



Research Article

Myocardial Depression and Inhibition of Positive Inotropic Effect of Digoxin by Rosiglitazone

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ABSTRACT

This study was taken to investigate the effect of rosiglitazone (RGN) on the contractility of the isolated rat atrium as well as its possible inhibitory effect on positive inotropic effect of digoxin. RGN significantly increased the dose of digoxin required to produce cardiac arrest in anesthetized rats. RGN (10, 20, 40, 60, 80 & 100 μ M) produced concentration dependant depressant effect on atrial contractility in both diabetic and non-diabetic animals with the depressant effect was more pronounced in diabetic rats than in non-diabetic ones. RGN also inhibited the positive inotropic effect of digoxin (0.1, 0.3 & 1 μ M) in isolated rat right atrium treated with 4 mg/kg orally daily for 21 days. Moreover, RGN also produced significant increase in serum K^+ and decrease in Na^+ levels in rats. These results indicate that RGN has a negative inotropic effect on the heart especially in diabetics and it inhibits the positive inotropic effect of digoxin.

Keywords: rosiglitazone, digoxin, right atrium, inotropic effect, diabetes mellitus

1. Introduction

Thiazolidinediones are class of compounds for treatment of type 2 diabetes mellitus. The efficacy of these drugs in decreasing plasma glucose level is well established. Rosiglitazone (RGN) a member of this class became available for clinical use in the United States in 1999 (Parulkar *et al.*, 2001). RGN requires insulin to be present for its action. RGN exerts its principal effects by increasing insulin sensitivity in peripheral tissue and also may lower glucose production by the liver. PPAR- γ agonist increase glucose transport into muscle and adipose tissue by enhancing the synthesis and translocation of specific forms of the glucose transporters. PPAR- γ agonist also can activate genes that regulate fatty acid metabolism in peripheral tissue (Havel, 2004; Davis, 2006).

There has been increasing discussion about whether thiazolidinediones affect cardiac function, with evidence suggesting both negative and positive effects on myocardial performance. Consequently, the practicing cardiologist is often faced with a difficult

decision about whether to initiate therapy with RGN because a large proportion of patients with diabetes often have coexisting ischemic and non-ischemic cardiomyopathy (Diep *et al.*, 2002; Wang *et al.*, 2003).

The precise mechanism for the risk of heart failure associated with RGN is not known till now. It is not known that whether the incidence of heart failure associated with RGN treatment is due to sodium retention and fluid accumulation or a direct cardiac depressant effect.

Digoxin inhibits plasma membrane Na^+ , K^+ -ATPase, leading to a decrease in the net cellular uptake of K^+ and a rise in intracellular Na^+ concentration. Na^+ , K^+ -ATPase in the heart serves as a principal molecular receptor for the digitalis compounds (Demiryurek and Demiryurek, 2005). The rise in intracellular Na^+ levels causes intracellular Ca^{2+} overload because of the reduction of Ca^{2+} efflux via the Na^+ - Ca^{2+} exchange system (Kalyoncu and Ozyavuz, 1999). The increase in releasable Ca^{2+} from the sarcoplasmic reticulum is the biological substrate through which cardiac glycosides enhance myocardial contractility. In addition, co-administration of digoxin with RGN may be needed in some cases of diabetes

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mellitus with congestive heart failure. This concomitant administration did not affect steady-state pharmacokinetics of digoxin (DiCicco *et al.*, 2000). Thus, pharmacodynamic interactions may be expected by direct or indirect effect on the heart. Therefore, this study was undertaken to study the possible cardiac effect of RGN as well as pharmacodynamic interactions between RGN and digoxin.

2. Materials and methods

2.1. Chemicals and drugs

Streptozotocin (STZ) and digoxin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). RGN was obtained from Glaxo Smith Kline Pharmaceuticals (Worthing West Sussex, UK). Thiopental sodium was obtained from Egyptian Int. Pharmaceutical Industries Co. (EIPICO, 10th Ramadan City, Egypt).

2.2. Animals

Adult male Sprague Dawley rats weighing 180-250 g were used in this study (purchased from Urology and Nephrology Center, Mansoura University, Egypt). The experimental protocol conducted in the study complies with the ethical guidelines and the principals of care, use and handling of experimental animals adopted by "The research Ethics Committee", Faculty of Pharmacy, Mansoura University, Egypt. The adopted guidelines are in accordance with "Principles of Laboratory Animals Care" (NIH publication No. 85-23, revised 1985). The study protocol was approved by members of "The Research Ethics Committee" and by the head of Pharmacology and Toxicology department, Faculty of Pharmacy, Mansoura University, Egypt.

