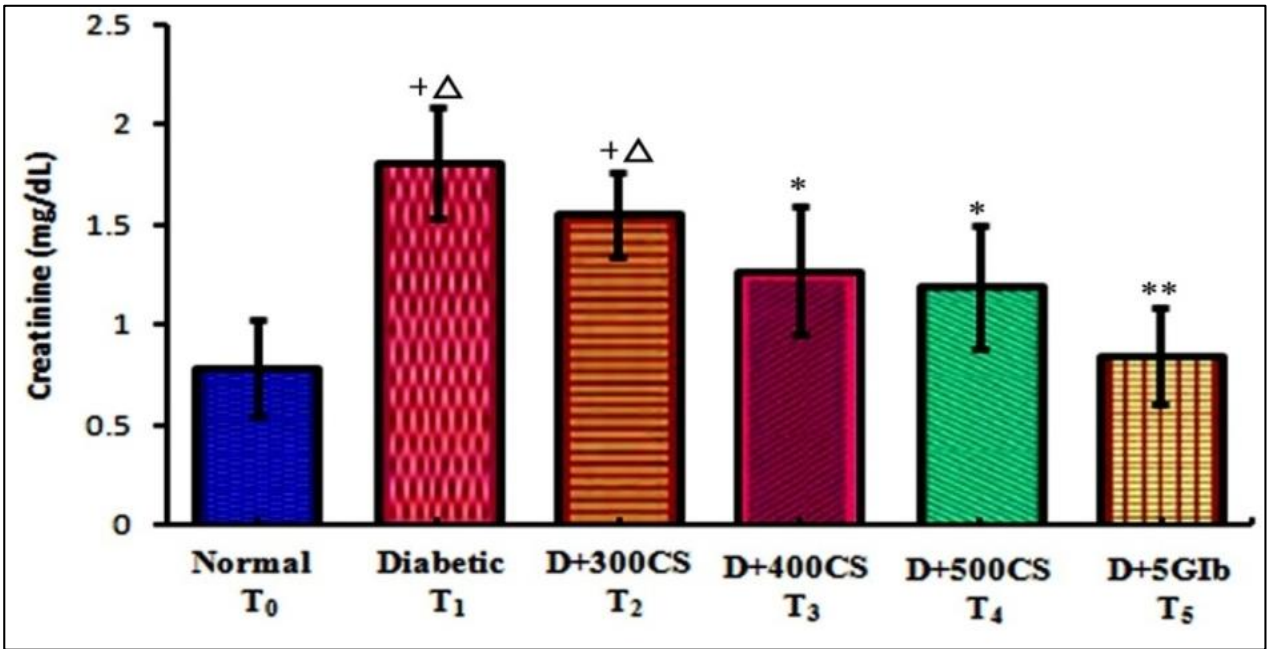

Fig 3: The effects of *Cinnamon extract* on Creatinine in STZ induced diabetic mice. *The results are expressed as mean ± SD (n =6). * – significant at p<0.05compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, + - significant at p<0.05 compared with normal control, Δ -significant at p<0.05 compared with Glibenclamide treated group, D-Diabetic, CS - Cinnamon extract.*

Effects of Cinnamon extract on Serum Urea: Figure 4 describes the effect of *Cinnamon extract* on serum urea in normal and diabetic mice. There were significant (p<0.05) increases in serum urea in diabetic group as compared to normal control. However, serum urea was reduced after the administration of *Cinnamon extract* at all concentrations and 5mg/kg Glibenclamide in treated diabetic groups as compared to diabetic group after treatment for three weeks.

