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## Effect of valsartan on isoproterenol induced myocardial infarction and histopathological in heart in diabetic rats

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### ABSTRACT

Present study was designed to evaluate Valsartan on isoproterenol induced myocardial infarction and histopathological in normal and Streptozotocin-Nicotinamide induced diabetic in rats. Valsartan (8 mg/kg, p.o) was administered for 28 days in rats injected with single dose of Streptozotocin (65 mg/kg, i.p, STZ) and Nicotinamide (110 mg/kg, i.p, NIC) and after isoproterenol (200mg/kg, s.c., ISO) induced myocardial infarction in rats on 29<sup>th</sup> and 30<sup>th</sup> day. At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and glycogen and nitrite carried out for further estimations. Administration of STZ–NIC in rats showed a significant ( $p<0.001$ ) increased in the levels of serum glucose, glycosylated heamoglobin (HbA1c), creatine kinase (CK), Glutamate oxaloacetate transferase (GOT), glycogen and nitrite whereas the levels of myocardial infarct size was found low to be significant ( $p<0.05$ ). Treatment with Valsartan no significantly change HbA1c, glucose level and glycogen but significantly reduced CK ( $P<0.05$ ), GOT ( $P<0.01$ ) and nitrite ( $P<0.01$ ) in compared to diabetic control group. The myocardial infarction in diabetic rats also led to severe splaying of muscle fiber, heavy neutrophil infiltration and cellular edema than non diabetic rats. The VAL treated diabetic rats exhibited reduction in necrosis with less fragmentation of fibres as compared to diabetic control groups, which reflects the cardio protective effect of VAL. This study concluded that VAL at 8 mg/kg may show reduce experimentally induced myocardial infarction in type 2 diabetic rats.

Keywords: Valsartan, cardioprotective, isoproterenol, Type 2 diabetic, Histopathology

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## INTRODUCTION

Three major metabolic abnormalities contribute to the development of hyperglycemia in Type 2 diabetes mellitus such as impaired insulin secretion in response to glucose, increased hepatic glucose production and decreased insulin-stimulated glucose uptake in peripheral tissues. The latter 2 abnormalities are primarily due to insulin resistance [1, 2]. Cardiovascular disease is one of the leading causes of death in the western world and diabetes mellitus has been identified as a primary risk factor [3], due to which there is alteration in vascular responsiveness to several vasoconstrictors and vasodilators [4]. Recently, a protective effect of Valsartan against oxidative stress in liver and kidney of diabetic rabbits [5] has been reported.

Recent evidence suggest that blockade of the rennin-angiotensin system ameliorates diabetes induced cardiac dysfunction. Because activation Valsartan (VAL) - Angiotensin II receptor (AT 1) blocker blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscles and the adrenal gland. Recent evidence suggests that blockade of the renin-angiotensin system ameliorates diabetes-induced cardiac dysfunction. Angiotensin receptor antagonists are widely used as antihypertensive in diabetic and non diabetic patients. Valsartan is reported for its renoprotective activity in diabetic rats.

So far the effect of Valsartan on experimentally induced myocardial infarction in type 2 diabetic rats has not been studied. Hence, the purpose of the present study was to instigate the effect of Valsartan treatment on serum heart marker, heart tissue parameter and histopathological alteration in Isoproterenol Induced myocardial infarction in type 2 diabetic rats.

## MATERIALS AND METHODS

### Drugs and Chemicals

Valsartan hydrochloride was obtained as a gift sample from Alembic Pharmaceuticals Pvt. Ltd., Baroda, India. STZ and NIC were obtained from SIGMA, St. Louis, MO, USA. All other chemicals and reagents used in the study were of analytical grade.

### Experimental Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Dharmaj Degree Pharmacy College, Anand. Sprague Dawley rats ( $210 \pm 15$  g) were housed in-group of 3 animals per cage and maintained under standardized laboratory conditions (12- h light/dark cycle,  $24^{\circ}\text{C}$ ) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt., Pune) and purified drinking water ad libitum.

