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EVALUATION OF RAMIPRIL ON BLOOD SUGAR LEVEL AND INTERACTION WITH THE ORAL ANTI-DIABETIC DRUGS IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT:

Aim: The present study investigates the effect of Ramipril on blood sugar level and interaction with the oral anti-diabetic drugs in alloxan induced diabetic rats.

Method: Rats were divided into 10 groups (n = 6) where Group I –II were normal and Group III –X were diabetic. Ramipril at the dose of 0.9 mg/kg body weight were administered to the normal and diabetic rats. In diabetic groups, the Ramipril was given concomitantly with the each of three Oral anti-diabetic drugs to find-out the effect on blood sugar 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 Ramipril slightly reduced the blood sugar level which was insignificant ($P>0.05$) except at '30' min time point. In contrast in, it exhibited the anti-hyperglycemic activity significantly ($P\leq0.01$) at all-time points in alloxan induced diabetic rats.

Conclusion: The present study has concluded that The Ramipril has significant anti-hyperglycemic activities which accentuate the effect of oral anti-diabetic drugs in alloxan-induced diabetic rats.

INTRODUCTION: Diabetes mellitus (DM) describes a metabolic syndrome of multiple etiologies characterized by chronic hyperglycemia with alteration of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Approximately 140 million people are suffered from diabetes worldwide [1-2]. The chronic hyperglycemia of DM is associated with long-term damage, dysfunction and failure of various vital organs, especially the heart, kidneys, nerves, eyes and blood vessels [3].

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Diabetic nephropathy (DN) is the leading cause of end-stage renal failure (ESRD) in western countries and carries a 20- to 40-fold increased risk for cardiovascular (CV) mortality. In the past 2 decades, there has been a continual increase in the incidence of ESRD among patients with diabetes, predominantly those with type 2 diabetes [4-5].

In diabetic patients, ACE inhibitors prevent the development and progression of incipient or established nephropathy [6-7] and delay the progression of diabetic retinopathy [8].

Recently, the Ramipril was demonstrated to reduce death, cardiovascular events (myocardial infarction and stroke), progression of diabetic nephropathy, and even the number of new cases of diabetes in high risk patients [9-10].

ACE inhibitors have been recommended as the treatment of choice for all patients with diabetic nephropathy regardless of diabetes type [11].

The pharmacological treatment of DM requires a continued monitoring for optimal blood sugar level in patients because strict blood sugar regulation can prevent diabetic retinopathy, neuropathy, nephropathy and vascular abnormalities. Avoidance of hypoglycemia is also important because it may cause convulsion, coma and death. It is therefore necessary to know interaction of various pharmacological drugs with anti-diabetic agents to avoid or minimize untoward side effects by modification of drug schedule or by using alternative drugs.

Diabetes and hypertension are both independent risk factors for heart disease and the risk further increases with nephropathy. Cardiovascular causes are responsible for >50% mortality associated with DN [12]. ACE inhibitors/Angiotensin Receptor Blockers (ARB) 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 sugar level is still not clear and Majority of the old studies were dealt with the Captopril that is why, the present study was planned to see interactions of Ramipril with various oral hypoglycemic agents in diabetic rats.

MATERIALS AND METHODS:

Animals: The study was conducted on albino Wistar rats of either sex weighing 150-250 gm. The animals were made available in the Central animal house, GSVM Medical College, Kanpur. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light /dark cycle, at room temperature 25± 3º C and 35-60% humidity), fed in standard pellet diet and water *ad libitum*.

The study was approved by the Institutional Animal Ethics Committee, GSVM Medical College, and Kanpur. The animal care and handling was done as per the guidelines set by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Induction of Experimental Diabetes: Diabetes was induced in albino rats by a single intra-peritoneal injection of aqueous alloxan monohydrate (135 mg/kg body weight) (Sigma Chemical Co. USA) [13]. Blood samples were collected before and after the administration of alloxan to know the status of diabetes. After two days, diabetes was confirmed by testing blood glucose level using glucometer and they were further maintained for four days for well establishment of diabetes. The animals with blood glucose level more than 200 mg/dl (moderate diabetes) were selected for the experiment.

Drugs and chemical agents: Alloxan- alloxan monohydrate (dose-135 mg/kg body weight/rat), ACE inhibitor- Ramipril (dose of 0.9 mg /kg body weight /day /rat), Oral anti-diabetics drugs- (a) Biguanides- Metformin (225 mg /Kg body weight /day /rat) (b) Peroxisome Proliferator-Activated Receptor-gamma (PPAR-γ) agonists-Pioglitazone (4 mg /kg body weight/day /rat) (c) Second generation Sulfonylureas- Gliclazide (22 mg/ Kg body weight/day /rat).

Experimental Design: Rats were divided into 10 groups (n = 6).

