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Research Article

The effect of Lisinopril on blood glucose level given alone and in combination with oral anti-diabetic drugs in alloxan-induced diabetic rats

Neeraj K. Agrawal [a,*] and Uma Gupta [b]

[a] *Department of Pharmacology, Maulana Azad Medical College, University of Delhi, India;*
[b] *Department of Zoology, University of Rajasthan, India.*

* **Corresponding author:** 701 Shanti Nagar, Durgapura Station, Jaipur 302018, Rajasthan, India; **Tel**: +91-999-9592142; **Email**: drneer80@yahoo.com

Background: In diabetic patients, ACE (Angiotensin converting enzyme) inhibitors prevent the development and progression of incipient or established nephropathy and delay the progression of diabetic retinopathy hence these drugs are routinely prescribed with oral anti-diabetic drugs in these conditions. The picture of actual impact of ACE inhibitors on blood glucose is controversial and still not clear. This study therefore aimed to evaluate the effect of Lisinopril on blood glucose level and the interaction with the oral anti-diabetic drugs metformin, gliclazide and pioglitazone in alloxan induced diabetic rats.

Method: Rats were classified into ten groups (n = 6). The Group I –II were normal and Group III –X were diabetic. The Lisinopril at the dose of 3.6 mg/kg body weight was administered to the normal and diabetic rats. In diabetic groups, the Lisinopril was also given with the oral anti-diabetic drugs to assess any alteration in blood glucose level. All drugs administered orally once a day for 13 days and at the end of the experimentation Oral Glucose Tolerance Test (OGTT) was conducted.

Results: It was observed that in normal rats the Lisinopril slightly reduced the blood glucose level at all time points which was significant only at 30 and 120 min time points ($P \leq 0.05$) but in alloxan-induced diabetic rats Lisinopril exhibited significant anti-hyperglycemic activity at all time points ($P \leq 0.01$) when given alone and even more significantly when given with the oral anti-diabetic drugs ($P \leq 0.001$).

Conclusion: The present study has showed that the Lisinopril has significant anti-hyperglycemic activities and also enhances the effect of oral anti-diabetic drugs in diabetic animal.

Keywords: ACE inhibitor, Lisinopril, Blood glucose, Alloxan-induced diabetic rats, Oral anti-diabetic drugs

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1. Introduction

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. In the United States, DM is the leading cause of end-stage renal disease (ESRD), non-traumatic lower extremity amputations, and adult blindness. It also predisposes to cardiovascular diseases. With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality for the foreseeable future (Alvinc, 2012). Hence there is a need for safe and effective hyperglycemic management to prevent further progression of vascular complications. This provides an interesting challenge to

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multi-disciplinary healthcare teams, particularly considering the need to maximize patient adherence in a group likely to be prescribed a multitude of drugs (Hamilton, 2012).

The drugs for treating DM fall into several categories. Firstly, drugs that primarily stimulate insulin secretion by binding to the sulfonylurea receptor: sulfonylureas remain the most widely prescribed drugs for treating hyperglycemia. The meglitinide analog repaglinide and the D-phenylalanine derivative nateglinide also bind the sulfonylurea receptor and stimulate insulin secretion. Secondly, drugs that primarily lower glucose levels by their actions on the liver, muscle, and adipose tissue: metformin works in the liver. The thiazolidinediones group includes pioglitazone, rosiglitazone etc. appear to have their main effect on skeletal muscle and adipose tissue. Thirdly, drugs that principally affect absorption of glucose: the α-glucosidase inhibitors acarbose and miglitol are such currently available drugs. Fourthly, drugs that mimic incretin effect or prolong incretin action: Glucagon-like peptide-1 (GLP-1) receptor agonists and DPP-4 (Dipeptidyl peptidase-4) inhibitors fall into this category. Others: Pramlintide lowers glucose by suppressing glucagon and slowing gastric emptying (John H, 2011).