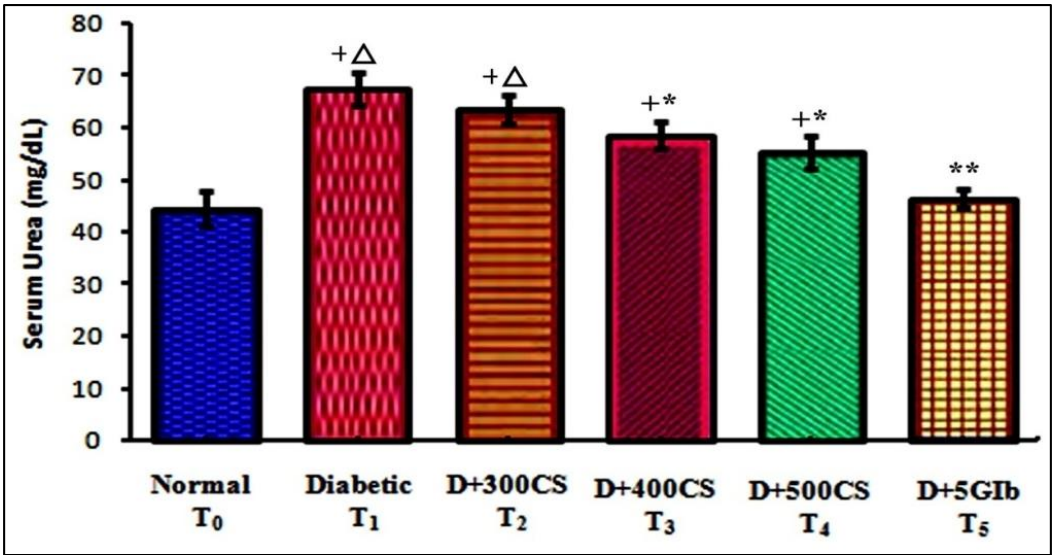

Fig 4: Effects of *Cinnamon extract* on serum urea in STZ induced diabetic mice. *The results are expressed as mean ± SD (n =6). * – significant at p<0.05 compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, + - significant at p<0.05 compared with normal control, Δ - significant at p<0.05 compared with Glibenclamide treated group, D-Diabetic, CS - Cinnamon extract*

Effect of Cinnamon extracton Acute Toxicity Test: In acute oral toxicity test, Cinnamon extract revealed no mortality at the 2000 mg/ kg body weight concentration in mice. The mice did not also show any toxic effects like changes in behavioral activities such as anxiety, polyuria, diarrhoea, seizures, and coma which received *cinnamon extract*. Thus, the *Cinnamon extract*, 2000 mg/Kg body weight of mice were found be a good safety margin indicator. Therefore, one-fifth (20%) of the safe doses were taken by the researcher for the experiments.

Discussion

Diabetes mellitus is now described as a disorder of multiple etiologies with abnormalities in carbohydrate, lipid as well as protein metabolism. Abnormalities in glucose metabolism and renal function are important risk factors for diabetes, cardiovascular and many other diseases.

STZ induced animal model has been described as a useful experimental model to study the effect of anti-diabetic agent such as Glibenclamide against T2DM. STZ is known to induce diabetes, hyperinsulinemia, or hyperglycemia by damaging the pancreatic β cells (Graham *et al.*, 2011) [4].

This study result revealed that Cinnamon extract treated groups did not show important body weight gain; this could support that Cinnamon to be an important for treatment of diabetes mellitus over convectional drugs (Glibenclamide) which are mostly known to cause body weight gain in diabetes mellitus treatment (Prabhakar and Doble, 2011) [11].

The increase in fasting blood glucose concentration is an important characteristic feature of T2DM. In this study, there were elevations in FBG level in diabetic treated group. However, the extract of Cinnamon reduced FBG level in diabetic mice. Glibenclamide (5mg/kg) led to reduction of FBG level by 46.03%, 61.30% and 62.12% on 8th, 15th and 21st days respectively as compared to diabetic group. These results of reduction in FBG are in agreement with pervious work on effect of Cinnamon extract on insulin release from pancreatic β cells in Streptozotocin-induced diabetic rats (Eidi *et al.*, 2009) [2].

Hence, when the concentrations of Cinnamon extracts increased, the FBG level was shown to have been decreased. The glycemic control was nearly similar between Glibenclamide and Cinnamon extracts treatment. Thus, the increment of the Cinnamon extracts concentration may further provide a similar result as Glibenclamide drug.

The present findings indicate the hypoglycemic and/or potential ant hyperglycemic effect of the extract. There were many possible explanations for this finding. The ant hyperglycemic effect of Cinnamon extracts may be due to restoration of insulin response via the presence of ant hyperglycemic, “insulin-releasing” and “insulin like” activity in Cinnamon bark (Gray and Flatt, 1999) [5]. It was also suggested that the anti-hyperglycemic effects of the Cinnamon extracts could be caused by high level of fiber which interfere carbohydrate absorption, increased peripheral uptake of glucose, improved sensitivity of insulin receptor, and regenerative effect of Cinnamon on pancreatic tissue (Byambaa *et al.*, 2010) [1].

