Anti-Diabetic Activity of Aliskiren, Spironolactone and Their Combination on STZ Induced Diabetes in Female Rats: In Vivo and In Vitro Study

Amal M. Mahfoz1*, Hekma A. Abd El-Latif2, Afaf A. Shoka2

1Department of pharmacology, National Organization for Drug Control and Research (NODCAR), Giza 35521, Egypt.
2Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

*Corresponding Author: Amal Mohamed Mahfoz, Department of pharmacology, National Organization for Drug Control and Research (NODCAR), Giza 35521, Egypt

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ABSTRACT

The present study aimed to investigate in vivo, in vitro antidiabetic activity of aliskiren, spironolactone, and their combination and characterize their possible mechanisms of action. Type 2 diabetes was induced in rats by streptozotocin (STZ)-nicotinamide (65mg/kg–110mg/kg; i.p.) administration. Diabetic rats were divided into 6 groups; normal, diabetic, diabetic treated with gliclazide (10mg/kg/day), diabetic treated with aliskiren (50 mg/kg/day) diabetic rats treated with spironolactone (20 mg/kg/day) and diabetic rats treated with combination of aliskiren (25 mg/kg/day) plus spironolactone (10 mg/kg/day). After one month treatment, rats were euthanized, serum was separated for determination of glucose and insulin. Besides, Islets of Langerhans were isolated from normal rats by collagenase digestion technique for in vitro study. As compared to gliclazide aliskiren, spironolactone, and their combination resulted in normalized hyperglycemia, increased insulin sensitivity and increased insulin level both in-vivo and in-vitro. However, the effects of each drug alone were better than their combination. Aliskiren and spironolactone have potential antidiabetic activity in type 2 diabetes. They can be used for treatment of hypertension in diabetic patients, with decreasing the dose of antidiabetic drugs. However, their combination does not give higher antidiabetic effect.

Keyword: Aliskiren; spironolactone; gliclazide; STZ; islets
INTRODUCTION

Diabetes is characterized by hyperglycemia, altered lipids, carbohydrates, and proteins metabolism which affect the patient quality of life in terms of social, psychological wellbeing as well as physical ill health [1, 2]. Two forms of diabetes (Types 1 and 2) differ in their pathogenesis; however both have hyperglycemia as a common hallmark. In type 2 diabetes, hyperglycemia caused due to impairment in insulin secretion combined with or without impairment of insulin action [3]. The World Health Organization reported that worldwide global population is in the midst of a diabetes epidemic. The people in Southeast Asia and Western Pacific are being under greater risk, and the majority of patients have type 2 diabetes. Insulin resistance typically precedes the onset of type 2 diabetes and is commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, and prothrombotic factors [4]. Aliskiren is a direct renin inhibitor that approved for lowering arterial blood pressure [5, 6]. Increasing evidence shows that aliskiren has an antiproteinuric effect in patients with diabetes also exerts renoprotective, cardioprotective, and anti-atherosclerotic effects in animal models independent of its blood pressure lowering activity [7–9]. But the data on the mechanisms of aliskiren effects on the progress of diabetes or its antidiabetic effect are still lacking.

Spironolactone is a synthetic steroidal antimineralocorticoid and antiandrogen. It belongs to potassium-sparing diuretics, and is used primarily as a diuretic and antihypertensive in the treatment of heart failure and hypertension [10]. Recent trials have suggested that adding an inhibitor of the aldosterone system as SPR to an angiotensin-converting enzyme inhibitor-based regime in patients with DN may further reduce proteinuria and thereby provide additional renal protection in type 1 and 2 diabetic rats [11, 12]. So this study aimed to evaluate the antidiabetic effects of aliskiren, spironolactone or their combination on STZ-induced type 2 diabetes in rats.