The Group I-II were normal and Group III-X was diabetic.

Group I (NC) received 1 ml sterile water and served as normal control.

Rats of Group II (N+R) received Ramipril only.

Group III (DC) received 1 ml sterile water and served as diabetic control.

Group IV (D+R) were treated with Ramipril only, Group V (D+M) with Metformin only, Group VI (D+G) with Gliclazide only and Group VII (D+P) with Pioglitazone only.

The oral anti-diabetic drugs treated groups, Group V, VI and VII served as positive control for the rest of the groups Group VIII, IX and X respectively. Group VIII (D+M+R) received both Ramipril and Metformin, Group IX (D+G+R) received both Ramipril and Gliclazide and Group X (D+P+R) received both Ramipril and Pioglitazone.

Rats of all groups received the doses orally for 13 consecutive days and at the end of the experimentation an Oral Glucose Tolerance Test (OGTT) [14] was conducted and blood glucose estimation was done in all groups.

Oral Glucose Tolerance Test: After an overnight fasting, 0 min blood samples (0.2 ml) were taken from the all groups by orbital sinus puncture[15]. 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.

Blood Glucose Estimation: Blood samples (0.2 ml) were collected through orbital sinus puncture with capillary tube and then centrifuged. The clear supernatant serum was taken for estimation of blood glucose level. The plasma blood glucose levels were determined by using GOD–POD method [16].

Span diagnostic reagent kit (Code no. B 0112) was used for estimation of blood glucose level.

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

RESULTS:

Effect on Normal Rats: Effect of Ramipril on blood glucose levels in normal rats presented in **Table 1**. It is observed that Ramipril in a dose of 0.9 mg/ kg body weight slightly reduced the blood glucose level at all-time points but it is significant only at '30' min time point ($P\leq0.05$). It means, it has insignificant hypoglycemic activity in normal rats.

TABLE 1: EFFECT OF RAMIPRIL ON BLOOD GLUCOSE LEVEL IN NORMAL RATS

S. No.	Groups	Serum glucose level (mg/dl)			
		0 min(Fasting)	30 min	60 min	120 min
I	I (NC)	78.33±3.19	100.66±2.71	91.50±1.66	85.66±2.98
2	II (N+R)	71.33±2.56 (8.93%)	94.00±1.23 [a] (6.61%)	88.33±1.11 (3.46%)	79.50±2.12 (7.19%)

Values are expressed as Mean ± S.E (% reduction); (n=6), Significance levels as compared to control ([a] $P\leq0.05$ [b] $P\leq0.01$ [c] $P\leq0.001$)

Effect on Alloxan induced Diabetic Rats: The effect of 13-day administration of Ramipril and Oral anti-diabetic drugs on blood glucose levels in alloxan induced diabetic rats is shown in Table 02. It is noteworthy to mention that Ramipril has showed significant anti hyperglycemic effect in diabetic rats.

The percentage of blood glucose reduction was much more at 120 min i.e. 30.45% as compare to rest of the studied hrs which were found to be 8.65% at 0 min, 7.21% at 30 min and 8.96% at 60 min. In statistical point of view, it was significantly decreased ($P < 0.05$) at 0 and 30 min .The higher level of significance was found at 60 min ($P < 0.01$) with more pronounced effect at 120 min ($P < 0.001$).

As per **Table 2**, Gliclazide, Pioglitazone and Metformin have significant hypoglycemic activity at all-time points in alloxan induced diabetic rats. The Interaction of Ramipril with the Oral hypoglycemic drugs in alloxan induced diabetic rats outlined in **table 3**. It revealed that blood glucose level is decreased with the drug Ramipril 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), Ramipril exhibited highest hypo-glycemic activity at '120' min time point with reduction of 40.33% whereas in group VI (D+G) & group IX (D+G+R) and group VII (D+P) & group X (D+P+R), there were highest impact on 'O' min (fasting) with reduction of 37.35% and 35.12% respectively.

TABLE 2: EFFECT OF RAMIPRIL AND ORAL ANTI DIABETIC DRUGS ON BLOOD GLUCOSE LEVEL IN ALLOXAN-INDUCED DIABETIC RATS

S.N.	Groups	Serum glucose level (mg/dl)			
		0 min(Fasting)	30 min	60 min	120 min
1	III (DC)	254.16±6.12	286.16±5.33	345.83±8.10	350.83±6.71
2	IV (D+R)	232.16±4.28 [a] (8.65%)	265.50±6.08 [a] (7.21%)	314.83±3.88 [b] (8.96%)	244.00±3.07 [c] (30.45%)
3	V (D+M)	224.00±3.69 [b] (11.86%)	254.83±8.85 [a] (10.94%)	288.83±6.30 [c] (16.48%)	228.50±5.28 [c] (34.86%)
4	VI (D+G)	226.66±5.03 [b] (10.81%)	256.83±9.11 [a] (10.24%)	290.16±0.83 [c] (16.09%)	229.50±6.09 [c] (34.58%)
5	VII (D+P)	226.83±5.38 [b] (10.75%)	256.56±8.63 [a] (10.34%)	293.00±2.39 [c] (15.27%)	230.33±5.25 [c] (34.34%)