2.3. Induction of diabetes

Hyperglycemia was induced in rats by I.P. injection of STZ (60 mg/kg) (Mori *et al.*, 2002), prepared in ice-cold 0.1 M citrate buffer. Forty eight hours after STZ administration, diabetes was detected by urinary glucosuria using urine glucose strips. One week after the onset of diabetes, blood samples were taken for determination of non-fasting (random) blood sugar. Animals with serum glucose level more than 350 mg/dl up to 520 mg/dl were used in this study (Khandoudi *et al.*, 2002). The day on which hyperglycemia had been confirmed was considered as day zero.

2.4. In-vitro effect of RGN on the contractility of isolated right atrium of non-diabetic and diabetic rats

The rats were anesthetized with ether. The heart was carefully removed and immediately placed in a Petri dish containing ice-cold oxygenated Krebs-Henseleit Solution (KHS) of the following composition (mM): NaCl 118.4, KCl 4.7, CaCl₂ 2.5, NaHCO₃ 25, Mg SO₄ 1.2, KH₂ PO₄ 1.2 and glucose 11.7 (pH = 7.4). After washing blood from the heart, the ventricular tissue, fat and connective tissue was trimmed off. The atrium tissue was removed and transferred to another dish containing cold fresh

KHS. The right atrium was then carefully dissected and suspended vertically in a 20 ml tissue bath with KHS. One end of the atrium was attached to a tissue holder and the other end was mounted by silk thread and attached to isometric transducer (Harvard Apparatus LTD, MA, USA) that was connected to a two channel oscillograph (Harvard Apparatus). The right atrium was allowed to beat spontaneously. The tissue preparations were equilibrated at 37°C for 60 min in KHS aerated with a mixture of 95% O₂ and 5% CO₂. During this period, the KHS in the tissue bath was replaced every 15 min. Resting tension of 1 g in the tissue preparation was maintained throughout the experiment. For tissues separated from non-diabetic and diabetic rats, after steady base-line recordings, a non-cumulative concentration-response curve was constructed for RGN at concentrations of (10, 20, 40, 60, 80 & 100 µM). The change in tension was measured and the % change in the contractility of the right atrium was calculated.

The effects of addition of equivalent volume of the vehicle (0.025% ethanol as a final bath concentration) on the contractility of isolated right atria of non-diabetic and diabetic rats were observed in 6 preparations for each group.

2.5. Effect of RGN incubation on digoxin-induced cardiotoxic effect on isolated right atria of non-diabetic and diabetic rats

Tissue preparations (containing 8 preparations), prepared as previously mentioned, were isolated from non-diabetic and diabetic animals. A cumulative concentrations response curve was constructed with regard to the inotropic effect of digoxin alone (0.1, 0.3 & 1 µM) after preincubation with the vehicle (0.025% ethanol) for 10 min. Another groups of tissue preparations from non-diabetic and diabetic rats (comprising 8 preparations), after a steady base-line recording, were used to study the inotropic effect of digoxin in preparations preincubated with RGN (40 µM) for 10 min.

2.6. Effect of daily treatment with RGN for 3 weeks on digoxin-induced cardiotoxic effect on isolated right atria of diabetic rats

One week after confirmation of diabetes, diabetic rats were randomly divided into 2 groups, in addition to control non-diabetic group:

Group (1): Control non-diabetic rats received only the vehicle (0.5 ml/kg of 10% ethanol, I.P.) once daily for 3 weeks.

Group (2): Control diabetic rats received only the vehicle (0.5 ml/kg of 10% ethanol, I.P.) once daily for 3 weeks.

Group (3): Diabetic rats received RGN (4 mg/kg, I.P.) once daily for 3 weeks.

On day 22, blood samples were withdrawn from the retro-orbital plexus under ether anesthesia and serum was separated for biochemical analysis after treatment. The right atrium was isolated from each group, as

previously mentioned. After a steady base-line recording, a cumulative concentrations response curve was constructed with regard to the inotropic effect of digoxin alone (0.1, 0.3 & 1 μ M).

2.7. Effect of acute treatment with RGN on digoxin induced cardiotoxicity in anesthetized rats

Rats were divided into 2 groups (8 animals each).

