

*Full Length Research Paper*

## Impact of liraglutide versus atorvastatin on cardiovascular changes in rat model of adenine induced chronic renal failure

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The prevalence of cardiovascular changes markedly increases with deterioration of patient's renal function and can stack up 65 to 70% in end-stage renal disease. A rapid fall in renal function is often associated with uncontrolled congestive heart failure. Beyond their lipid-lowering effect, statins have been shown to protect the heart in different diseases. Liraglutide is a glucagon-like peptide-1 (GLP-1) analogue, used to control diabetes. It improves cardiac dysfunction in non-diabetics, but underlying mechanisms remain to some extent, unclear. Fifty two male Sprague-Dawley rats weighing 200 to 250 g were grouped into negative control, positive control, liraglutide treated group (0.3 mg/kg), atorvastatin treated group (10 mg/kg), liraglutide and atorvastatin treated group (0.3 and 10 mg/kg, respectively), liraglutide and atorvastatin treated group (0.15 and 5 mg/kg, respectively). Combination of both drugs significantly reduce creatine phosphokinase isoenzyme (CK-MB) in both doses ( $p < 0.008$ ,  $p < 0.002$ ) and lactate dehydrogenase (LDH) ( $p < 0.04$ ,  $p < 0.01$ ). Liraglutide alone or in combination with atorvastatin in both doses ( $p < 0.02$ ,  $p < 0.01$  and  $p < 0.01$ ) significantly increased superoxide dismutase (SOD). Atorvastatin alone ( $p < 0.02$ ) or in combination with liraglutide in both doses ( $p < 0.001$ ,  $p < 0.001$ ) induced a significant decrease of malondialdehyde (MDA). Atorvastatin alone ( $p < 0.01$ ) or in combination with liraglutide in both doses induced a significant amelioration of nitric oxide (NO) and cholesterol ( $p < 0.01$  and  $p < 0.02$ ). Significant improvements in blood urea nitrogen (BUN) and glucose profile were seen with all tested drugs either single or in combination. Combined implementation of both drugs improves histopathological changes of cardiac muscle fibres. Liraglutide has promising effects on cardiovascular changes in adenine induced chronic nephropathy through modulation of LDH, CK-MB and improvement of NO and SOD. Also, it can mitigate fibrosis and cardiac tissue changes. Its effects markedly increase in conjunction with atorvastatin. Combined administration of liraglutide and atorvastatin in a high dose has a reliable effect on improving outcome in biochemical and histopathological cardiac muscle fibers changes.

**Key word:** Atorvastatin, liraglutide, adenine, renal failure.

## INTRODUCTION

Increased incidence of cardiovascular system dysfunction is associated with chronic kidney disease (CKD) (Serizawa et al., 2015). Alterations in the kidneys and heart occur in CKD (Hernández-Reséndiz et al., 2015; Thaug et al., 2015). Studies on these structures in this disease have so far not been addressed. We are confronted by an alarmingly increasing number of patients with progressive renal disease.

Adenine is used for induction of renal failure. Only after 2 days of its administration, it is immediately metabolized to 2,8-dihydroxyadenine, which precipitate as crystals in the microvilli and the apical region of the proximal tubular epithelia (Adachi et al., 1993). The crystals were deposited at these tissues, induced degenerative changes in the cells and caused renal dysfunction. These structural alterations were associated with increased levels of serum creatinine and inorganic phosphate, and decreased levels of serum calcium (Yokozawa et al., 1986).

Statins had antiproliferative, anti-inflammatory, anti-thrombotic and anti-oxidant effects in previous studies (Wang et al., 2008). Statin clinical trials showed a reduction in stroke rate in patients with coronary heart disease (CHD) or atherosclerotic risk factors (John et al., 2005; Kucera et al., 2014). Atorvastatin is one of important statins that cause regression of lipid profile. It acts through inhibition of 3-hydroxy-3-methylglutaryl (HMG) Co-A reductase that is considered as a key enzyme in cholesterol synthesis. It is used as primary and secondary prevention of cardiovascular disease and can protect against nuclear damage in coronary artery diseases (Gundapaneni et al., 2016).

Liraglutide is glucagon-like peptide-1 (GLP-1) analogue and it exerts cardioprotective effects in animals and patients with or without diabetes (Nikolaidis et al., 2005; Sokos et al., 2006 Poornima et al., 2008). A large body of evidence has indicated that GLP-1 may have a beneficial effect on cardiovascular system; however, the mechanism is not fully understood, especially in non-diabetics. So, in this work, the authors evaluated its potential impact on cardiovascular changes occurring in chronic renal failure induced in rats in comparison to atorvastatin.