In diabetic patients, ACE inhibitors prevent the development and progression of incipient or established nephropathy (Lewis et al, 1993) and delay the progression of diabetic retinopathy (Parving et al 1989). Some clinical studies have detected inhibition of diabetic retinopathy by ACE inhibitors. The EURODIAB Control Trial of Lisinopril in Insulin Dependent Diabetes Study Group investigated the effect of the ACE inhibitor, lisinopril, on retinopathy in normotensive type I diabetic patients. They found a 50% reduction in the progression of retinopathy in lisinopril treated subjects compared to controls (Chaturvedi et al, 1998). The ACE inhibitors have been recommended as the treatment of choice for all patients with diabetic nephropathy regardless of diabetes type (American Diabetes Association, 2001) .

The pharmacological treatment of DM requires a continued monitoring for optimal blood sugar level in patients because it can prevent many of the complications of diabetes like retinopathy, neuropathy, nephropathy and vascular abnormalities. Avoidance of hypoglycemia is very important because that is also dangerous and can lead to convulsion, coma and death. It is therefore necessary to know interaction of various pharmacological agents with anti-diabetic agents because knowledge of such an interaction enables the clinician to avoid or minimize the unacceptable reactions by modification of doses and schedule of drug administration or by using an alternative drugs.

DM and hypertension are both independent risk factors for cardiovascular disease and the risk is even more when nephropathy is present. The cardiovascular causes account for more than half of the mortality associated with nephropathy (Pyorola, 1987). As ACE inhibitors are first line drugs for hypertension in diabetes and routinely prescribed with the anti-diabetic drugs, we must know the interaction between them. The actual impact of ACE inhibitors on blood glucose is doubtful because some clinical studies have showed the hypoglycemic activity but some are in favor of neutral effect. Majority of the studies were dealt with the Captopril which is the prototype of the ACE inhibitor that is why, the present study was planned to see interactions of one popular ACE inhibitor Lisinopril with various oral hypoglycemic agents in experimental animal.

2. Materials and Methods

2.1 Animals

The study was conducted on healthy albino wistar rats of either sex weighing 150-250 gm. The animals were made available in the central animal house, Ganesh Shankar Vidyarthi Memorial (GSVM) Medical College, Kanpur, Uttarpradesh, India. The rats were housed in polypropylene cages and maintained under standard conditions (12 hr light /dark cycle, 35-60% humidity and at room temperature 25 ± 3 °C), fed in standard pellet diet and water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee (IAEC), GSVM Medical College, Kanpur which is constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Ethical guidelines were strictly followed during all the experiments.

2.2 Drugs and chemical agents

Drug/chemical	Dose
Alloxan monohydrate (Sigma)	135 mg/kg body wt
ACE inhibitor	
Lisinopril (GSK)	3.6 mg /kg body wt/day
Oral anti-diabetics	
Metformin (Bristol-Myers Squibb)	225 mg/kg body wt/day
Gliclazide (Torrent)	22 mg/ kg body wt/day
Pioglitazone (Zydus Cadila)	4 mg /kg body wt/day

2.3 Induction of Experimental Diabetes

Diabetes was induced in albino rats of either sex by a single intraperitoneal injection of aqueous Alloxan monohydrate (135 mg/kg body weight) (Sigma Aldrich Co. USA) (Anita et al, 2005). The fasting blood samples were collected 0, 24 & 48 hrs after the administration of Alloxan to know the status of diabetes. Diabetes was confirmed by testing blood glucose level using a glucometer (Accu-Chek). The animals with blood glucose level more than 200 mg/dl (moderate diabetes) were selected for the experiment.

