Modulatory Effect of Lovastatin on Therapeutic Efficiency of Conventional Antidiabetics on Pancreatic and Cardiovascular Complications of Diabetes

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Abstract

The aim of the present study is to assess the possible modulatory effect of lovastatin (LOVA) on metformin (MET) and gliclazide (GLIC) on pancreatic and cardiovascular complications in rats with experimentally induced diabetes. Male Swiss rats were randomly assigned to seven groups. Group 1 served as normal control group. Group 2 served as diabetic control group, treated with streptozotocin (STZ) alone. Groups 3, 4, 5, 6 and 7 received LOVA, MET, GLIC, LOVA plus MET and LOVA plus GLIC, respectively, for 30 consecutive days after treatment with STZ. To evaluate the modulatory effect of LOVA, blood glucose level was assessed coupled with pancreatic tissue levels of oxidative stress markers including thiobarbituric acid reactive substances (TBARS) production, glutathione reduced (GSH) stores, total nitrite (NO₂⁻) production as well as glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) activities. Degree of lipid derangement was evaluated by measuring serum levels of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, triglycerides (TGs), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C). Serum levels of C-reactive protein (CRP) and interleukin-6 (IL-6), as well as blood fibrinogen (FBG) level and white blood cells (WBCs) count were evaluated to assess generalized inflammatory and hematological disorders. Combination of LOVA with either MET or GLIC significantly corrected STZ-induced alterations of serum levels of glucose, HMG-CoA reductase, TGs, TC, LDL-C, HDL-C, CRP and IL-6, as well as blood fibrinogen (FBG) level and white blood cells (WBCs) count were evaluated to assess generalized inflammatory and hematological disorders. Combination of LOVA with either MET or GLIC significantly corrected STZ-induced alterations of serum levels of glucose, HMG-CoA reductase, TGs, TC, LDL-C, HDL-C, CRP and IL-6, tissue contents of TBARS, GSH, NO₂⁻, GST and SOD, as well as blood FBG level and WBCs count as compared to either drug alone. LOVA combination with MET but not with GLIC could modulate serum HDL-C level, whilst LOVA combination with GLIC but not with MET could modulate pancreatic tissue CAT activity. These results show that combination of LOVA with MET or GLIC significantly modulates their effects on diabetes-induced pancreatic and cardiovascular complications, including hyperglycemia, oxidative and nitrosative stress, dyslipidemia, inflammatory disorders, hyperfibrinogenemia and leucocytosis.

1 Introduction

Diabetes, a serious metabolic disorder of carbohydrates, fats and proteins, affects a large number of populations in the world. Diabetes is either pancreatic or extra-pancreatic in origin and causes many complications on nearly all body tissues, including cardiovascular system, kidney, liver and others. Microvascular complications are behind a massive percentage of diabetes morbidity and mortality cases, including retinopathy, neuropathy and nephropathy, and macrovascular complications, including heart attack, stroke and peripheral vascular disease, with cardiovascular disease (CVD) being the main cause of mortality in diabetic patients.

The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, lovastatin (LOVA), is a well-known inhibitor of cholesterol synthesis and, along with second generation statins, is considered the first line treatment for hypercholesterolemia. Since dyslipidemia mediates many diabetic complications, specially, cardiovascular and
hematological complications, statins may be of great value in reducing such complications\(^5\). Statins have also been demonstrated to exert anti-inflammatory effects\(^3\).

The standard antidiabetic biguanide drug metformin (MET) is widely popular. It acts as a cellular AMP-activated protein kinase activator, a well-known cellular metabolic sensor. The antihyperglycemic properties of metformin are mainly attributed to its suppression of hepatic glucose production, especially hepatic gluconeogenesis, and slightly increased peripheral tissue insulin sensitivity\(^4\). MET, independent of its antidiabetic effect, was reported to offer protection against vascular and other inflammatory complications\(^5\).

The second generation sulfonylurea agent, gliclazide (GLIC), is a common antidiabetic acting by stimulating pancreatic insulin release through binding to specific ATP-sensitive potassium channels\(^6\). It was reported to possess good antioxidant activity not related to its antidiabetic potential\(^7\).

Based on the facts listed above, the aim of the present study was to assess the possible modulatory effect of LOVA on therapeutic efficiency of MET and GLIC against experimental streptozotocin (STZ)-induced diabetes in rats concerning pancreatic and vascular complications. For fulfillment of this purpose, serum level of glucose and pancreatic tissue levels of thiobarbituric acid reactive substances (TBARS), glutathione reduced (GSH), total (NO\(^3\)), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) were measured. Degree of lipid derangement was evaluated by measuring serum levels of HMG-CoA reductase, triglycerides (TGs), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C). To assess inflammatory and hematological abnormalities, serum C-reactive protein (CRP) and interleukin-6 (IL-6) levels, in addition to white blood cells (WBCs) count were measured.