## MATERIALS AND METHODS

### The chemicals

Adenine was obtained from Sigma and was prepared freshly every day. Liraglutide (victoza<sup>®</sup>) 6 mg/ml from Novo Nordisk; Atorvastatin

(lipitor 40 mg<sup>®</sup>) was dissolved in 4 ml methylcellulose (0.05%) to make a concentration of 10 mg/ml.

### The animals

Fifty two male Sprague-Dawley rats (Urology and Nephrology Center, Mansoura University, Egypt), weighing 200 to 250 g were housed under conditions of controlled temperature and 12 h lighting cycle and fed with standard diet ad libitum. The study was approved by Institutional Ethics Committee for the use of laboratory animals. The animals were divided into 6 groups of 8 animals each. 4 rats died during induction. Group 1: Negative control received 0.5 ml methylcellulose by gavage feeding for 4 weeks; Group 2, Positive control: adenine was injected i.p. (100 mg/kg for four weeks) (Al Za'abi et al., 2015). Group 3: was given adenine as in above mentioned dose plus liraglutide (0.3 mg/kg subcutaneously) injected twice daily (Zhang et al., 2015) for 4 weeks; Group 4: was given adenine as in the above mentioned dose plus atorvastatin (10 mg/kg orally 6 days/week) for 4 weeks (Mehrzadi et al., 2016); Group 5: was given adenine as in the above mentioned dose plus combination of liraglutide (0.3 mg/kg subcutaneously) injected twice daily for 4 weeks and atorvastatin (10 mg/kg orally) 6 days/week for 4 weeks; Group 6: was given adenine as in the above mentioned dose plus combination of liraglutide (0.15 mg/kg subcutaneously) injected twice daily for 4 weeks and atorvastatin (5 mg/kg orally) 6 days/week for 4 weeks.

### Parameters of the study

Endothelial dependent factors; endothelin-1 (ET-1) enzyme measured by immunoassay kits were supplied by R and D systems IncMcKinley place N.E., Minneapolis, MN, USA (Suzuki et al., 1989). Nitric oxide (NO) was determined by Griess reagent (Amano and Noda, 1995).

Serum creatinine was determined by the method described by Henry (1974). Blood urea nitrogen was measured according to Patton and Crouch (1977); using urea kits (Diamond diagnostics company, Egypt).

### Serum indices of cardiotoxicity

Lactate dehydrogenase (LDH) and creatine phosphokinase isoenzyme (CK-MB) were determined kinetically at 340 nm using commercially available kits (Stanbio laboratory, INC. USA).

### Preparation of cardiac tissue

The heart samples were dried, weighed, homogenized in 50 mM ice cold phosphate-buffered saline (pH 7.4) and centrifuged for 5 min at 5000 g. The samples were stored at -80°C for biochemical estimations.

### Cardiac oxidative stress; MDA, SOD, glutathione

The superoxide dismutase (SOD) activity was determined spectrophotometrically according to the method of Lowry et al. (1955). Malondialdehyde (MDA) was measured using the

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**Table 1.** Effect of liraglutide and atorvastatin on serum levels of cardiotoxicity indices in adenine induced renal failure in rats (Mean±SEM).

	CK-MB (U/L)	LDH (U/L)
Gp1 (Negative control)	350.5±3.97	303.25±3.35
Gp2 (Positive control)	399.5±3.27 <sup>P</sup>	330.875±3.41 <sup>P</sup>
Gp3 (Liraglutide treated group)	387.8±8.6 <sup>P</sup>	319.63±4.06
Gp4 (Atorvastatin treated group)	388.6±6.3 <sup>P</sup>	316.5±4.21
Gp5 (Liraglutide 0.3 mg/kg plus atorvastatin 10 mg/kg)	383.3±7.3 <sup>P1</sup>	311.375±5.51 <sup>P1</sup>
Gp6 (Liraglutide 0.15 mg/kg plus atorvastatin 5 mg/kg)	381.1±8.6 <sup>P1</sup>	309±4.88 <sup>P1</sup>

P = significant difference when compared with negative control group, P1 = significant difference when compared with positive control.

**Table 2.** Effect of liraglutide and atorvastatin on levels of cardiac oxidative stress parameters in adenine induced renal failure in rats (mean ± SEM).