MATERIALS AND METHODS

Animals

One hundred female Wistar rats weighing 180–200 g were obtained from National Organization for Drug Control and Research animal house (Giza, Egypt). Rats were housed under controlled temperature (25 ± 2°C) and constant light cycle (12 h light/dark) and allowed free access to a standard rodent chow diet and water ad libitum. Animal use and handling were done under protocols approved by National Institute of Health (NIH).

Drugs and Chemicals

STZ, Gliclazide, SPR and all study chemicals were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Aliskiren was provided as 150 mg tablets from Novartis Company. For in vivo experiments, gliclazide and SPR were suspended in 2% tween 80 and administered i.p. in a dose of 10 mg/kg/day and 20 mg/kg/day respectively for 1 month. Aliskiren tablets were crushed, dissolved in normal saline, filtered and administered in a dose of 50 mg/kg/d i.p. for 1 month.

In Vivo Study

Experimental Type 2 Diabetes Induction

Type 2 diabetes was induced by injection of freshly prepared streptozotocin (STZ 65mg/kg; i.p.) in cold citrate buffer (0.1M, pH 4.5), 15 minutes after the administration of nicotinamide (NIC—110mg/kg; i.p.) in overnight-fasted rats [2]. Diabetes induction was assured after 72 h by measurement of blood glucose levels by glucose meter.
Glucocard 01-mini, Arkray Factory Inc., Japan). To stabilize the blood glucose level, diabetic rats were kept under standard laboratory condition up to 14 days. Blood glucose was again determined on day 14, and diabetic rats showing blood glucose $>200\text{mg/dL}$ were selected to assess the antidiabetic activity of aliskiren, spironolactone and their combination.

**In vivo Study Design**

Two weeks after STZ injection diabetic rats were divided into 4 groups; 1st normal control group, 2-4th groups, STZ-diabetic rats. The 2nd group served as a diabetic control group and was treated with normal saline, the 3rd group treated with gliclazide (10 mg/kg i.p.), and the 4th group treated with SPR (20 mg/kg i.p.). Drugs and saline treatments were done daily for 1 month. At the end of the experiment, animals were euthanized 1 h after the last drugs dose.

**Biochemical Analysis**

i. **Fasting serum glucose level:**

Fasting serum glucose level was determined at the end of the experiment just after euthanizing animals. It was determined colorimetric at 546 nm according to the method of Trinder, 1969 [13] using a commercial reagent kit (Biodiagnostic, Germany) and was expressed as mg/dl.

ii. **Insulin immunoassay**

Fasting rats serum insulin level and insulin concentration in supernatant of isolated islets were determined using a commercial ELISA kit (Li KaShing Faculty of Medicine, The University of Hong Kong, AIS) and were expressed as µU/ml serum and µU/hr/islet respectively. Serum samples were collected after euthanizing animals and kept frozen at – 20 till insulin measurements.

**In vitro study:**

**Isolation of rat pancreatic islet:**

Pancreatic islets were isolated following collagenase digestion technique according to the method of lacy & kostianovsky, (1967) [14].

**Procedure:**

Adult female rats weighing 250 – 300 g were used. Islets were isolated from non-fasting rats since fasting would diminish the responsiveness of the islets to stimulation of insulin secretion in-vitro. Rats were pretreated with intraperitoneal injection of pilocarpine nitrate (20mg/kg) 2-3 hours prior to islets isolation. Pilocarpine allows the depletion of zymogens from the exocrine pancreatic tissues and thus minimizes the destruction of islets membrane structure which could occur during collagenase digestion of the tissue.

**Isolation of pancreas:**

Rats were anaesthized with ether until death then the skin and the underlying muscular layer of the abdomen were removed. The duodenum was clamped near the stomach. The common bile duct was clamped adjacent to the liver and then the acinar tissue was disrupted by injecting 10 ml of Krebs -Ringer - HEPES (KRH) solution into the pancreatic duct via the common bile duct. The pancreas was separated from the small intestine, stomach, spleen, liver and placed in Petri dish containing KRH solution and washed twice with fresh KRH solution.