## 2 Materials and Method

### 2.1 Animals

Adult male swiss rats (150-180 g) were procured from central animal house, Faculty of Medicine, Assiut University (Assiut, Egypt). The animals were acclimatized to the animal room condition for at least a week at 25 ± 2 °C with 12/12-hour light/dark cycles prior to the experiment. All animals were supplied with commercial pellet food and water ad libitum. All animal housing and handling were conducted according to the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23).

### 2.2 Drugs and Chemicals

Test agents LOVA, GLIC and STZ as well as thiobarbituric acid (TBA), 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), N-(1-Naphthyl) Messia et al. Modulatory Effect of Lovastatin on Therapeutic Efficiency ethylenediamine dihydrochloride (NEDD) and glutathione reduced (GSH) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and protected from sunlight. MET was obtained from Chemical Industries Development CO. (CID; Giza, Egypt). HMG-CoA reductase ELISA kit was obtained from Wuhan El-Aab Science Co. (St. Biopark, Wuhan, China). C-reactive protein (CRP) was obtained from Assaypro LLC (St. Charles, MO, USA). Serum glucose kit, erum triglyceride (TG), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C) kits were obtained from Biodiagnostic Co. (Cairo, Egypt). Serum interleukin-6 (IL-6) kit was obtained from Glory Science Co. (St, Del Rio, USA). Analytical grade was a necessity in all other chemicals and solvents.

### 2.3 Experimental design

Male Swiss rats were randomly assigned to seven groups, each of 6 to 7 rats. Group 1 served as normal control group, receiving only vehicles. Group 2 received a single i.p. dose of streptozotocin (STZ; 50 mg/kg)\(^8\) dissolved in cold citrate buffer (0.1 M, pH = 4.5) and served as diabetic control. Groups 3, 4, 5, 6 and 7 received LOVA (15 mg/kg/day)\(^9\), MET (100 mg/kg/day)\(^10\), GLIC (20 mg/kg/day)\(^11\), LOVA plus MET and LOVA plus GLIC, respectively, by oral gavages for 30 consecutive days starting on 4th day after treatment with STZ.

### 2.4 Methodology

#### 2.4.1. Induction of experimental diabetes

Experimental diabetes was induced in 12-hour fasted rats by single i.p. injection of STZ (50 mg/kg). To prevent initial hypoglycemic effect of STZ, animals were given 20% glucose solution for the first 24 hours. Induction of diabetes was confirmed 72 hours after STZ injection through assessment of tail vein blood glucose, after 16 hours of fasting, using a portable glucometer (Accu-Chek, Roche, Germany). Only animals having fasting blood glucose exceeding 250 mg/dl, coupled with diabetic symptoms like polyphagia, polydipsiapolyuria, and weight loss were considered diabetic and included in the study.

After 30 consecutive days of daily drug treatments, rats were lightly anesthetized with diethyl ether and sacrificed by cervical decapitation. Blood samples were collected in tubes containing EDTA to determine fibrinogen (FBG) and white blood cell (WBCs) count. Serum was obtained after centrifugation at 3000 x g for 20 minutes using a centrifuge (Beckman GS-6 centrifuge, USA), then quickly stored at -80°C and used for assessment of serum levels of triglycerides (TGs), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), C-reactive protein (CRP) and interleukin-6 (IL-6). Pancreas was excised immediately, washed with chilled isotonic saline and stored at -80°C for preparation of tissue homogenate and slides for
Histopathology. Tissue homogenates (10% w/v) were prepared in ice cold conditions in a homogenizer (Cole-Parmer instrument, USA) using glass tube and teflon pestle, in phosphate buffer solution. The homogenate was centrifuged at 1000 g for 10 minutes in refrigerated centrifuge and the supernatants obtained were used for assessment of pancreatic tissue levels of thiobarbituric acid reactive substance (TBARS), glutathione reduced (GSH), total nitrite (NO2-), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT). Autopsy samples were taken from pancreas of rats in different groups and fixed in 10% formalin solution in saline to be used for preparation of liver sections for histopathological study.