	SOD (U/ mg protein)	MDA (nmol/mg protein)	GSH (µ mol/gm tissue)
Gp1 (Negative control)	35.9±1.6	116.38±4.45	4.36±0.25
Gp2 (Positive control)	23±1.52 <sup>P</sup>	179.63±7.84 <sup>P</sup>	2.8±0.34 <sup>P</sup>
Gp3 (Liraglutide treated group)	30.9±1.5 <sup>P1</sup>	156.25±5.51 <sup>P</sup>	3.3±0.23
Gp4 (Atorvastatin treated group)	29.6±1.97	151.87±6.35 <sup>P,P1</sup>	3.09±0.16 <sup>P</sup>
Gp5 (Liraglutide 0.3 mg/kg plus atorvastatin 10 mg/kg)	31.4±2.02 <sup>P1</sup>	134.75±5.64 <sup>P1</sup>	3.63±0.27
Gp6 (Liraglutide 0.15 mg/kg plus atorvastatin 5 mg/kg)	34.13±1.03 <sup>P1</sup>	137.625±5.28 <sup>P1</sup>	3.47±0.22

P = significant difference when compared with negative control group, P1 = significant difference when compared with positive control.

thiobarbituric acid (TBA) (Bernheim et al., 1948). The level of glutathione (GSH) was determined according to Beutler et al. (1963). Blood glucose was determined by enzymatic method according to Trinder (1969) (Bio Med-Glucose, Germany).

### Cholesterol and TG

Serum TG was estimated according to the method of Fossati and Principe (1982) while enzymatic determination of serum total cholesterol was determined according to the method of Tietz (1976). They were measured spectrophotometrically with the use of Spinreact kits.

### Histological study

Paraffin sections of cardiac tissues (5 µm thickness) were prepared, and then stained with haematoxylin and eosin (H&E) (Kiernan, 1999; Bancroft and Gamble, 2002).

### Statistical analysis

All data are expressed as means ± SEM. Comparisons between different groups were analyzed by one-way ANOVA followed by bonfernie multiple comparison tests. In all cases, a probability error of 0.05 was selected as the criterion for statistical significance. Graphs were drawn using SPSS (version 21 for Windows).

## RESULTS

In Table 1, liraglutide insignificantly reduce CK-MB as

well as atorvastatin as compared to adenine induced nephropathy group. Abrogating effect of combination of both drugs on CK-MB in both doses (liraglutide 0.3 mg/kg plus atorvastatin 10 mg/kg or liraglutide 0.15 mg/kg plus atorvastatin 5 mg/kg) were significant when compared with positive control group ( $p < 0.008$  and  $p < 0.002$ , respectively).

As regard LDH, only combination of both drugs can exert a significant reduction in its levels when compared with positive control group ( $p < 0.04$  and  $p < 0.01$ , respectively).

In Table 2, there were significant changes in SOD as regard tested drugs either liraglutide alone ( $p < 0.02$ ) or in combination with atorvastatin in both doses ( $p < 0.01$  and  $p < 0.01$ , respectively). On the other hand, atorvastatin alone ( $p < 0.02$ ) or in combination with liraglutide in both doses induced a significant decrease of MDA as compared to positive control group ( $p < 0.001$  and  $p < 0.001$ , respectively).

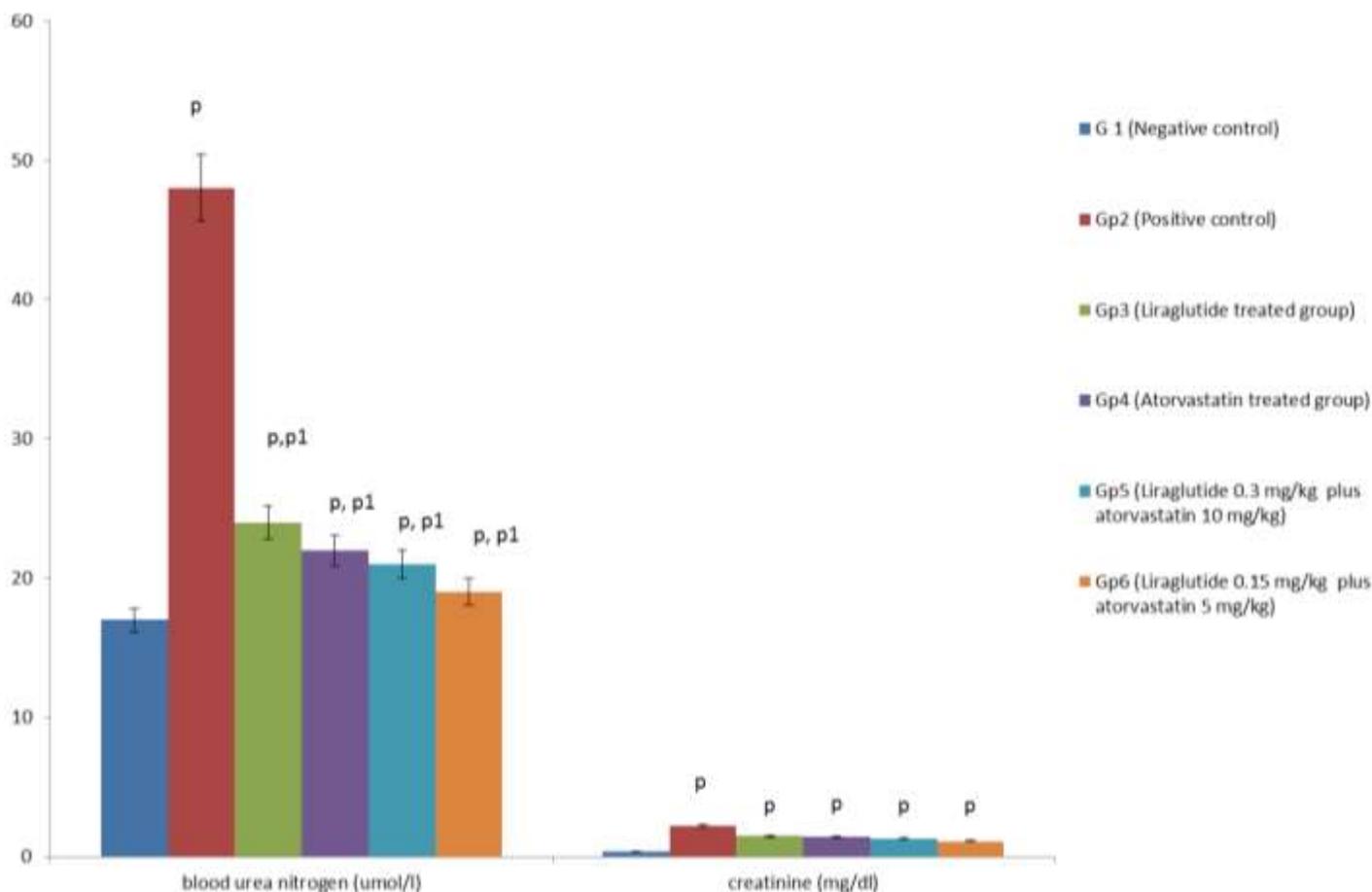
In Table 3, there were insignificant changes in endothelin as regard tested drugs either single or in combination. On the other hand, atorvastatin alone ( $p < 0.01$ ) or in combination with liraglutide in both doses induced a significant amelioration of NO as compared to the positive control group ( $p < 0.01$  and  $p < 0.02$ , respectively).