Group (1) : Control rats received the vehicle (0.5 mL/kg 10% alcohol, I.P.) 1 hr before infusion of digoxin.

Group (2): Treated rats received RGN (4 mg/kg) by I.P. injection 1 hr before infusion of digoxin.

After anesthesia with thiopental sodium (40 mg/kg, I.P.), the jugular vein was exposed and cannulated for digoxin intravenous (I.V.) infusion at a flow rate of 0.25 ml/min (25 μ g/min) for one min every 5 min interval and the electrocardiogram (ECG) was recorded at standard lead II limb leads using hypodermic needle electrodes and a single channel ECG (Fukuda Cardisunny model B-501 III, Japan). The electrocardiograph was standardized before each tracing so a 1mV pulse produces a square 10-mm high. The paper (chart) speed was 50 mm/sec. The threshold dose of digoxin required to cause death from fetal ventricular fibrillation or from complete heart block was determined.

2.8. Estimation of serum glucose, potassium and sodium levels

Glucose, potassium and sodium were determined in rat serum according to manufacturer instructions (Biomerieux Company, France).

3. Results

3.1. In-vitro effect of RGN on the contractility of isolated right atria of non-diabetic and diabetic rats

As shown in fig. 1 & 2, the incubation of the right atria of non-diabetic rats with different concentrations of RGN (10, 20, 40 & 60 μ M) produced little non-significant increase in the contractility followed by significant decrease in the contractility at higher concentrations of RGN (80 μ M & 100 μ M) when compared with vehicle treated group. While incubation of the right atria of diabetic rats with different concentrations of RGN produced significant decrease in the contractility in all tested concentrations (10, 20, 40, 60, 80 & 100 μ M) when compared with vehicle treated group. The vehicle (at final bath concentration of 0.025% ethanol) did not produce any significant effect on the contractility of the isolated right atria of non-diabetic and diabetic rats.

3.2. In-vitro effect of RGN on digoxin-induced increase in the contractility of isolated right atria of non-diabetic rats

As shown in fig. 3 & 4, the preincubation of isolated right atria of non-diabetic rats with RGN (40 μ M) for 10

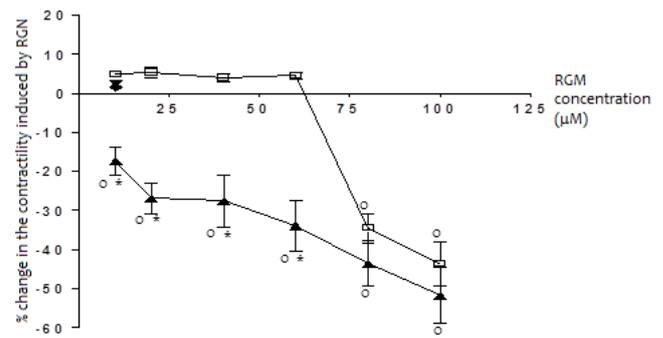


Figure 1. In-vitro effect of RGN on the contractility of isolated right atria of non-diabetic and diabetic rats. Values represent the mean \pm SEM of 8 preparations for RGN treated groups and 6 preparations for the vehicle treated group

*Significantly different from non-diabetic rats when compared together using unpaired Student's t-test ($P < 0.05$).

° Significantly different from the corresponding mean value of the vehicle (0.025% ethanol) when compared together using unpaired Student's t-test ($P < 0.05$)

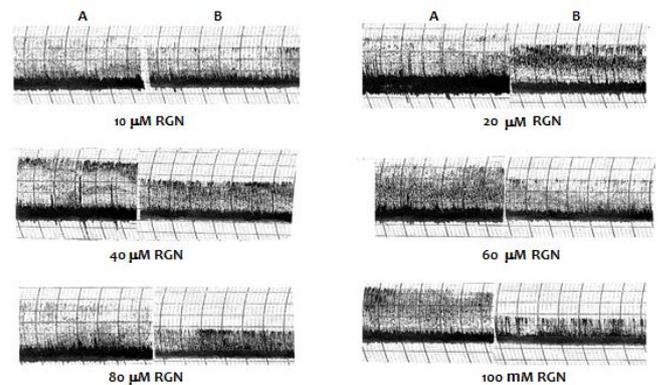


Figure 2. The contractility response induced by Rosiglitazone (RGN) (10, 20, 40, 60, 80 & 100 μ M) on isolated right atrium of A) non-diabetic rat, B) diabetic rat