### Experimental Induction of Type 2 Diabetes in Rats

Type 2 Diabetes was induced in rats by a single intraperitoneal (i.p) injection of Streptozotocin (65 mg/kg, STZ) in overnight fasting rats or mice followed by the i.p administration of Nicotinamide (110 mg/kg, NIC) after 15 minutes. STZ was dissolved in citrate buffer (pH 4.5) and NIC was dissolved in normal saline. After 7 days following STZ and NIC administration, blood was collected from retro-orbital puncture and serum samples were analyzed for blood glucose [6]. Animals showing fasting blood glucose higher than 300 mg/dL were considered as diabetic and used for the further study. Valsartan (8 mg/kg, p.o) was administered for 28 days in diabetic rats and after isoproterenol induced myocardial infarction in rats on 29<sup>th</sup> and 30<sup>th</sup> day.

At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and carried out for further estimations.

## Experimental Protocol

Animals were divided into following groups, each group containing 6 animals and the treatment period for whole study was 4 weeks.

- Group 1: Non-diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks and (ND-CON)] and normal saline subcutaneously on 29<sup>th</sup> and 30<sup>th</sup> day.
- Group 2: Non-diabetic control treated with VAL (8 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (ND-VAL)] and normal saline subcutaneously on 29<sup>th</sup> and 30<sup>th</sup> day.
- Group 3: STZ-NIC diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks (D-CON)] and received ISO (200mg/kg, s.c.) on 29<sup>th</sup> and 30<sup>th</sup> day in normal saline.
- Group 4: STZ-NIC diabetic rats treated with VAL (8 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (ND-VAL)] and received ISO (200mg/kg, s.c.) on 29<sup>th</sup> and 30<sup>th</sup> day in normal saline.

## Biochemical estimations

### Characterization of Type 2 Diabetes Model

Type 2 diabetes was confirmed by measuring fasting serum glucose using standard diagnostic kit (SPAN diagnostics Pvt., India) and the degree of uncontrolled diabetic state was confirmed by measuring HbA1c (Ion Exchange Resin method). After 4 weeks, diabetes was confirmed by measuring glucose and HbA1c as mentioned above.

### Estimation of Serum Markers

On 4<sup>th</sup> week blood samples were collected from retro-orbital plexus under light ether anesthesia and centrifuged at 2500 rpm for 20 minutes to separate serum. Glucose, HbA1c, CK and GOT were estimated using diagnostic kits (SPAN Diagnostics Pvt. India). In vitro quantitative determination of the activity of myocardial glycogen and myocardial nitrite [7] levels.

### Histological Examination

After decapitation, the heart was rapidly dissected out and washed immediately with saline and fixed in 8% buffered formalin. Hearts which were stored in 8% formalin were embedded in paraffin, sections cut at 5  $\mu$ m and were stained with haematoxyline and eosin. The sections of the heart were observed under microscope (Olympus BX8) for histological changes.

### Statistical Analysis

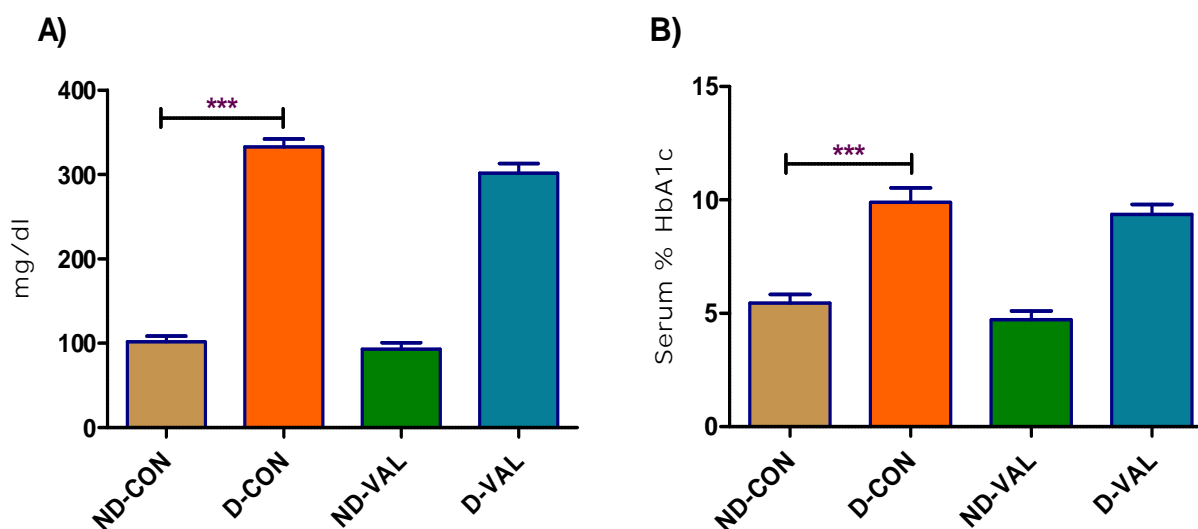
All of the data are expressed as mean  $\pm$  SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when  $p < 0.05$ .