**Isolation of the islets:**

Pancreas was chopped into small nearly equal pieces with a sharp scissor. The chopped pancreas was aspirated from the bottom of the petri dish using needle less syringe and transferred to another Petri dish containing fresh (KRH) solution. This step was repeated several times until complete removal of any remaining fatty tissues. The chopped pancreas was transferred with 10 ml of (KRH) solution to a small flask containing collagenase (30 mg/pancreas), mixed well, covered and placed
in a shaking water bath (140 strokes/min) at 37°C for 18-20 minutes for complete digestion. The collagenase concentration and the incubation time for digestion are dependent on the activity of the enzyme batch preparation and should be determined for each batch. The tissue was considered completely digested if the digest appeared as a light brown coloration with no clumps and it is considered over digested if it appeared as a very fine powdery suspension. After complete digestion, the flask was removed from the water bath and the enzymatic activity was immediately stopped by the addition of 10 ml of ice-cold KRH solution. The digested pancreatic pieces were well mixed and centrifugated gently for two minutes using manual centrifuge. The supernatant was discarded and fresh buffer was added. This process was repeated three times till clear supernatant was obtained. The remaining sediment was diluted with KRH mixture thoroughly, transferred in portions to a black bottom Petri dish and examined with stereomicroscope. The free islets were picked up into fresh medium by a micropipette.

Preincubation of islets:
The islets were transferred into Wassermann tube containing fresh KRH solution and incubated at 37°C for 30 minutes using shaking water bath in order to adapt the islets to the in vitro system before determining the effect of different agents on insulin secretion.

Incubation of islets:
After the preincubation period the contents of the tube were poured in a Petri dish with a black bottom and diluted with fresh KRH solution. Batches of 5 islets were picked up and transferred into small tubes each containing 1 ml KRH buffer supplemented with 0.5% bovine serum albumin and glucose 3 mmol/l (basal concentration) and the test agent under study was added. The tubes were covered and incubated at 37°C in shaking water bath for 1 hour with intermittent hand shaking every 15 minutes. At the end of incubation period the tubes were transferred into ice bath and mixed with vortex mixer and then aliquots of 0.5 ml were taken and kept frozen at -20 °C for insulin determination [14].

Physiological solution:
The basic physiological solution Krebs-Ringer-HEPES (KRH) was supplemented with 0.5% bovine serum albumin and 0.54 mg/ml glucose (3 mmol/l) or 3 mg/ml glucose (16.7 mmol/l).

Preparation of (KRH) buffer solution:
The buffer has the following constituents (mmol/l)
NaCl ..................... 118.4
KCl ........................ 4.8
CaCl₂ ..................... 2.51
MgSO₄ .................... 1.2
KH₂PO₄ ................... 1.2
HEPES ................... 20

For its preparation, three main stock solutions were prepared.

Stock solution A:
Stock solution A was prepared as follows:
NaCl ..................... 69.2 g
KCl ........................ 3.58 g
MgSO₄ .................... 1.44 g
KH₂PO₄ ................... 1.63 g
Bidistilled water ad ........1000 ml

Stock solution B:
Stock solution B was prepared as follows:
HEPES...................... 47.66 g
Bidistilled water ad........ 1000 ml

Stock solution C:
Stock solution C was prepared as follows:
CaCl₂ ..................... 2.77 g
Bidistilled water ad ........ 1000 ml
The stock solutions were kept at 4°C until mixed and diluted for KRH solution.
Preparation of the working solution:
The following parts of the three stock solutions were mixed as follows just before use:
Stock solution A …………………………..One part.
Stock solution B …………………………...One part.
Stock solution C……………………………One part.
Bidistilled water …………………………...Seven parts.
The formed buffer was supplemented with 0.5% bovine serum albumin and glucose 3 or 16.7 mmol/l.