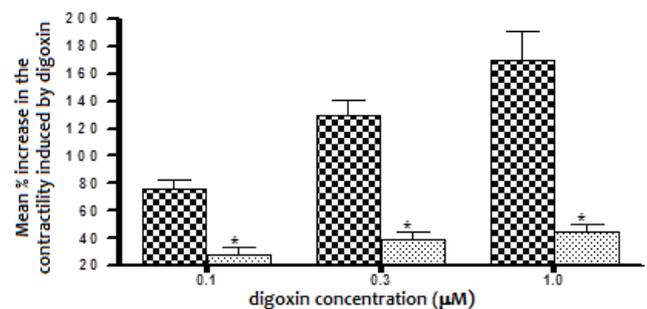


Figure 3. In-vitro effect if rosiglitazone (RGN) on digoxin iduced increase in the contractility of isolated right atria of non-diabetic rats. Values represent the mean \pm SEM of 8 preparations.

*Significantly different from that treated with vehicle when compared using unpaired Student's t-test ($P < 0.05$)

▨ Vehicle ▩ RGN (40 μ M)

min produced significant decrease in the cardiotoxic effect induced by digoxin in all tested concentrations (0.1, 0.3 & 1 μ M) when compared with the corresponding effect produced with digoxin in atrium preincubated with vehicle (0.025% ethanol).

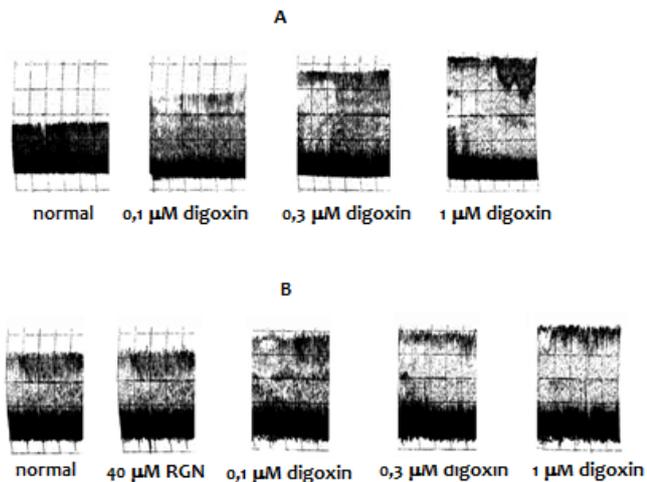


Figure 4. The contractility response induced by digoxin (0.1; 0.3; and 1 μM) on right atrium isolated from non-diabetic rat A) incubated with vehicle, B) pre-incubated with 40 μM RGN for 10 min

3.3. Effect of digoxin treatment on the contractility of isolated right atria of non-diabetic and diabetic rats

As shown in fig. 5 and 8, the increase in the contractility of the atria of the control diabetic rats induced by digoxin showed significant decrease when compared with the corresponding value of control non-diabetic animals in all tested concentrations (0.1, 0.3, and 1 μM)

3.4. In-vitro effect of RGN on digoxin-induced increase in the contractility of isolated right atria of diabetic rats

As shown in fig. 6 and 8, the preincubation of diabetic rats right atria with RGN (40 μM) for 10 min before digoxin treatment produced significant lowering effect in the contractility when compared with that produced by digoxin in atria preincubated with the vehicle for 10 min in all tested digoxin concentrations (0.1, 0.3 & 1 μM).

3.5. Effect of daily treatment for 3 weeks with RGN on digoxin-induced increase in the contractility of isolated right atria of diabetic rats

As shown in fig. 7 and 8, the treatment of diabetic rats with RGN (4 mg/kg, I.P., once daily for 3 weeks) showed significant decrease in the inotropic effect produced by digoxin in all tested concentrations (0.1, 0.3 & 1 μM) when compared with that of control diabetic animals.