In Figures 1 and 2, there were insignificant changes in creatinine and TG as regard tested drugs either single or

**Table 3.** Effect of liraglutide and atorvastatin on levels of endothelial dependant factors; endothelin-1 (ET-1) and nitric oxide (NO) (mean  $\pm$  SEM).

	Endothelin-1 (ET-1) (pg/ml)	Nitric oxide (NO) (umol/ul)
Gp1 (Negative control)	1.57 $\pm$ 0.11	125.5 $\pm$ 4.8
Gp2 (Positive control)	3.38 $\pm$ 0.27 <sup>P</sup>	90.44 $\pm$ 2.89 <sup>P</sup>
Gp3 (Liraglutide treated group)	3.26 $\pm$ 0.25 <sup>P</sup>	105.11 $\pm$ 3.5 <sup>P</sup>
Gp4 (Atorvastatin treated group)	3.07 $\pm$ 0.21 <sup>P</sup>	107.37 $\pm$ 3.08 <sup>P,P1</sup>
Gp5 (Liraglutide 0.3 mg/kg plus atorvastatin 10 mg/kg)	2.93 $\pm$ 0.18 <sup>P</sup>	107.33 $\pm$ 2.62 <sup>P,P1</sup>
Gp6 (Liraglutide 0.15 mg/kg plus atorvastatin 5 mg/kg)	3.09 $\pm$ 0.11 <sup>P</sup>	106.19 $\pm$ 2.7 <sup>P,P1</sup>