Preparation of drug solutions:
i. Gliclazide: A stock solution in a concentration of 1 mmol/l was prepared by dissolving 0.16 g gliclazide in 10 ml saline. A volume of 20 or 40 µl of the stock solution was added to specific volume of the incubation medium and completed to 1 ml to get the specified final concentrations.

ii. Aliskiren: A stock solution in a concentration of 100 nM was prepared by dissolving 150mg aliskiren in 54.5 ml saline. A volume of 20 or 40 µl of the stock solution was added to specific volume of the incubation medium and completed to 1 ml to get the specified final concentrations.

iii. Spironolactone: A stock solution in a concentration of 5µg/ml was prepared by dissolving 25 mg of spironolactone in 100 ml saline. A volume of 20 or 40 µl of the stock solution was added to specific volume of the incubation medium and completed to 1 ml to get the specified final concentrations.

In vitro study design
Isolated β cells distributed as 5 per tube, then divided into 12 groups (each one 6 tubes); normal control, 20 µl gliclazide (40 µmol/L), 20µl aliskiren, 20µl aliskiren plus 20µl gliclazide, 20 µl SPR (5 µg/ml), 20 µl gliclazide + 20 µl SPR and the same groups using higher drugs volume (40 µl). All drugs were incubated with isolated islets for 1 h then the supernatants were separated and kept frozen for measurement of insulin concentration. Number of experiments pre-specified as 6 per group before start of the experimental series.

Statistical analysis:
Values throughout the text are expressed as mean ± standard error [SEM] using a computer software program Statistical Package for the Social Sciences “SPSS” (Version 16.0.). For multiple comparison (i.e. more than one mean), the “One-Way Analysis of Variance” (ANOVA or F test) followed by Tukey-Kramer multiple comparison post hoc test was used for comparing the means of the different groups [15]. The level of significance was fixed at P=0.05. If the P ≥ 0.05 this indicates non-significant difference while if P < 0.05 this indicates significant difference.

RESULTS
Results of effect of one month treatment with gliclazide, aliskiren, spironolactone and aliskiren-spironolactone combination on serum glucose, insulin and insulin sensitivity in STZ-induced diabetic nephropathy in female rats are presented in Table 1.

Table 1: Effect of one month treatment with gliclazide, aliskiren, spironolactone and aliskiren-spironolactone combination on serum glucose, insulin and insulin sensitivity in STZ-induced diabetic nephropathy in female rats.
Each value represents mean of 8 rats ± SEM. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparisons test.

a Significantly different from Normal control at p < 0.05.
b Significantly different from Diabetic control at p < 0.05.
c Significantly different from gliclazide treated group at p < 0.05.

### Serum glucose
The mean value of normal serum glucose level was 120±0.5. Induction of type 2 diabetes resulted in significant increase in serum glucose level to about 400±2.2. Treatment with gliclazide (10 mg/kg) daily for four weeks significantly decreased serum glucose to about 36.7% compared to diabetic control. Treatment with aliskiren (50 mg/kg) or SPR (20 mg/kg) for four weeks after induction of diabetes significantly decreased serum glucose to about 30.8% and 41.2% respectively as compared to diabetic control rats. Aliskiren only significantly decreased serum glucose as compared to gliclazide treated group. The combination of aliskiren (25mg/kg) plus SPR (10mg/kg) resulted in a significant decrease in serum glucose to about 67.9% as compared to diabetic control rats. However combination resulted in significant increase in serum glucose as compared to gliclazide treated group. That is the effect of each drug alone is better than combination.

### Serum insulin
The mean value of serum insulin concentration of normal control was 61.17±0.79. Induction of diabetes resulted in significant decrease in serum insulin concentration to about 23.5±0.43. Treatment with gliclazide (10 mg/kg) daily for four weeks after induction of diabetes significantly increased serum insulin concentration to about 256% compared to diabetic control. Treatment with aliskiren (50 mg/kg) or SPR (20 mg/kg) daily for four weeks significantly increased serum insulin concentration to about 256% compared to diabetic control. Treatment with aliskiren (50 mg/kg) or SPR (20 mg/kg) daily for four weeks significantly increased serum insulin concentration to about 41.2% respectively compared to diabetic control rats,
also significantly decreased serum insulin as compared to gliclazide treated group. Combination of aliskiren (25mg/kg) and SPR (10mg/kg) resulted in significant increase in serum insulin concentration to about 258 % as compared to diabetic control rats. However the effect of combination was less than each drug alone.