3.6. Effect of treatment with RGN on digoxin induced cardiotoxicity in anesthetized rats

As shown in fig. 9, the infusion of digoxin in control group (0.5 ml/kg 10% ethanol, I.P., 1 hr before digoxin infusion) produced gradual decrease in heart rate till complete heart block and cardiac arrest with lethal dose of about (2.285 ± 0.126 mg/kg), while infusion of digoxin

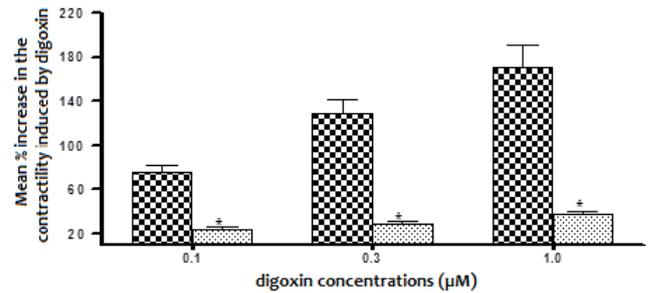


Figure 5. Effect of digoxin treatment on the contractility of isolated right atria of non-diabetic and diabetic rats. Values represent the mean \pm SEM of 6 preparations

*Significantly different from non-diabetic rats when compared using unpaired Student's t-test ($P < 0.05$)

■ Non-diabetic group ● diabetic group

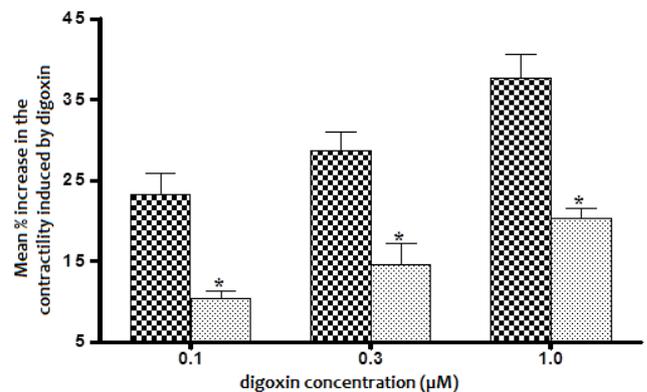


Figure 6. In-vitro effect of rosiglitazone (RGN) on digoxin-induced increase in the contractility of isolated right atria of diabetic rats. Values represent the mean \pm SEM of 6 preparations

*Significantly different from that treated with vehicle when compared using unpaired Student's t-test ($P < 0.05$)

■ Vehicle ● RGN (40 μM)

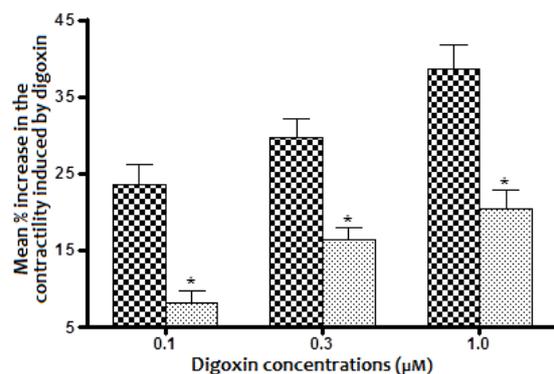


Figure 7. Effect of daily treatment for 3 weeks with RGN treatment on digoxin-induced increase in the contractility of isolated right atria of diabetic rats. Values represent the mean \pm SEM of 6 preparations

*Significantly different from control diabetic atria when compared using unpaired Student's t-test ($P < 0.05$)

■ Non-diabetic group ● diabetic group

in RGN treated group (4 mg/kg, I.P., 1 hr before digoxin infusion) produced a significant increase in the lethal dose of digoxin (4.09 ± 0.22 mg/kg) needed to induce cardiac arrest.