## RESULTS

### Characterization of Type 2 Diabetes

Single intraperitoneal (i.p) injection of Streptozotocin (65mg/kg) followed by i.p administration of Nicotinamide (18 mg/kg) to rats produced severe hyperglycemia and increased HbA1c in 70 to 80 % the animals (Figure 1).

Figure 1. Effect of Valsartan (8 mg/kg/day, p.o) on changes in serum glucose and HbA1c level in normal and STZ-NIC induced diabetic rats.



### Body Weight and Heart Weight

Final body Weight of control animals was significant ( $P < 0.05$ ) increased as compared to initial body weight. There was a significant reduction in final body weight as compared to initial body weight of D-CON diabetic group (Table 1). Valsartan treatment had no significant effect on the body weight of D-CON group animals. There was a significant ( $P < 0.05$ ) increased in heart weight of diabetic rats (D-CON). VAL treatment could prevent increase in heart weight in diabetic rats (D-CON). Heart to body weight ratio of the entire group is show in (Table 1).

### Effect of VAL on serum enzymes

There was a significant ( $P < 0.05$ ) increase in serum CK and GOT ( $P < 0.001$ ) level after myocardial infarction in D-CON group as compared to ND-CON group (Fig. 1). Treatment of VAL in STZ-NIC diabetic rats (D-VAL) as well as in non diabetic rats (ND-VAL) could reduce elevated levels of serum CK and GOT as compared to D-CON group and respectively (Figure 2).

### Effect of VAL on myocardial tissue parameter

There was a significant ( $P < 0.001$ ) increase in myocardial glycogen level in D-CON group as compared to ND-CON group after myocardial infarction. VAL treatment could not reduce glycogen deposition in diabetic animal (D-VAL) as compared to diabetic control group (ND-VAL) (Figure 3A). There was a significant ( $P < 0.01$ ) increase in

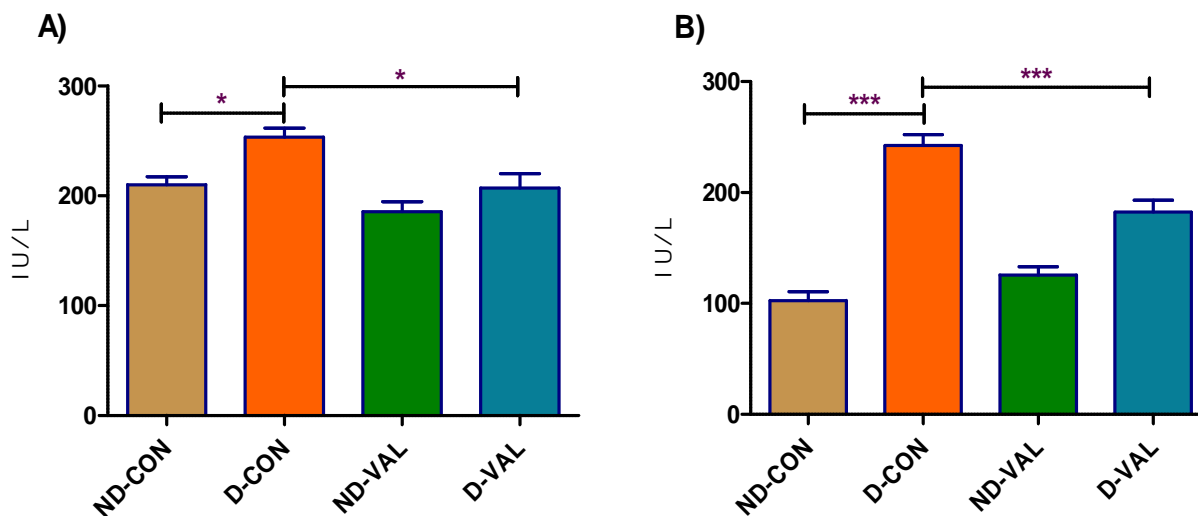
myocardial nitrite level in D-CON group as compared to ND-CON group after myocardial infarction. VAL treatment in diabetic rats (D-CON) and non diabetic rats (ND-CON) significantly ( $P < 0.05$ ) reduced nitrite level in heart in diabetic animal (D-VAL) as compared to diabetic control group (D-CON) and non diabetic rats (ND-VAL) respectively (Figure 3B).