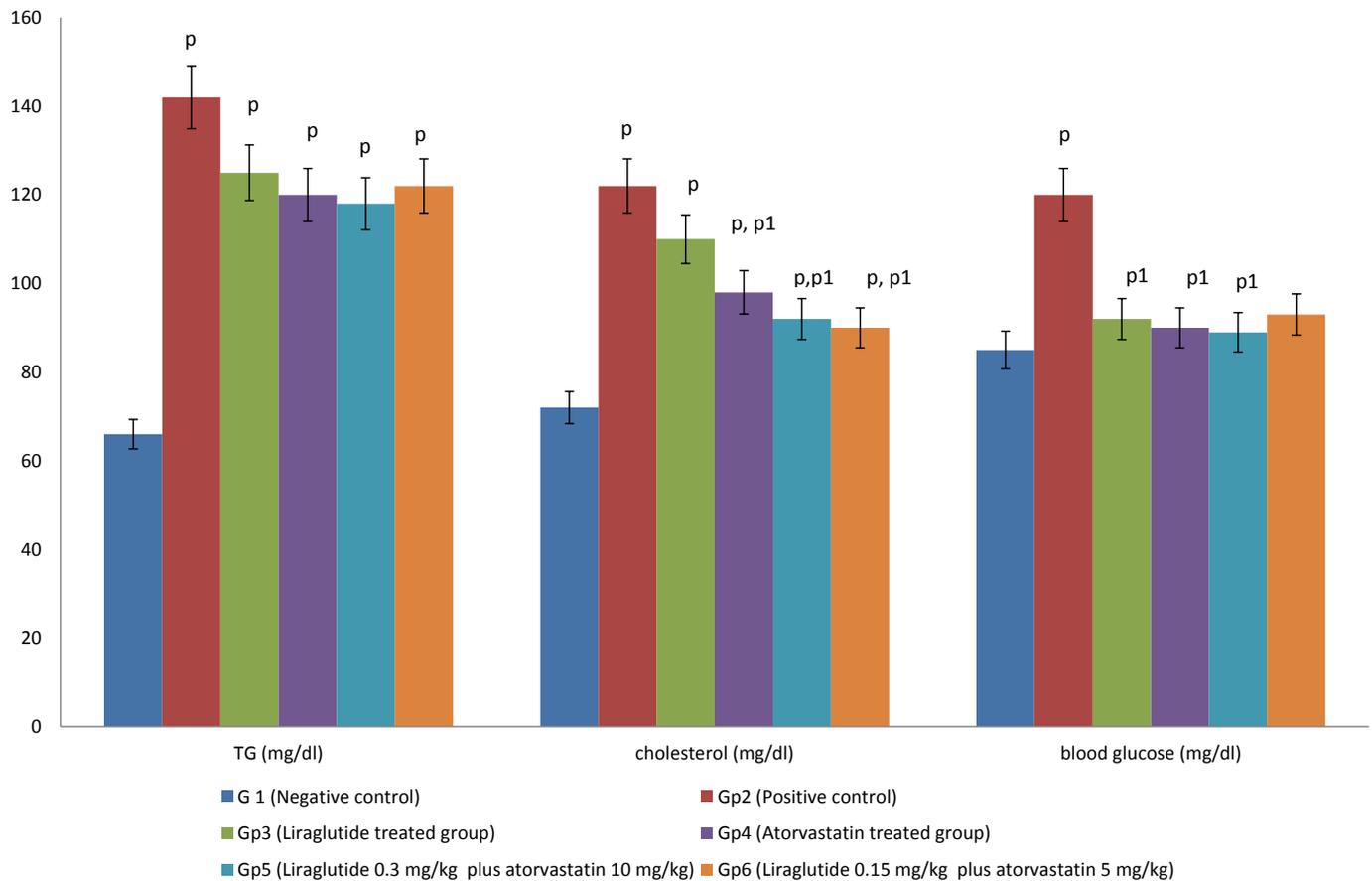
P = significant difference when compared with negative control group, P1 = significant difference when compared with positive control.

**Figure 1.** Effect of liraglutide and atorvastatin on creatine (mg/dl), blood urea nitrogen ( $\mu\text{mol/L}$ ). P = significant difference when compared with negative control group, P1 = significant difference when compared with positive control.

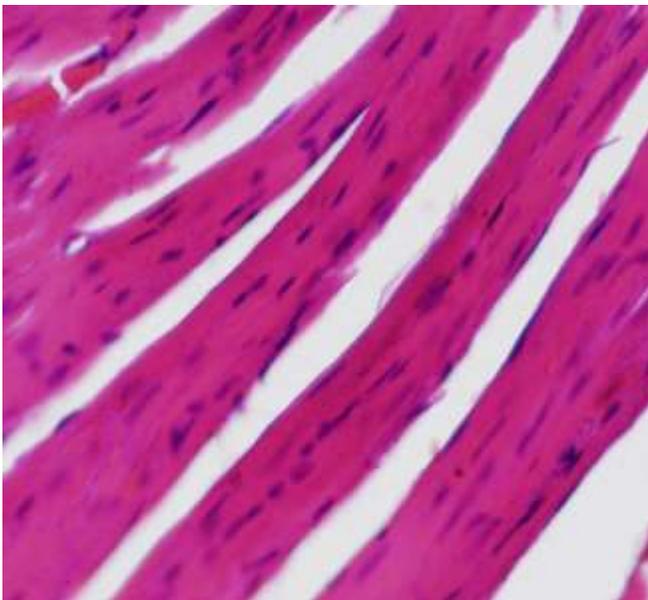
in combination. On the other hand, there were significant improvements in BUN with all tested drugs either single or in combination ( $p < 0.001$ ). Also, there were significant changes in glucose profile with all tested drugs when compared with positive control group ( $p < 0.05$ ). As regard cholesterol, there were significant changes attributed to atorvastatin alone ( $p < 0.01$ ) or in combination with liraglutide ( $p < 0.006$  and  $p < 0.004$ , respectively).