**Insulin sensitivity (insulin / glucose) (pmol / mmol)**
The mean value of insulin sensitivity of normal control was 64±1. Induction of diabetes resulted in significant decrease in insulin sensitivity to about 7.3±1. Treatment with gliclazide (10 mg/kg) daily for four weeks after induction of diabetes significantly increase insulin sensitivity to about 705% compared to diabetic control. Treatment with aliskiren (50 mg/kg) or SPR (20 mg/kg) daily for four weeks after induction of diabetes significantly increased insulin sensitivity to about 2102% and 774% respectively as compared to diabetic control rats. Aliskiren only significantly increased insulin sensitivity as compared to gliclazide treated group. Combination of aliskiren (25mg/kg) plus SPR (10mg/kg) resulted in significant increase in insulin sensitivity to about 379% as compared to diabetic control rats. However the effect of combination was less than each drug alone.

**Effect of 20µl of gliclazide, aliskiren, aliskiren + gliclazide, spironolactone or spironolactone + gliclazide on insulin secretion from isolated pancreatic islets of normal female rats.**

Results are illustrated in figure (1). The mean value of insulin secreted from isolated islets incubated in 3mM glucose was 37.5±1.2. Incubation of islets for 1 hour with 20 µl gliclazide (1 mmol/l) significantly increased insulin secretion to about 253% compared to normal control. Incubation of islets for 1 hour with 20 µl aliskiren (100 nM) significantly increased insulin secretion to about 206% compared to normal control. Its combination with gliclazide significantly increased insulin secretion to about 345% compared to normal control. Incubation of islets for 1 hour with 20 µl SPR (5µg/ml) did not significantly change insulin secretion while its combination with gliclazide significantly increased insulin secretion to about 209% compared to normal control.
Fig. 1: Effect of 20µl of gliclazide, aliskiren, aliskiren + gliclazide, spironolactone or spironolactone + gliclazide on insulin secretion from isolated pancreatic islets of normal female rats

Each value represents means ± SEM.
Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparisons test.

\textit{a} Significantly different from Normal control at $p < 0.05$.

\textit{b} Significantly different from Gliclazide at $p < 0.05$.

Effect of 40µl of gliclazide, aliskiren, aliskiren + gliclazide, spironolactone or spironolactone + gliclazide on insulin secretion from isolated pancreatic islets of normal female rats.

Results are illustrated in figure 2. The mean value of insulin secreted from isolated islets incubated in normal medium (normal control) was 37.5±1.2. Incubation of islets for 1 hour with 40 µl gliclazide (1 mmol/l) significantly increased insulin secretion to about 488% compared to normal control. Incubation of islets for 1 hour with 40 µl aliskiren (100 nM) or 40 µl spironolactone (5 µg/ml) significantly increased insulin secretion to about 310% and 245% respectively compared to normal control. Combination of aliskiren or spironolactone with gliclazide significantly increased insulin secretion to about 919% and 852.6% respectively as compared to normal control.
Fig. 2: Effect of 40µl of gliclazide, aliskiren, aliskiren + gliclazide, spironolactone or spironolactone + gliclazide on insulin secretion from isolated pancreatic islets of normal female rats. Each value represents means ± SEM.

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparisons test.

\( ^a \) Significantly different from Normal control at p < 0.05.

\( ^b \) Significantly different from Gliclazide at p < 0.05.