Table 1. Effect of Valsartan (8 mg/kg/day, p.o) on changes in body weight, heart weight and heart to body weight ratio after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Groups	Body Weight						Heart Weight (gm)			Heart to Body Weight ratio		
	Initial			Final								
ND-CON	240.6	±	12.5	261.6	±	15.4 <sup>#</sup>	0.872	±	0.021	0.00333	±	0.00064
D-CON	249.2	±	17.4	224.4	±	16.1 <sup>#</sup>	0.973	±	0.019*	0.00433	±	0.00027*
ND-VAL	237.5	±	18.4	245.6	±	12.4	0.854	±	0.033	0.00347	±	0.00029
D-VAL	239.1	±	16.8	250.2	±	16.4	0.957	±	0.064*	0.00382	±	0.00046

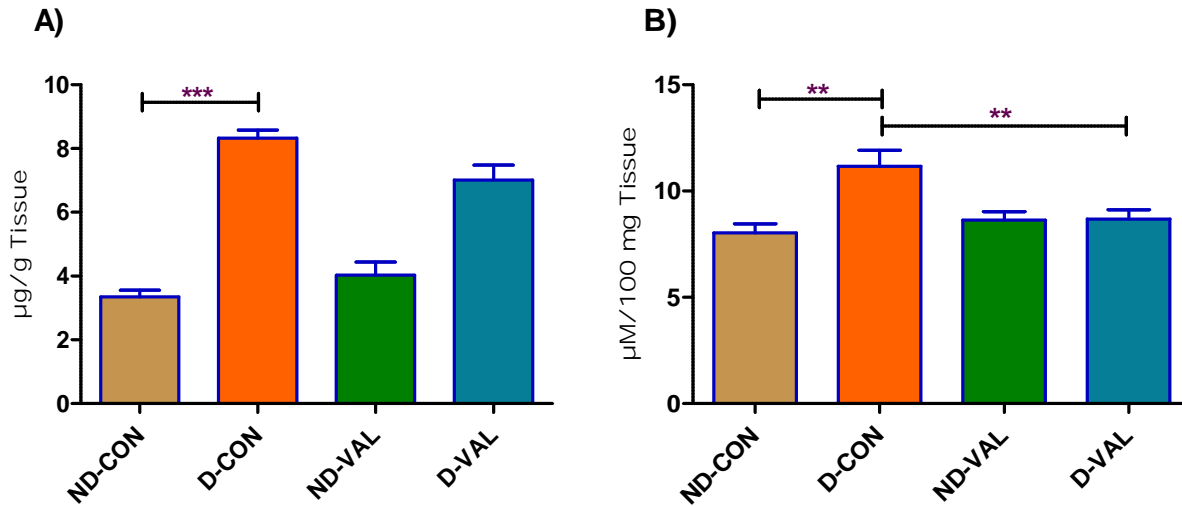
Values are expressed as mean  $\pm$  SEM for six animals in the group. \*  $P < 0.05$  compared to respective control group and <sup>#</sup>  $P < 0.05$  compared to initial weight.