Type 2 diabetes mellitus also causes renal damage due to abnormal glucose regulation including elevated glucose and glycosylated protein tissue level, hemodynamics changes within the kidney and oxidative stress. Both negative balance of nitrogen and lowered protein synthesis leads to increased level of serum urea and creatinine that indicates progressive renal damage in diabetic mice (Musabayane *et a*l; 2012) [9].

Nevertheless, the Cinnamon extracts reduced both serum urea and creatinine in diabetic treated mice. These reductions of serum urea and creatinine may show the beneficial effects of the Cinnamon extracts on the kidney function of diabetic mice. Thus, this renephroprotective function could be mediated via antioxidant and/or free radical scavenging activities as they possess high concentration of flavonoids and alkaloids (Udayakumar *et al.*, 2009) [14].

Conclusion and Recommendation

In this study, hydro-ethanolic extracts of Cinnamon extract showed a reduction on fasting blood glucose level in STZ induced diabetic mice. This could be due to ‘’insulin like’’ and insulin releasing activities of the extract. Thus, it may be concluded that Cinnamon extracts has potent hypoglycemic effect and provided better glycemic control in STZ induced diabetic mice. Cinnamon extracts also showed a decrease of serum urea and creatinine which indicates restoring properties on the function of kidney of diabetic mice. Generally, from the above findings, it is possible to conclude that extract of Cinnamon has anti-hyperglycemic, and restoring the function of kidney in STZ induced diabetic Swiss albino mice. Hence, it could be conclude that 500mg/kg concentration of Cinnamon extract have a better anti-diabetic capacity, and almost equipotent with Glibenclamide drug. Cinnamon extract solution possesses antihyperglycemic and antioxidant effects in diabetic animals.

References

1. Byambaa E, Zeynep O, Erdembileg A, Lars B. Postprandial Lipoproteins and Cardiovascular Disease Risk in Diabetes Mellitus Curr Diab Rep. 2010; 10:61-69.
2. Eidi M, Eidi A, Saeidi A, Saadat M, Massih B, Kamal B. Effect of Coriander leaf Ethanol Extract on Insulin Release from Pancreatic β Cells in Streptozotocin-induced Diabetic Rats Phytother. Res. 2009; 23:404-406
3. Fawcett J, Scott J. A rapid and precise method for the determination of urea J Chim. Pathol. 1960; 13:156
4. Graham M, Jody L, Jessica A, Bernhard J, Henk-Jan S. The Streptozotocin- Induced Diabetic Nude Mouse Model: Differences between Animals from Different Sources: American Association for Laboratory Animal Science. 2011; 61(4):356-360
5. Gray AM, Flatt PR. Insulin-releasing and insulin-like activity of the traditional anti- diabetic plant Coriandrum sativum (coriander). Br J Nutr. 1999; 81(3):203-9.
6. Jahadar L. Plants with hypoglycemic effects. Cesk. Farm, 1993; 42(b):251-259.
7. Mukherjee K. Medical laboratory technology: Tata McGraw Hill. 1988; 3:991-993.
8. Murrag RK, Granner DK, Mayes PA, Rodwell VW. Harper's biochemistry. ALONGE Medical Book., 24ed. 1996, 582.
9. Musabayane CT. The effects of medicinal plants on renal function and blood pressure in diabetes mellitus Cardiovasc J Afr. 2012; 23:462-468.
10. Peake M, Whiting M. Measurement of Serum Creatinine-Current Status and Future Goals Clin Biochem Rev. 2006; 27:173-184.
11. Prabhakar PK, Doble M. Interaction of phytochemicals with hypoglycemic drugs on glucose uptake in L6 myotubes. Phytomedicine. 2011; 18(4):285-91. doi:10.1016/j.phymed. 2010.06.016
12. Preuss HG, Echard B, Polansky MM, Anderson RA. Whole cinnamon and aqueous extract a meliorate sucrose induced blood pressure elevations in spontaneously hypertension rats. J Am Coll. Nutr. 2006; 25(2):144-150.
13. Sachin A, Kumar O, Divya V. Characterization of Streptozotocin Induced Diabetes Mellitus in Swiss Albino Mice Global J Pharmacol. 2009; 3(2):81-84.
14. Udayakumar R, Kasthurirengan S, Mariashibu TS, Rajesh M. Hypoglycemic and hypolipidaemic effects of *Withania somnifera* root and leaf extracts on alloxan induced diabetic rats. International Journal of Molecular Sciences. 2009; 10(5):2367-82.