**DISCUSSION**

Screening of antidiabetic activity of various drugs is performed in experimental animal models after induction of diabetes by several methods. To induce non-insulin-dependent type 2 diabetes in animals, streptozotocin-nicotinamide is commonly used which produces moderate hyperglycemia with clinical symptoms similar to type 2 diabetes [16]. STZ causes alkylation of pancreatic deoxyribonucleic acid by entering to β-cell through glucose transporter 2 and induces activation of poly (ADP ribosylation), that causes depletion of cellular nicotinamide adenine dinucleotide (NAD+) and adenosine triphosphate. As a result, generation of free radicals causes pancreatic β-cells necrosis [17]. Nicotinamide administration along with STZ can act as a weak polyn ADP ribosylation, and precursor for the coenzyme NAD+ which is necessary for the cellular function and metabolism. Thus, nicotinamide prevents pancreatic damage caused by STZ mediated cytotoxicity and produces a diabetic condition in rats similar to human type 2 diabetes [18]. In the present study, induction of type 2 diabetes resulted in significant increase in blood glucose level and decrease in insulin level and insulin sensitivity in vivo and in vitro compared to control rats which confirm the induction of diabetes, and it may be due to partial necrosis of pancreatic β-cell by STZ. In type 2 diabetes, peripheral insulin resistance and impaired insulin secretion from pancreatic β-cells are two important mechanisms. Insulin resistance in peripheral tissues as liver, skeletal muscle, and adipose tissue is commonly
The occurrence of cardiovascular diseases in type 2 diabetic patients mainly due to insulin resistance mediated hyperglycemia and dyslipidemia. Drug which diminishes insulin resistance will effectively control hyperglycemia, normalize lipid metabolism in type 2 diabetes, and hence it will prevent the diabetes-mediated cardiovascular complications [19]. Antidiabetic drugs like metformin and pioglitazone will ameliorate insulin resistance and control the hyperglycemia and abnormal lipid metabolism. This class of drugs has adverse effects such as lactic acidosis, gastrointestinal disturbance, liver toxicity, and cardiovascular risk [20]. Thus, decreasing the dose of antidiabetic drugs will be useful for the long-term treatment in type 2 diabetes.

TREATMENT WITH GLICLAZIDE (10 mg/kg) FOR FOUR WEEKS NORMALIZED HYPERGLYCEMIA AND INCREASED SERUM INSULIN CONCENTRATION AND INSULIN SENSITIVITY AS COMPARED TO DIABETIC CONTROL RATS AND THESE EFFECTS EMPHASIZED BY THE IN VITRO MEASUREMENTS OF INSULIN SECRETION FROM ISOLATED β CELLS OF PANCREAS IN NORMAL RATS. AS A SULFONYLUREA COMPOUND, Gliclazide lower blood glucose in normal and in diabetic animals by stimulating insulin release from pancreatic β cells and by peripheral utilization of glucose [21].

TREATMENT WITH ALISKIREN (50 mg/kg) DAILY FOR FOUR WEEKS NEARLY NORMALIZED HYPERGLYCEMIA AND INCREASED SERUM INSULIN CONCENTRATION AND INSULIN SENSITIVITY AS COMPARED TO DIABETIC CONTROL RATS. THESE EFFECTS WERE SUPPORTED BY THE IN VITRO STUDY WHERE ALISKIREN STIMULATED INSULIN SECRETION FROM β CELLS OF NORMAL RATS WITH 20 µL OF 100 nM AND INSULIN RELEASE INCREASE MORE WITH THE 40µL DOSE LEVEL, also aliskiren significantly synergized gliclazide induced insulin secretion in this IN-VITRO STUDY. THE RESULTS OF THE PRESENT STUDY ARE CONFIRMED BY GANDHI ET AL., (2013) [22] WHO FOUND THAT ALISKIREN-TREATED DIABETIC RATS SHOWED IMPROVED LIVER AND MUSCLE GLUTOTRANSPORTER EXPRESSION LEVELS QUALITATIVELY. THESE RESULTS QUALIFY THE IMPROVED INSULIN SENSITIVITY BY THE ANTI-RENIN ALISKIREN. THIS was EXPLAINED BY A PREVIOUS STUDY WHICH DEMONSTRATED THAT RENIN INHIBITION ATTENUATES INSULIN RESISTANCE AND IMPROVES SYSTEMIC INSULIN SENSITIVITY IN TRANSGENIC REN2 RATS THAT OVER-EXPRESS RENIN [23]. Thus, a possible link between renin activation and insulin resistance was suggested.