Figure 2. Effect of Valsartan (8 mg/kg/day, p.o) on changes in serum Creatine kinase (CK) and Glutamate oxalatoacetate transferase (GOT) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.



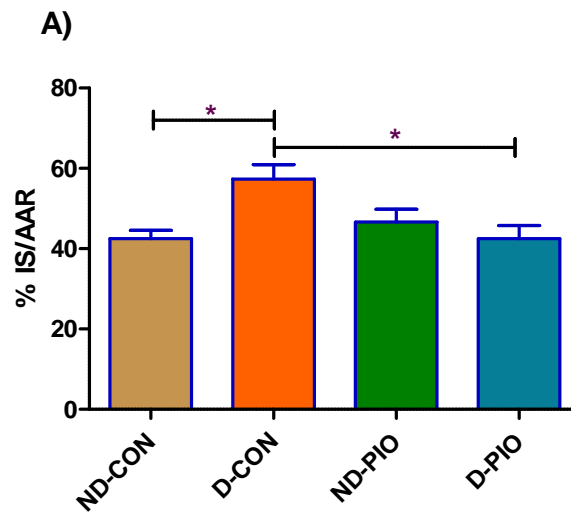
Values are expressed as mean  $\pm$  SEM for six animals in the group. \*  $P < 0.05$ , \*\*  $P < 0.001$ , \*\*\*  $P < 0.001$  considered statistically significant as compared to respective Control group.

Figure 3. Effect of Valsartan (8 mg/kg/day, p.o) on myocardial changes in Glycogen (A) and Nitrite (B) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.



Values are expressed as mean ± SEM for six animals in the group. \* P<0.05, \*\*P<0.001, \*\*\*P<0.001 considered statistically significant as compared to respective Control group.

Figure 4. Effect of Valsartan (8 mg/kg/day, p.o) on myocardial infarct size changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.



Values are expressed as mean ± SEM for six animals in the group. \* P<0.05, \*\*P<0.001, \*\*\*P<0.001 considered statistically significant as compared to respective Control group.

### Myocardial Infarct Size

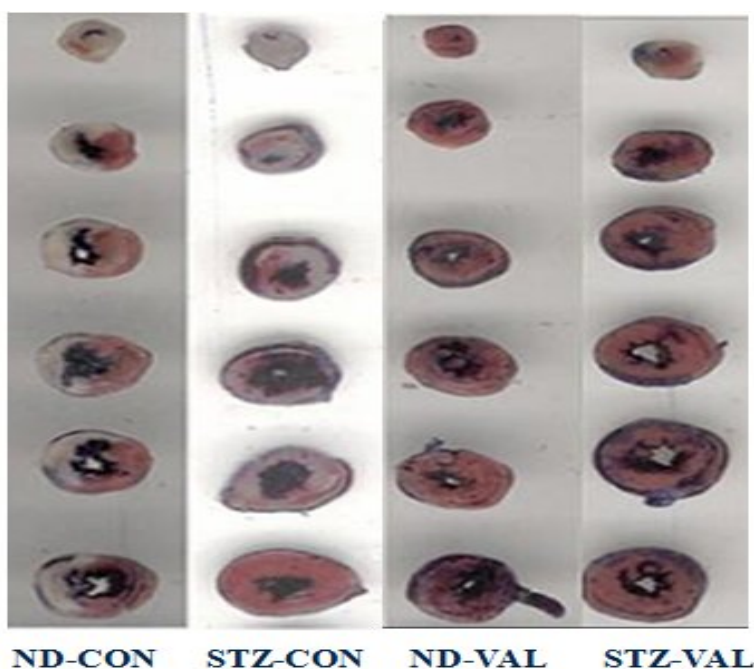
There was a significant (P < 0.05) increase in infarct size after myocardial infarction in diabetic rats (D-CON) as compared to ND-CON. VAL treatment significantly (P < 0.05) reduced infarct size in D-VAL group as

compared to D-CON group (Figure 4, 5). However, treatment with VAL could not reduce infarct size in non diabetic rats (ND-VAL) as compared to ND-CON group (Figure 4, 5).