Values are expressed as Mean ±□ S.E (% reduction); (n=6), Significance levels as compared to control ([a] P ≤0.05, [b] P≤0.01, [c] P≤0.001)

TABLE 3: INTERACTION OF RAMIPRIL WITH THE ORAL ANTI DIABETIC DRUGS IN ALLOXAN INDUCED DIABETIC RATS

S. No.	Groups		Serum glucose level (mg/dl)		
		0 min(Fasting)	30 min	60 min	120 min
I	V (D+M)	224.00±3.69	254.83±8.85	288.83±6.30	228.50±5.28
2	VIII (D+M+R)	136.00±4.61 [c] (39.28%)	170.83±3.70 [c] (32.96%)	208.50±5.45 [c] (27.81%)	136.33±4.80 [c] (40.33%)
3	VI (D+G)	226.66±5.03	256.83±9.11	290.16±0.83	229.50±6.09
4	IX (D+G+R)	142.00±2.47 [c] (37.35%)	180.16±4.71 [c] (29.85%)	220.16±4.02 [c] (24.12%)	161.16±6.49 [c] (29.77%)
5	VII (D+P)	226.83±5.38	256.56±8.63	293.00±2.39	230.33±5.25
6	X (D+P+R)	147.16±5.55 [c] (35.12%)	185.83±6.79 [c] (27.56%)	210.16±6.74 [c] (28.27%)	155.83±4.35 [c] (32.34%)

Values are expressed as Mean ±□ S.E (% reduction); (n=6), Significance levels as compared to control ([a] P ≤0.05, [b] P≤0.01 [c] P≤0.001)

DISCUSSION: Our study has evaluated the significant interaction of Ramipril with Oral anti-diabetic drugs in alloxan induced diabetic rats. Alloxan is a well- known diabetogenic agent widely used to chemically induce Type 2 diabetes in experimental animals [17]. Diabetogenicity and pancreatic β cell destruction is due to the redox cycling and the generation of toxic reactive oxygen species (ROS), alloxan's hydrophilicity and its molecular shape which is similar to glucose [18].

In light of results, the study indicates that the Ramipril 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 where it enhanced the hypoglycemic activity of all three Oral anti-diabetic drugs (Metformin, Gliclazide and Pioglitazone).

Metformin shows anti-hyperglycemic effect primarily by suppressing hepatic gluconeogenesis [19]. The "average" person with type 2 DM has 3 times the normal rate of gluconeogenesis; metformin reduces this by over one third [20]. It activates AMP-activated protein kinase (AMPK), an enzyme that plays an important role in insulin signal pathway, metabolism of glucose and fats and energy balance [21]. Gliclazide is a second generation sulfonylurea and it acts on β cells of pancreas and stimulate insulin secretion by reducing the permeability of potassium ion. In addition, it increases the sensitivity of peripheral tissue to insulin.

Pioglitazone increases insulin sensitivity and decreases insulin resistance in Type-2 DM by activation of PPAR-γ (Peroxisome-proliferator-activated Receptor Gamma) nuclear receptor [22].

The present findings suggest that Ramipril might have insulin sensitivity properties in type 2 DM. Moreover, it enhanced the anti-hyperglycemic activities of Metformin, Glicalzide and pioglitazone, probably by decreasing insulin resistance in peripheral tissue. The results are strengthened by previous data which exhibited the potential for ACE inhibitor-associated hypo-glycemia but most of them are with the Captopril [23-24]. As far as the mechanism of ACE inhibitor-induced hypoglycaemia is concerned it is proposed that the increase in bradykinins may have some role to increase insulin sensitivity [25-26]. Opposite to this, Wiggam *et al* has postulated that ACE inhibitors have no impact on hepatic or peripheral insulin sensitivity [27].

Although the present findings confirm the anti-hyperglycemic potential of Ramipril but the specific mechanism of action requires further studies for appropriate elucidation.

CONCLUSION: The present study shows that Ramipril lower blood sugar level in healthy and alloxan induced diabetic rats. Also, these drugs augment the blood glucose lowering effect of Metformin, Gliclazide and Pioglitazone. It is therefore need the use of Ramipril with anti-diabetic drugs carefully with modification of dose schedule to avoid adverse hypoglycemic episodes in diabetic individuals. Further studies are necessary to substantiate the above observation and to work out the exact mechanism of action involved in the anti-hyperglycemic activity of Ramipril.

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