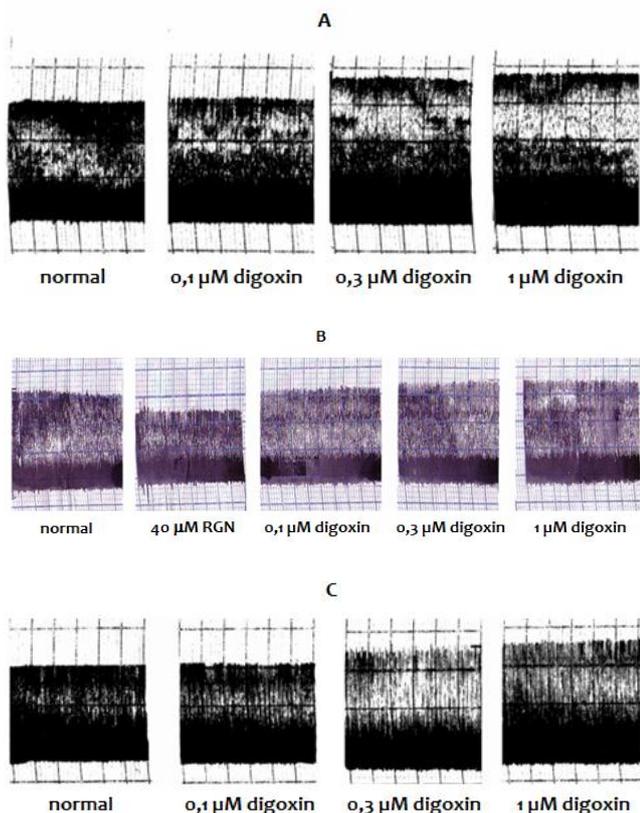


Figure 8. The contractility response induced by digoxin (0.1, 0.3, and 1 μM) on right atrium isolated from diabetic rat A) vehicle pretreatment, B) RGN pre-incubation with 40 μM RGN for 10 min, C) treated with RGN (4mg/kg I.P., once daily for 3 weeks).

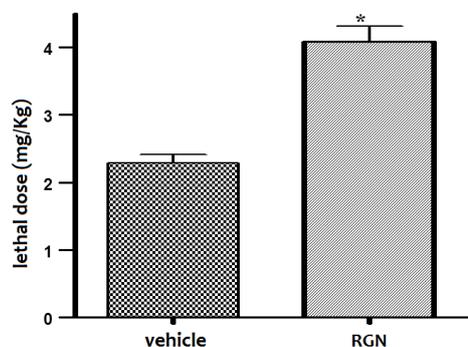


Figure 9. Effect of treatment with rosiglitazone (RGN) on the lethal dose of digoxin. Values represent the mean ± SEM of 8 preparations. *Significantly different from control group received vehicle (0.5 mL/kg 10% ethanol) before digoxin infusion when compared using unpaired Student's t-test (P<0.05)
 [checkered box] Vehicle [dotted box] RGN (4 mg/Kg I.P.)

3.7. Effect of once daily treatment for 3 weeks with RGN on serum glucose level in diabetic rats

As shown in fig. 10, mean serum glucose level in control diabetic group was significantly higher than its initial value and that of control non-diabetic animals. RGN treatment showed a significant decrease in serum glucose level when compared with control diabetic value and control non-diabetic value.

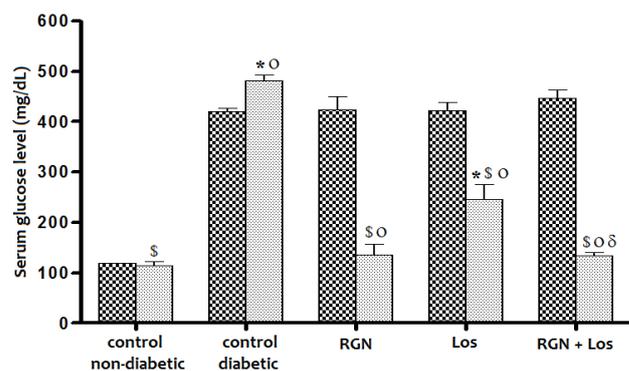


Figure 10. Effect of once daily treatment for 3 weeks with rosiglitazone (RGN), losartan (Los) and their combination once daily for 3 weeks on serum potassium level in diabetic rats. Values represent the mean ± SEM of 6 rats.
 *§ significantly different from control non-diabetic and control diabetic rats respectively using unpaired Student's t-test (P<0.05)
 o significantly different from its initial value using paired Student's t-test (P<0.05)
 δ significantly different from the corresponding mean value of losartan (2 mg/kg, orally) treated diabetic animal using unpaired Student's t-test (P<0.05)
 [checkered box] before treatment [dotted box] after treatment

3.8. Effect of treatment with RGN once daily for 3 weeks on serum potassium level in diabetic rats

As shown in fig. 11, serum potassium level in control diabetic rats was significantly less than both its initial value and control non-diabetic animals, but it was not significantly different from control non-diabetic value. Treatment of diabetic rats with RGN showed significant increase in serum potassium level when compared with its initial, control diabetic and control non-diabetic values.