### Histopathology of Heart

The photomicrographs revealed that induction of myocardial infarction caused more necrotic damage along with focal loss and fragmentation of muscle fibres of myocardial in diabetic rats (D-CON) than non diabetic rats (ND-CON) (fig. 6). The myocardial infarction in diabetic rats (D-CON) also led to severe splaying of muscle fiber, heavy neutrophil infiltration and cellular edema than non diabetic rats (ND-CON). The VAL treated diabetic rats (D-VAL) exhibited reduction in necrosis with less fragmentation of fibres as compared to D-CON groups, which reflects the cardio protective effect of VAL (Fig. 6). However, VAL treatment could protect myocardial infarction against in non diabetic rats (ND-VAL).

Figure 5. Effect of Valsartan (8 mg/kg/day, p.o) on TTC stained myocardial sections changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.



### DISCUSSION

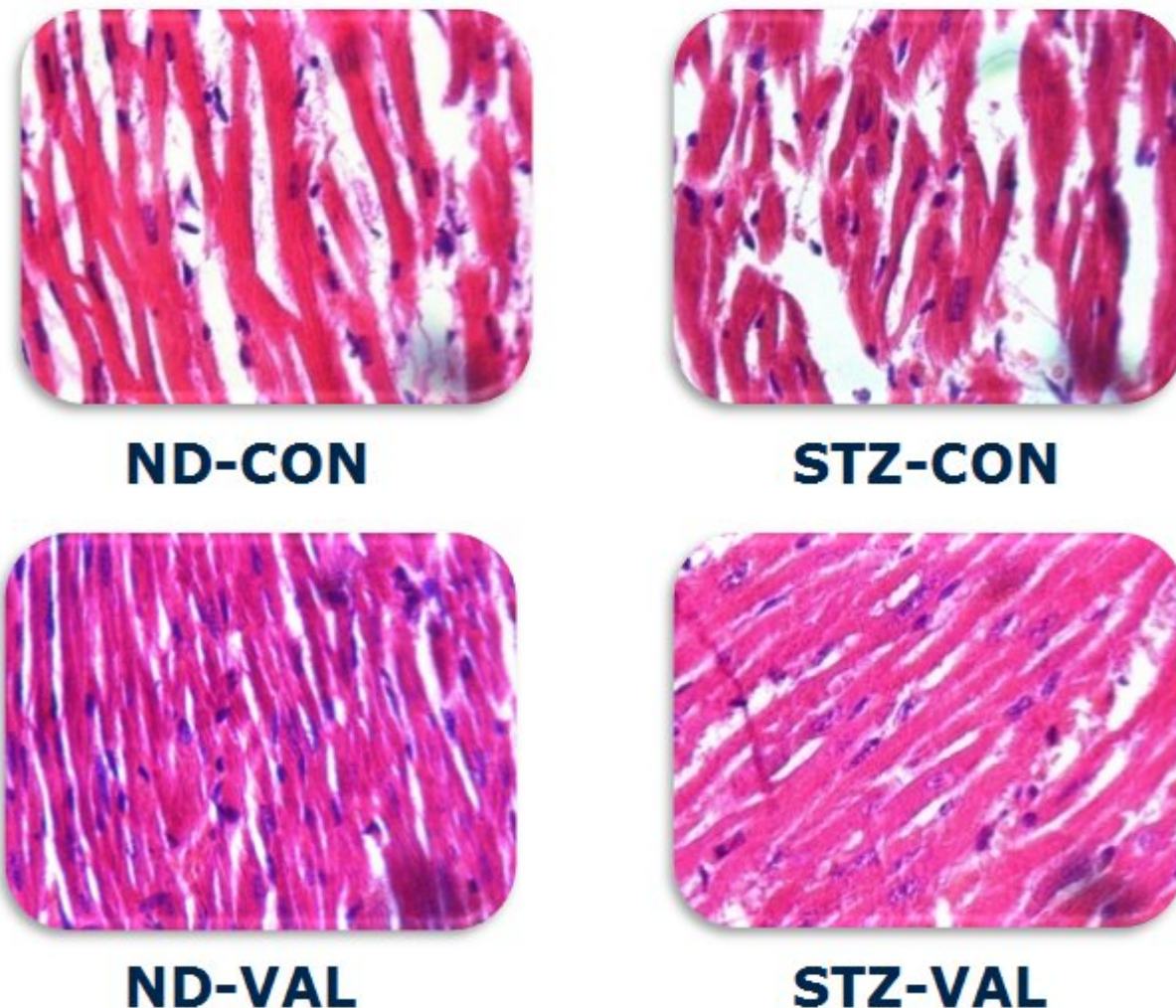
The present study was under taken with the objective of exploring the Valsartan Reduces on experimently induced myocardial infarction in diabetic rats. Recent studies have suggested that prevalence of type 2 diabetes is rapidly increasing. Heart failure of myocardial infarction or ischemic origin is more frequent and severe in patients with diabetes. Diabetes is an independent risk factor for cardiac failure [8], although its detrimental impact on the myocardium remains to be identified. The significant amount of myocytes loss in this model of non insulin dependent diabetes mellitus is consistent with a greater vulnerability of the diabetic heart to cardiac processes.