### Histological examination of cardiac tissues

In the negative control group (Figure 3), normal striated cardiac muscle fibers was observed between fine collagen fibres with single centrally located oval nuclei and intercalated discs by light microscope. In semithin section, a clear zone surround central vesicular nuclei with visible cross striations were present.



**Figure 2.** Effect of liraglutide and atorvastatin on blood glucose (mg/dl), cholesterol (mg/dl) and triglyceride (mg/dl) P = significant difference when compared with negative control group, P<sub>1</sub> = significant difference when compared with positive control.

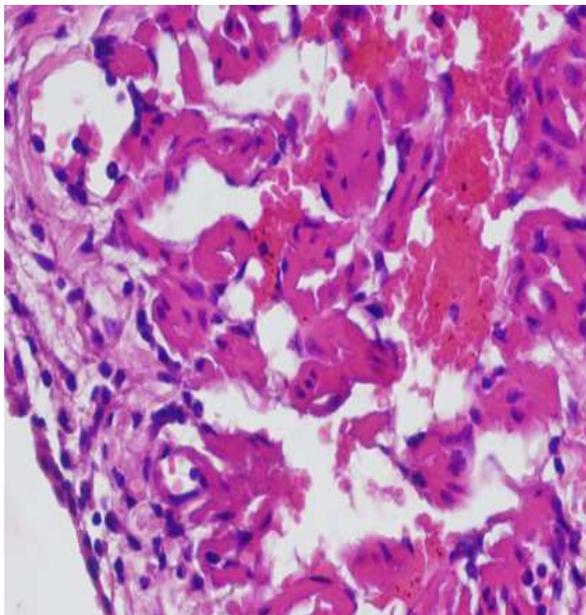


**Figure 3.** Group (1): cardiac muscle fibres showing striated muscle fibres with single centrally located oval nuclei and intercalated located oval nuclei (H & E, 400x).

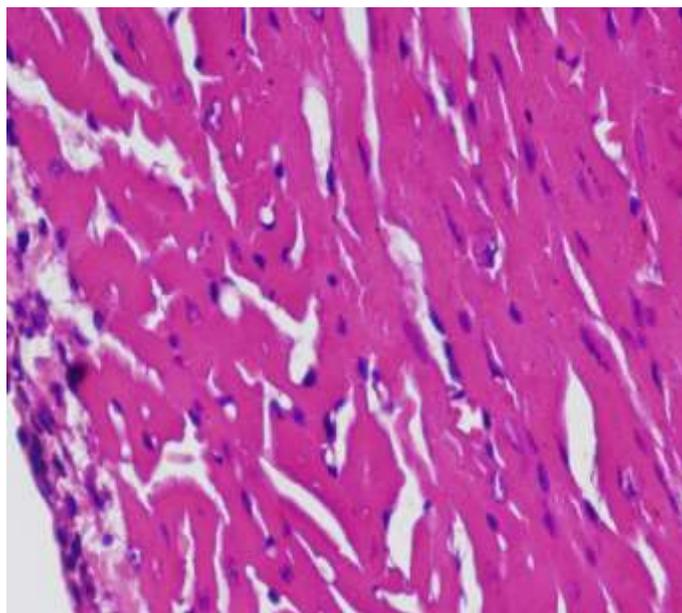
Positive control group is shown in Figure 4. LM showed distorted striation of separated muscle fibres with vesicular nuclei and myofibroblast. Collagen fibres moderately and significantly increase in the cardiac muscle as compared to the negative control group ( $P < 0.05$ ). Loss of muscle fibres striation and some nuclei, congested blood vessels and presence of myofibroblasts were seen in semithin sections.

With regards to the liraglutide treated group (Figure 5), LM illustrated splitted muscle fibres with patchy loss of striation, some nuclei become pyknotic with chromatin margination, congested blood vessels and lymphocytic infiltration. Focal areas of collagen fibres deposition were also seen in the cardiac muscle but significantly less in comparison with positive control group.

Atorvastatin treated group (Figure 6). LM demonstrated restored striation of cardiac muscle fibers together with pyknotic nuclei, plenty of myofibroblasts and lymphocytic infiltration. Mild significant decrease in collagen fibres in cardiac muscle were observed in comparison with positive control. Semithin sections revealed myofibroblast and lymphocytic infiltration. Combined high dose group (Figure 7) (LM) revealed significant restoration of near