Another study of Kang et al., (2010) [24] found an improvement in insulin resistance by aliskiren treatment. They explained this effect by the significant decrease in plasma levels of the homeostasis model assessment index (HOMA-IR), lipid abnormalities, and insulin sensitivity confirmed by insulin tolerance test with aliskiren treatment.

However, the observed antidiabetic effect of aliskiren in the present study is different from that obtained by Zhou et al., 2015 who found that aliskiren not decrease blood glucose level in uninephrectomized mice [25].

TREATMENT WITH SPIRONOLACTONE (SPR) (20 mg/kg) DAILY FOR FOUR WEEKS NEARLY NORMALIZED HYPERGLYCEMIA AND INCREASED SERUM INSULIN CONCENTRATION AND INSULIN SENSITIVITY AS COMPARED TO DIABETIC CONTROL RATS. THESE EFFECTS ARE ALSO SUPPORTED BY THE IN-VITRO STUDY OF EFFECT OF SPR ON INSULIN SECRETION FROM ISOLATED β-CELLS OF PANCREATIC ISLETS OF NORMAL RATS. THERE was STIMULATION OF INSULIN SECRETION FROM ISOLATED β-CELLS IN PRESENCE OF 20 µL OF SPR (5µg/ml) AS COMPARED TO NORMAL CONTROL AND THIS EFFECT INCREASED WITH THE 40µL DOSE LEVEL.

Also SPR SYNERGIZED GLICLAZIDE-STIMULATED INSULIN SECRETION IN THIS IN-VITRO STUDY. THE ANTIDIABETIC EFFECT OF SPR WAS PARTIALLY DUE TO PROMOTED GLUT4 TRANLOCATION IN RAT MUSCLE CELLS. SINCE ALDOSTERONE IMPAIRS INSULIN SIGNALING, it is CONCEivable THAT SPR MIGHT BE EFFECTIVE LOWER OF SERUM GLUCOSE THROUGH INHIBITING ALDOSTERONE INDUCED INSULIN RESISTANCE [26, 27].

Another study found that treatment with SPR effectively ameliorated impaired glucose and...
lipid metabolism, reduced epididymal fat weight, and improved histological changes of fatty liver in mice fed a 60% high-fat diet with 30% fructose water. Thus, an MR antagonist might be a beneficial novel option for the treatment of patients with diet-induced metabolic syndrome and/or Nonalcoholic fatty liver disease, in addition to its wide usage as an antihypertensive agent and diuretic [28]. This also confirms the results of our study. The effect of increased insulin sensitivity by SPR can further be explained by its effect to ameliorate the elevation of circulating levels of triglyceride, inhibiting proinflammatory cytokine production and decreases adiponectin expression in 3T3-L1 cultured preadipocyte cells as observed previously [28, 29].

CONCLUSION
Aliskiren and spironolactone have potential antidiabetic activity in diabetic rats. In vitro study results scientifically supported the in vivo antidiabetic activity. They can be used for treatment of hypertension in diabetic patients, with decreasing the dose of antidiabetic drugs. However, their combination does not give higher antidiabetic effect.

CONTRIBUTION STATEMENT
HA and AM conceived and designed the study and conducted the systematic review. AM conducted the statistical analysis and drafted the paper. AM and LA contributed to interpreting the findings and revised the paper. NH, AS and HA are involved in critical revision of the paper. All authors edited and approved the final version of the manuscript to be published. AM had full access to data in the study and had final responsibility for the decision to submit for publication.

CONFLICT OF INTEREST STATEMENT
The authors declare that they have no conflict of interests.

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