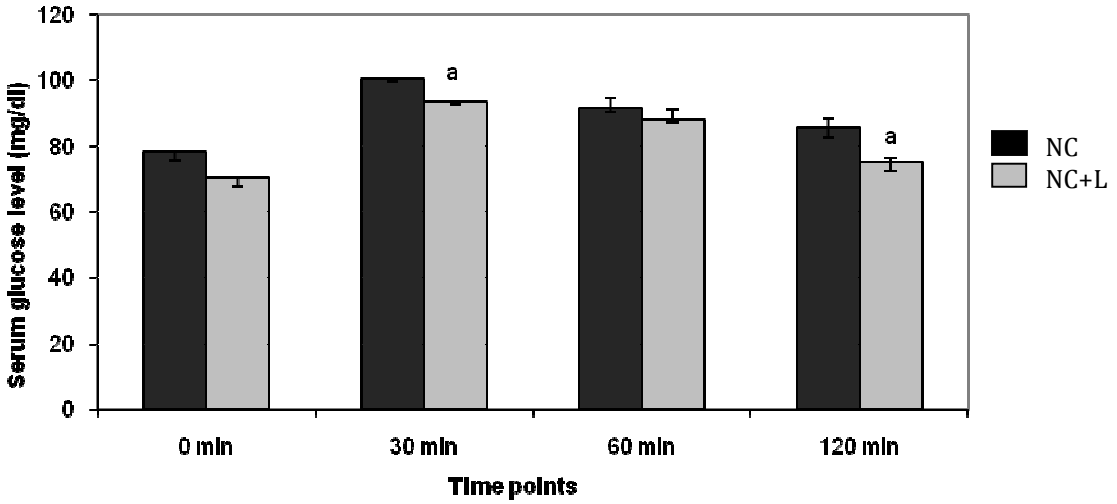
2.4 Experimental Design

Rats were classified into ten groups (n = 6). The Group I –II were normal and Group III –X were diabetic. Group I were given 1 ml sterile water and served as normal control (NC). Group II (NC+L) received drug Lisinopril only. Rats of diabetic Group III (DC) were administered with 1 ml sterile water served as diabetic control. Rats of diabetic Group IV (DC+L) were treated with drug Lisinopril only, Group V (D+M) with drug Metformin

only, Group VI (D+G) with drug Gliclazide only and Group VII (D+P) with drug Pioglitazone only. The oral anti-diabetic drugs treated groups (Group V – VII) served as positive control for the rest of the groups Group VIII, IX and X respectively. Rats of diabetic Group VIII (D+M+L) were treated with drugs Lisinopril and Metformin both, Group IX (D+G+L) with drugs Lisinopril and Gliclazide both and Group X (D+P+L) with drugs Lisinopril and Pioglitazone both. The animals of all groups were received the doses orally for 13 consecutive days and at the end of the experimentation an Oral Glucose Tolerance Test (OGTT) (Whittington et al, 1991) was conducted and blood glucose estimation was done in all groups.

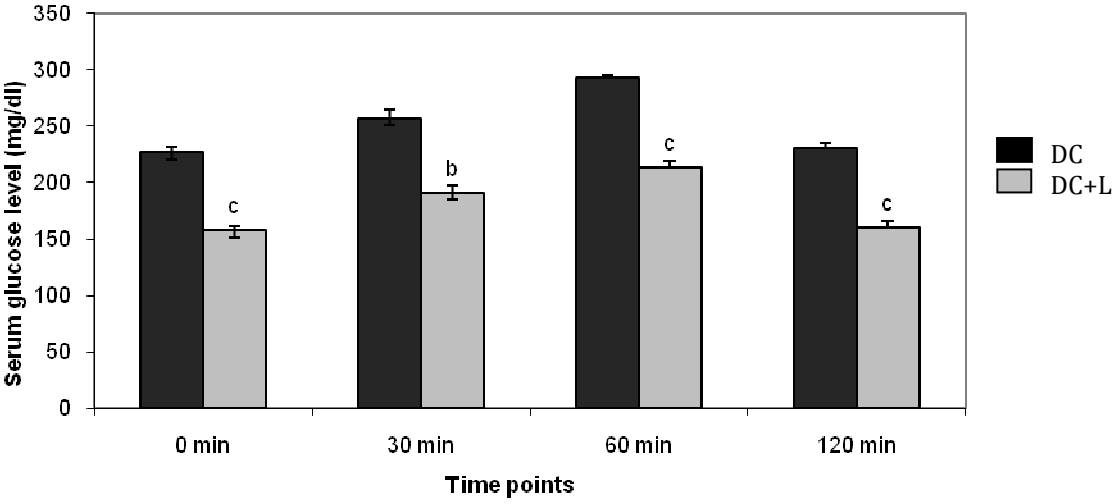
2.5 Oral Glucose Tolerance Test

Following the above treatments (**Section 2.4**) and after overnight fasting, 0 min blood samples (0.2 ml) were taken from the all groups by orbital sinus puncture (Waynforth, 1980). Glucose solution (2 g/kg of 25% w/v) was administered orally in OGTT. Three more samples were taken at 30 min, 60 min and 120 min after glucose administration.