Acutely after STZ-NIC administration there is an up regulation of the local RAS in the heart. The expression of renin and AT1 receptor in myocytes was enhanced at 3 days, and this response was coupled with an increased synthesis of AT II. ACE and AT2 receptor are apparent, but their quantities do not change with diabetes. Valsartan treatment in diabetic as well as non diabetic animals show cardioprotective effect without influencing glycemic control and dyslipidemia associated with STZ diabetes. Moreover Valsartan may not have beneficial effect in



glucose homeostasis or altered energy metabolism but it may have direct cardioprotective effect against cardiomyopathy and ischemic injury.

Figure 6. Effect of Valsartan (8 mg/kg/day, p.o) on light micrographs of histopathological section of heart changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.



In the present study, an increase in the levels of serum glucose and HbA1c in STZ-NIC treated rats confirmed the induction of diabetes mellitus. No significant was observed in the glucose and HbA1c level in diabetic rats after treatment with VAL (8 mg/kg) when compared with D-CON rats at the end of experimental period. There was a significant increase in heart weight in STZ-NIC diabetic rats which may be due to cardiomyopathy associated with diabetes. It was reflected by increase in serum CK and GOT levels along with heart weight to body weight ratio. Valsartan could protect the heart from cardiomyopathy associated with STZ-NIC diabetes. This may be the reason for decreased serum CK and GOT level in D-VAL group.

Myocardial infarction causes further reduction in nitric oxide due to endothelial dysfunction. Valsartan reduced myocardial infarct size in STZ-NIC diabetic rats. The glycogen deposition in heart is increased in STZ-NIC diabetic rats which may be due to reduction in glucose utilization. VAL reduced cardiac glycogen content in STZ-NIC diabetic rats (D-VAL) by increasing glucose utilization after myocardial infarction. Therefore, another possibility for cardioprotection by VAL may be shifting of energy substrate metabolism from fatty acid to glucose.



The serum CK, GOT levels, cardiac nitrite level along with histopathological studies suggest cardioprotective role of Valsartan against myocardial infarction in diabetic and non diabetic rats. The cardioprotective mechanism may be one or more from inhibition of angiotensive – II mediated detrimental effects, reduction in NO destruction, direct coronary vasodilation and thus improvement in oxygen supply to the myocardium.

There may be several mechanisms for cardioprotective by VAL against myocardial infarction. It may be due to improvement in NO availability in STZ-NIC diabetic rats. Administration of STZ caused increase in serum CK, GOT and Valsartan (8 mg/kg, p.o) could reduce them. This study concluded that VAL at 8 mg/kg may show reduced on experimentally induced myocardial infarction in diabetic rats.

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