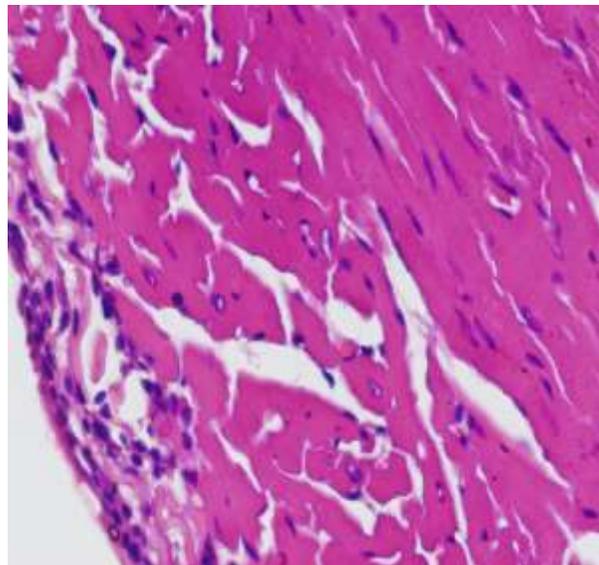


**Figure 4.** Group (2) cardiac muscle fibres showing distorted fibres striation, muscle fibres separation with vesicular nuclei and myofibroblasts (H & E, 400x).

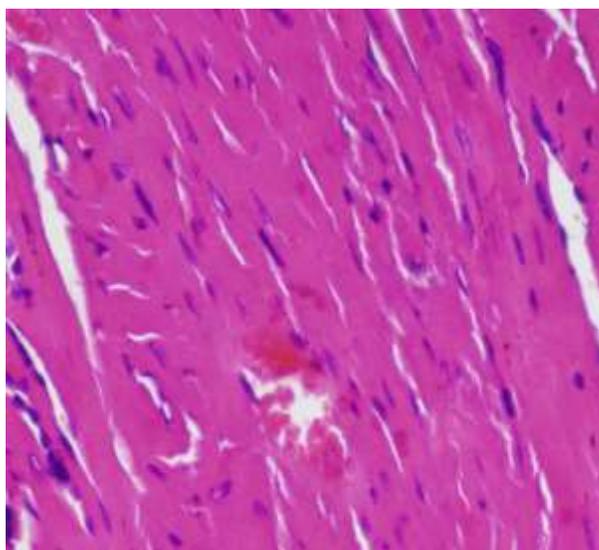


**Figure 5.** Group 3: Cardiac muscle fibres showing splitted muscle fibres, congested blood vessels lymphocytic infiltration and pyknotic nuclei (H & E, 400x).

normal cardiac striation with decreased collagen fibres in comparison with positive control, liraglutide treated, atorvastatin group and combined low dose treated group. With regards to combined low dose group (Figure 8), LM showed that cardiac muscle fibres restored their normal



**Figure 6.** Group 4: Cardiac muscle fibres showing restored striation, pyknotic nuclei, lymphocytic infiltration and increased myofibroblasts (H& E, 400x).

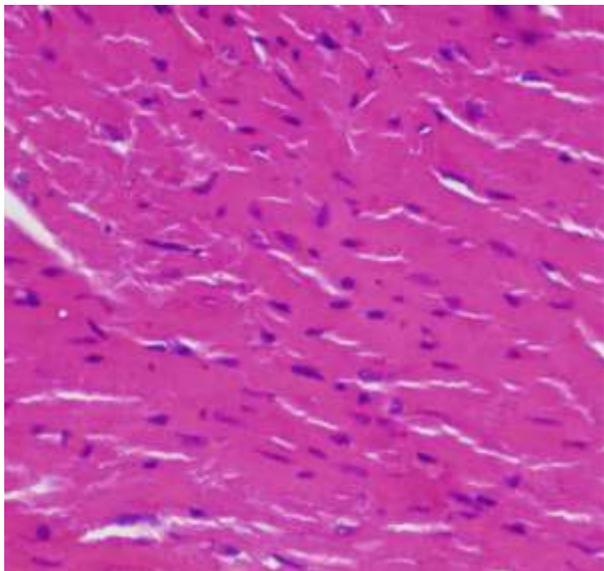


**Figure 7.** Group 5: Cardiac muscle fibres showing near normal striation with few collagen fibres (H&E, 400x).

striation and scattered myofibroblasts and collagen fibres in the cardiac muscle were observed but significantly less in comparison with positive control group, liraglutide treated and atorvastatin groups.

## DISCUSSION

GLP-1 receptor agonist may have potential multiple effects in patients with cardiovascular disease beyond their effect in managing diabetes. Little studies shows



**Figure 8.** Group 6: Cardiac muscle fibres showing myofibroblast with scattered collagen fibres (H&E 400x).

that GLP-1 receptor agonist may be effective for cardiac disorders in patients without diabetes mellitus.