Significance levels as compared to control (a: P ≤0.05, b: P ≤0.01; c: P ≤0.001)
Error bar represent ± SEM
***NC**: Normal Control group*
***NC+L**: Normal Control plus Lisinopril group*

Figure 1: Effect of Lisinopril on blood glucose level in normal rats.



Significance levels as compared to control (a: P ≤0.05, b: P ≤0.01; c: P ≤0.001)
Error bar represent ± SEM
***DC**: Diabetic Control group*
***DC+L**: Diabetic plus Lisinopril group*

Figure 2: Effect of Lisinopril on blood glucose level in Alloxan-induced diabetic rats.

The blood samples were then centrifuged at 3000 rpm for 10 min. The clear supernatant plasma was taken for estimation of blood glucose level. The plasma blood glucose levels were determined by using Glucose-oxidase-peroxidase (GOD–POD) method (Trinder, 1969). Span diagnostic reagent kit (Code No. B 0112) was used for estimation of blood glucose level.

2.6 Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by independent t-test. Results were considered to be significant when P values were less than 0.05 ($P<0.05$).

3. Results

3.1 Effect on Normal Rats

Effect of Lisinopril on blood glucose levels in normal rats exhibited in **Figure 1** which is also showing the standard error and significance level. It is observed that the drug Lisinopril in a dose of 3.6 mg/ kg body weight reduced the blood glucose level as 9.99% at 0 min, 6.95% at 30 min, 3.65% at 60 min and 12.05% at 120 min but it is significant only at 30 and 120 min time points ($P\leq0.05$). It means, it has some hypoglycemic activity in normal rats which is not overall significant.

3.2 Effect on Alloxan Induced Diabetic Rats

The effect of 13-day administration of Lisinopril on blood glucose levels in alloxan induced diabetic rats is shown in **Figure 2**. It is noteworthy to mention that Lisinopril has showed significant anti-hyperglycemic effect in diabetic rats. The percentage of blood glucose reduction was much more at 120 min i.e. 27.27% as compare to rest of the studied hrs which were found to be 12.52% at 0 min, 9.02% at 30 min and 11.37% at 60 min. In statistical point of view, it was significantly decreased ($P < 0.01$) at 30 min .The higher level of significance was found at rest of the time points ($P < 0.001$).

The Interaction of Lisinopril with the oral hypoglycemic drugs in alloxan induced diabetic rats illustrated in **Figures 3-5** which are showing standard error and significance which is expressed with respect to positive control groups. It revealed that blood glucose level is decreased with the drug Lisinopril at all time points in all groups in highly significant manner ($P\leq0.001$). In comparison of group V (D+M) and group VIII (D+M+R), group VI (D+G) & group IX (D+G+R) and group VII (D+P) & group X (D+P+R), Lisinopril exhibited highest hypoglycemic activity at '0' min time point with reduction of 37.35%, 34.77% and 30.56% respectively.

4. Discussion

Alloxan is a well- known diabetogenic agent that is used to induce Type I diabetes in experimental animals (Viena et al, 2001). It selectively destroys the insulin-producing beta-cells found in the pancreas; hence it is used to induce diabetes in laboratory animals. The toxic action of alloxan on pancreatic beta cells involve oxidation of essential sulphydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis (Dunn et al, 1943; Szkudelski, 2001)