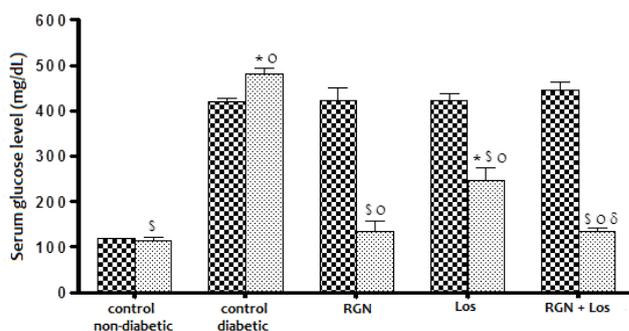


Figure 11. Effect of treatment with rosiglitazone (RGN), losartan (Los) and their combination once daily for 3 weeks on serum potassium level in diabetic rats. Values represent the mean ± SEM of 6 rats.
 *§ significantly different from control non-diabetic and control diabetic rats respectively using unpaired Student's t-test (P<0.05)
 o significantly different from its initial value using paired Student's t-test (P<0.05)
 μ δ significantly different from the corresponding mean value of RGN (4 mg/kg, I.P.) losartan (2 mg/kg, orally) treated diabetic animal using unpaired Student's t-test (P<0.05)
 [checkered box] before treatment [dotted box] after treatment

3.9. Effect of daily treatment for 3 weeks with RGN on serum sodium level in diabetic rats

As shown in fig. 12, mean serum sodium level in control diabetic group was significantly higher than its

initial value and that of control non-diabetic animals. RGN treatment showed a significant decrease in serum sodium level when compared with control diabetic value, while it was not significantly different from control non-diabetic value.

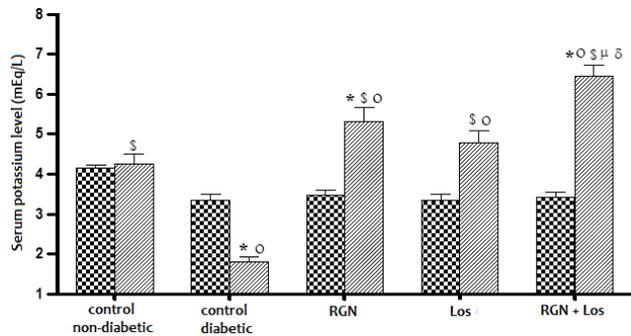


Figure 12. Effect of daily treatment for 3 weeks with rosiglitazone (RGN), losartan (Los) an their combination on serum sodium level in diabetic rats. Values represent the mean \pm SEM of 6 rats.

*§ significantly different from control non-diabetic and control diabetic rats respectively using unpaired Student's t-test ($P < 0.05$)

o significantly different from its initial value using paired Student's t-test ($P < 0.05$)

μ significantly different from the corresponding mean value of RGN (4 mg/kg, I.P.) treated diabetic animal using unpaired Student's t-test ($P < 0.05$)

■ before treatment ▨ after treatment

4. Discussion

In the present study, the significant stable moderate hyperglycemia was produced in rats using streptozotocin. Mild to moderate diabetes but not severe diabetes is required in this study because RGN (an insulin sensitizer) is active only if some insulin is still produced by the pancreatic β -cell (Malinowski and Bolestal, 2000). Treatment of diabetic rats with RGN produced significant decrease in serum glucose level. This observed lowering in serum glucose level indicated the insulin sensitizing effect of RGN which promotes insulin-dependent glucose uptake into target tissues such as liver, adipose tissue and skeletal muscle, and enhances insulin-mediated suppression of hepatic glucose production (Umrani *et al.*, 2002; Wu *et al.*, 2004).

In non-diabetic rat atria, RGN produced little non significant increase in the contractility at low RGN concentrations (10, 20, 40 & 60 μ M), but at high concentrations (80 & 100 μ M) significant depression in the contractility was produced when compared with vehicle treated group. Similarly, it was reported that both troglitazone and RGN have positive inotropic effect but not pioglitazone in normal isolated perfused rat heart (Furuse *et al.*, 2001). The little rise in the contractility was suggested to be due to Ca^{2+} sensitizing action of PPAR- γ agonists. RGN produced a concentration dependent reduction in the contractility of right atria of diabetic rats. This depressant effect may be attributed to any of the following actions of RGN: a- It was reported that PPAR- γ activation by RGN increased NO production which activate Maxi K^+ channel (Kim and Cheon, 2006). The opening of Maxi- K^+ channel lead to

hyperpolarization of membrane potential and thus closing of voltage-dependent Ca^{2+} channels responsible for Ca^{2+} influx (Knock *et al.*, 1999; Eto *et al.*, 2001). b- RGN inhibits (L-type) Ca^{2+} current this effect occurred over a higher concentration range (Knock *et al.*, 1999). c- Augmentation of delayed rectifier K^+ current causes dramatic shortening of action potential duration and hence to the negative inotropic response in atria (Ono, 2003). RGN inhibit delayed rectifier potassium channels (Knock *et al.*, 1999; Eto *et al.*, 2001).