In this study, combination of both drugs in both doses had significant effects on CK-MB and LDH. These findings were collaborated by Liu et al. (2016) who stated that pre-treatment with liraglutide in human neuroblastoma cell line exposed to beta-amyloid A $\beta$  decrease LDH leakage and cellular apoptosis. In the same direction, Sharma et al. (2014) illustrated that liraglutide reduce LDH and apoptosis. It was found that liraglutide suppressed NADPH oxidase and pro-inflammatory signals, and reduced collagen deposition in ischemia reperfusion model and obesity (Inoue et al., 2015; Wang and Yang, 2015).

In the present study, liraglutide alone or in combination with atorvastatin in both doses enhance SOD. Atorvastatin alone or in combination with liraglutide in both doses induced a significant decrease of MDA. In other studies on myocardial ischemia, atorvastatin exerts significant cardioprotective effects through increase of SOD and decreased MDA content (Sun et al., 2015). In the same direction, Mehrzadi et al. (2016) stated that atorvastatin enhance the renal SOD activity however, not reduce MDA. Rats pretreated with atorvastatin 2 or 5 mg/kg/day challenged with acetaminophen, showed higher hepatic SOD activities, lower MDA levels (Frag et al., 2015). Additionally, atorvastatin increase intrarenal activities of SOD and decrease MDA content (Zhou et al., 2014). Gao et al. (2015) show that liraglutide significantly increased SOD levels in non-alcoholic fatty liver disease. Also, it prevented brain edema, suppressed neuro-inflammation following intracerebral hemorrhage (Hou et al., 2012).

In the present study, atorvastatin alone or in combination with liraglutide in both doses induced a significant amelioration of NO and no effect on endothelin was reported. This work is supported by Nakata et al. (2007) who show that atorvastatin upregulates vascular nNOS through NF-kappa B pathway. However, other studies like Cetinkaya et al. (2013) mentioned that atorvastatin significantly decreased the levels of endothelin-1 in aged ovariectomized rats. Also, it was found that atorvastatin pretreatment attenuated endothelin 1 alterations and diminished histological injury scores (Chang et al., 2010; Cámara-Lemarrroy et al., 2014). Contrary to this work, studies of Dai et al. (2013) showed that liraglutide inhibit nuclear factor kappa B (NF- $\kappa$ B) phosphorylation that culminates in suppression of ET-1 expression. However, it was found that liraglutide (30  $\mu$ g/kg twice daily) reversed insulin resistance, obesity-induced perturbations in cardiac endothelial nitric oxide synthase in high fat diet mice (Noyan-Ashraf et al., 2013).

In the present study with all tested drugs either single or in combination, they improve significantly BUN and glucose profile; also, they improve creatinine. Zhou et al. (2014) reported that liraglutide decreased total cholesterol, blood urea nitrogen, serum creatinine in streptozotocin-induced diabetic rats. Atorvastatin effectively reduced creatinine and may carry a good approach as potential antidiabetic effect and serve as the therapeutic drug for diabetic kidney disease management in streptozotocin-induced diabetic rats (Liao et al., 2016). On other hand, Mehrzadi et al. (2016) mentioned that atorvastatin could not reduce elevated serum creatinine concentration, kidney weight, renal ROS and MDA levels.

Little studies are available on liraglutide and atorvastatin effects on structure of the cardiac muscle fibres. The obtained results in the present study revealed indistinct and distorted striation in the cardiac muscle fibers with separation, congested and dilated blood vessels, vesicular nuclei and loss of some nuclei, and presence of myofibroblast in positive control group. These results are in agreements with El Rabey et al. (2013). In our work, liraglutide partially improved histology of cardiac tissue and combined treatment of liraglutide and atorvastatin in both doses improved cardiac tissue as reflected by restoration of the striation and myofibroblast and scattered collagen fibres in the cardiac muscle. The evaluated data in this study declared a significant difference between all groups in collagen fibres. These results are in agreements with Deshmukh et al. (2012) who reported similar results. Also, Liu et al. (2013) reported that liraglutide significantly enhance cardiac structure and decreased the parameters of LV posterior wall and its end-diastolic diameter. However, in other studies, only minor cardiovascular effects of GLP-1 despite increased insulin levels and reduced plasma glucose concentration in compensated diabetic heart failure was reported (Halbirk et al., 2010). Atorvastatin

significantly reduced hyperplasia of fibrotic tissue in the model of heart failure and significantly reduced expression of type I and III collagen (An et al., 2013). Similarly, atorvastatin improve radiation-induced cardiac fibrosis and significantly reduced the expression of TGF- $\beta$ 1, Smad3/P-Smad3 in rats (Zhang et al., 2015).

## Conclusion

This study show promising effects of liraglutide and atorvastatin on cardiac muscle fibres. They not only depend on their role in modulating glucose and lipid profile. Combined administration of liraglutide and atorvastatin in a high dose has a reliable effect on improving outcome in biochemical and histopathological cardiac muscle fibers changes.

## Conflict of interest

The authors declare that there is no conflict of interest.

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