Our study has evaluated the significant interaction of Lisinopril with the different groups of Oral hypoglycemic drugs in Alloxan induced diabetic rats. In light of results, the study indicates that the Lisinopril did not exhibit significant reduction in blood glucose levels at all time points in normoglycemic rats but it showed significant anti-hyperglycemic activity in diabetic rats. Moreover, it enhanced the hypoglycemic activity of all three Oral anti-diabetic drugs (Metformin, Gliclazide and Pioglitazone) in highly significant manner. As far as the mechanism of action concerned, the Metformin delays the absorption of glucose from the gastrointestinal tract, increases the insulin sensitivity of cells, suppresses hepatic gluconeogenesis (Cusi et al, 1996; Fery et al 1997) and enhances glucose transport in fat and muscle (Lenhard et al, 1997). It does not usually lower blood glucose concentrations in non-diabetic subjects. The Gliclazide induce insulin secretion in the pancreatic beta cells and inhibit glycogenolysis and gluconeogenesis in the liver. Through improving insulin binding to surface receptors, they also enhance the insulin sensitivity of target cells. Furthermore, there is in vitro evidence for an increased number of insulin receptors in tissues of action (Defronzo, 1991). The pioglitazone caused improvements in insulin sensitivity and the ability to lower fasting plasma glucose levels (Diamant and Heine 2003).

The present findings suggest that Lisinopril may have some insulin sensitivity potentiating properties in type 2 diabetes mellitus but not in normoglycemic rats. Eleven studies document the potential for ACE inhibitor-associated hypoglycemia, and a majority of these are with the use of captopril (Murad et al, 2003; Herings et al 1995). Though the mechanism for this drug-induced hypoglycaemia is not well defined, it is proposed that the increase in bradykinins associated with ACE inhibitor use may cause an increase in insulin sensitivity (Pandit et al, 1993; Vuorinen and Jarvinen 1995).One study has demonstrated that ACE inhibitors have no impact on hepatic or peripheral insulin sensitivity (Wiggam et al, 1998).

Although the present findings confirm the anti-hyperglycemic potential but the precise mechanism of its action requires further studies for appropriate elucidation. The ACE inhibitors are the drug of choice of diabetic nephropathy and diabetic hypertension and are frequently used with oral anti-diabetic drugs. They attack a fundamental abnormality in the hypertensive kidney disease and provide nearly uniform renal protective effect along with blood pressure control (Weidmann, 1993; lewis et al, 1993). Hence it is important to find-out clear cut picture of effect of ACE inhibitors on blood glucose level in large sample size with highly sensitive modern scientific techniques which is lacking in our study. It will help out the clinician to reduce the hypoglycemic episode in diabetic individuals who are taking the both drug concomitantly.

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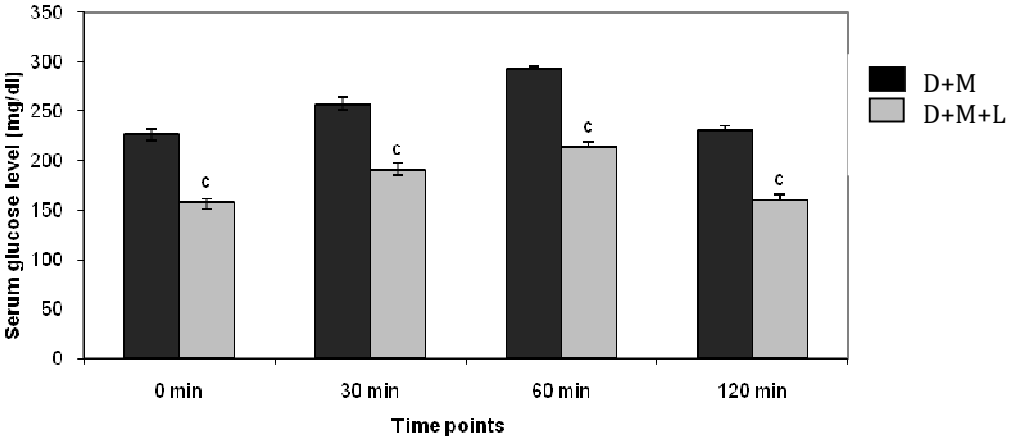
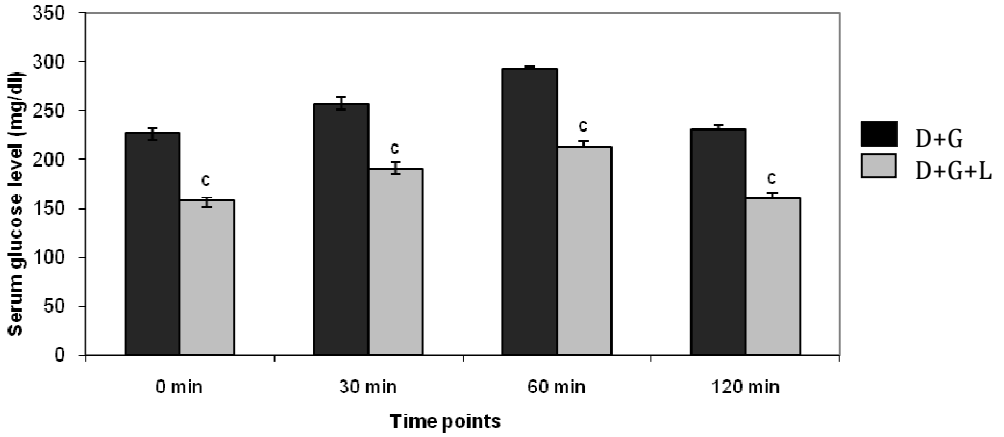