Our data revealed that preincubation of non diabetic atrial preparation by RGN (40 μ M) showed significant reduction in the atrial contractility induced by digoxin when compared with its corresponding vehicle. This inhibitory effect of RGN on digoxin-induced inotropic effect may be due to the effect of RGN on some ion channels (Knock *et al.*, 1999; Eto *et al.*, 2001; Kim and Cheon, 2006).

The result showed that digoxin-induced positive inotropic action in atria of diabetic rats was significantly reduced when compared with atria from non-diabetic animals. These data was in agreement with (Ku and Sellers, 1982; Grassby and McNeil, 1988). The low basal Na^+ , K^+ -ATPase activity and the reduced binding of cardiac glycosides to Na^+ , K^+ -ATPase can explain the diminished response to them in tissue removed from chronically diabetic rats (McCullough and McNeil, 1983; Grassby and McNeil, 1988). Also, the low thyroid status of diabetic animals may influence myocardial responsiveness to cardiac glycosides by reducing Na^+ , K^+ -ATPase activity (Grassby and McNeil, 1988).

In this study, the significant reduction in the atrial contractility induced by digoxin in diabetic rats right atria preincubation with RGN (40 μ M) for 10 min or right atrium separated from diabetic rats treated with RGN (4 mg/kg, I.P., daily for 3 weeks) comparing with the corresponding vehicle of each preparation (Knock *et al.*, 1999; Eto *et al.*, 2001). The effects of RGN on certain ion channels (Knock *et al.*, 1999; Eto *et al.*, 2001) may affect electrolytes balance that reduces the digoxin toxicity (Sundar *et al.*, 1983). Also RGN restored the activity of ATP-sensitive K^+ channels reduced by high glucose (Kinoshita *et al.*, 2008). Activation of ATP-sensitive K^+ channels in the heart *in-vitro* produced a negative inotropic and chronotropic actions (Kocic and Korolkiewicz, 1998).

The effect of RGN on digoxin-induced cardiotoxicity in anesthetized rats showed significant increase in the lethal dose of digoxin needed to induce cardiac arrest when rats injected with RGN (4 mg/kg, I.P., 1 hr before digoxin infusion). Since RGN does not alter the pharmacokinetics of digoxin (DiCicco *et al.*, 2000) and this inhibition of action occurs also *in-vitro* (isolated atria), accordingly pharmacodynamic interactions are expected. The exact mechanism of this interaction is unknown, but it may be due to the following mechanisms: increase in serum potassium level (hyperkalemia). This increase in serum K^+ level is accompanied with cardiac depressant effect (Glazier *et al.*, 1984) and was reported to inhibit digoxin-induced

arrhythmia (Sundar *et al.*, 1983; Finnegan *et al.*, 1984). The effect of RGN on myocardial potassium channels may cause a prolongation of action potential duration (APD) and may explain to some extent the antagonism of arrhythmogenic action of digoxin.

Diabetic rats showed a significant decrease in serum potassium level and increase in serum sodium level when compared with the control non-diabetic value (Tsuchida *et al.*, 2001). Diabetic rats produced a significant decrease in serum potassium level and increase in serum sodium level in comparison with their initial value. This may be explained by the duration of diabetes. Also, it was concluded that hypokalemia might induce tubulointerstitial injury that would result in alterations in local vasoactive mediators that would favor Na⁺ retention (Suga *et al.*, 2001). It was reported that in hyperglycemia, glucose enters the cell, it takes potassium with it to form glycogen or animal starch as a result dangerous, potentially lethal, low serum potassium can result (Knochel, 1977). Our data showed that treatment with rosiglitazone with significant decrease in serum sodium level and significant increase in serum potassium level when compared with control diabetic value. It was concluded that the normalization of blood glucose level after RGN treatment is accompanied by the restoration of the ability of dopamine to significantly inhibit the activity of Na⁺, K⁺-ATPase in proximal tubules and normalization of the upregulation of D1-receptor that was affected by hyperglycemia (Tsuchida *et al.*, 2001). It was demonstrated that chronic treatment of pre-diabetic Zucker rats with common insulin sensitizing drug, RGN, will maintain blood pressure in the normal range, reduce hyperglycemia, albuminuria and increase natriuretic ability.

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