Significance levels as compared to control (a: P ≤0.05, b: P ≤0.01; c: P ≤0.001)
Error bar represent ± SEM
***DC**: Diabetic plus Metformin Control group*
***DC+L**: Diabetic plus Metformin plus Lisinopril group*

Figure 3: Effect of Lisinopril plus Metformin on blood glucose level in Alloxan-induced diabetic rats.



Significance levels as compared to control (a: P ≤0.05, b: P ≤0.01; c: P ≤0.001)
Error bar represent ± SEM
***DC**: Diabetic plus Gliclazide Control group*
***DC+L**: Diabetic plus Gliclazide plus Lisinopril group*

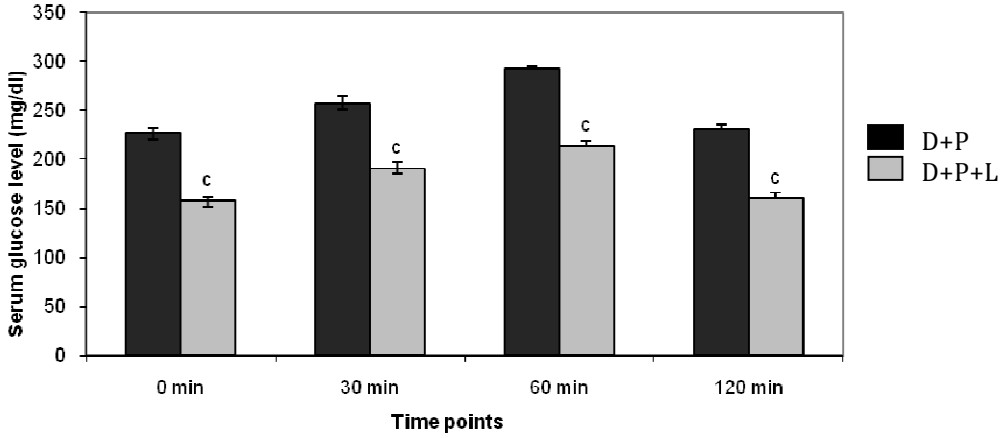


Figure 4: Effect of Lisinopril plus Gliclazide on blood glucose level in Alloxan-induced diabetic rats

Significance levels as compared to control (a: P ≤0.05, b: P ≤0.01; c: P ≤0.001)
Error bar represent ± SEM
***DC**: Diabetic plus Pioglitazone Control group*

***DC+L**: Diabetic plus Pioglitazone plus Lisinopril group*

Figure 5: Effect of Lisinopril plus Pioglitazone on blood glucose level in Alloxan-induced diabetic rats

5. Conclusion

The present study shows that Lisinopril lower blood glucose level in healthy and alloxan induced diabetic rats. Also, it enhance the blood glucose lowering effect of Metformin, Gliclazide and Pioglitazone It is therefore advisable to prescribe and use of ACE inhibitors with anti-diabetic drugs carefully with necessary dose adjustment to avoid adverse hypoglycemic episodes in diabetic individuals. But further studies are necessary to substantiate the above observation or analyze the exact mechanism of action involved in the anti-hyperglycemic activity of Lisinopril.

Conflict of Interest declaration

The authors declare no conflict of interest

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