2.4.2 Estimation of Biochemical parameters

Serum glucose was estimated by the method of Trinder12. TBARS was measured according to the method described by Mihara and Uchiyama13. GST activity was measured by using the method of Beutler14. GST activity was measured by using the method of Habig15. Total nitrite (NO2-) was done by the method of Montgomery and Dymock16. Antioxidant enzymes SOD and CAT were measured by the method of Marklund and Fridrid17. Serum HMG-CoA reductase activity and CRP level were assessed according to kit manufacturer instructions. Estimation of serum TGs was done according to the method of Fassati and Preni18. Estimation of serum TC was done as previously described19. Estimation of serum LDL-C was done by using the formula of Friedewald et al., stating that LDL-C = [TC – HDL-C – TG/S]20. Estimation of serum HDL-C was done according to the method of Burstein21. Serum IL-6 was estimated by immunosassay as described earlier22. Blood FBG level was measured by the method of Goodwin23. Estimation of white blood cells (WBCs) count was done by the method of Berkson24. Tissue manipulation for preparation of slides for histopathology was performed according to the method described by Bancroft and Steven25.

2.5 Statistical analysis

Results are presented as mean ± standard error of mean (SEM). All the statistical analyses were performed using one way analysis of variance (ANOVA) with turkey’s post hoc comparison test applied across treatment groups. Significance was based on p value < 0.05. Data analysis was accomplished using the computer software program statistical package for social sciences (SPSS; version 20).

3 Results

Administration of STZ to experimental rats resulted in significant elevations of serum levels of glucose, HMG-CoA reductase, TGs, TC, LDL-C, CRP and IL-6, pancreatic levels of, blood level of FBG and blood count of WBCs, as compared with normal rats. Conversely, STZ administration significantly reduced serum level of LDL-C and pancreatic levels of GSH, GST, SOD and CAT, as compared with normal rats.

Treatment of STZ-subjected rats with LOVA, MET, GLIC, LOVA plus MET or LOVA plus GLIC significantly corrected parameters related to pancreatic damage, including serum glucose level and pancreatic tissue levels as compared to STZ control values. Combination of LOVA with MET or GLIC resulted in significantly better restorations of serum glucose and pancreatic tissue TBARS, GSH, NO2-, GSH and SOD levels as compared with MET or GLIC monotherapies. Pancreatic tissue CAT level was significantly corrected only in LOVA plus GLIC group as compared to STZ control group (Table 1).

Serum markers of dyslipidemia, namely HMG-CoA reductase, TGs, TC, LDL-C and HDL-C, were significantly corrected by LOVA, MET, GLIC, LOVA plus MET or LOVA plus GLIC treatments when compared with STZ control rats. HMG-CoA reductase activity returned back to normal levels with MET, LOVA plus MET and LOVA plus GLIC treatments. HDL-C level was increased above normal control level, except for the GLIC group which was not significantly different from normal control group. Addition of LOVA to MET significantly corrected levels of TGs, TC, LDL-C and HDL-C to degrees better than LOVA or MET monotherapy. Moreover, addition of LOVA to GLIC significantly corrected TGs and LDL-C levels as compared to either drug alone (Table 2).

Administration of LOVA, MET, GLIC, LOVA plus MET or LOVA plus GLIC to rats injected with STZ significantly corrected serum levels of CRP and IL-6, in addition to blood FBG level and WBCs count as compared to rats injected with STZ alone. LOVA plus GLIC group showed blood FBG level not significantly different from that of normal control group. Combination of LOVA with MET significantly corrected CRP, IL-6, FBG and WBCs count values to degrees significantly better than LOVA or MET monotherapies. Similarly, combined effect of LOVA and GLIC on CRP, IL-6, FBG and WBCs count was significantly different from individual effects of LOVA and GLIC monotherapies (Table 3).

Results of histopathological study showed that STZ administration caused atrophy of islets of Langerhans in addition to perivascular edema and inflammatory infiltration. Treatment with either LOVA, MET or GLIC alone corrected such histopathological features partially, but combination of LOVA with MET or GLIC normalized pancreatic histological architecture (Fig. 1-7).

4 Discussion

In the present investigation, the modulatory effect of LOVA, a well-known statin used for treatment of hyperlipidemia, on the beneficial effects of traditional antidiabetics, namely the biguanide drug MET and the sulfonylurea drug GLIC, was studied on adult male Swiss
rats with experimental STZ-induced diabetes mellitus (DM) regarding pancreatic and cardiovascular complications. Results of the present work showed that a single i.p. dose of STZ in rats resulted in pancreatic injury evidenced by elevation of blood glucose level coupled with oxidative injury of pancreatic tissue represented as elevated TBARS and NO\textsuperscript{2-} levels and suppressed GSH, GST, SOD and CAT levels (Table 1). STZ-induced diabetes was coupled with inflammatory and hematological disorders represented as elevations of serum CRP and IL-6 levels in addition to elevations of blood FBG level and WBCs count (Table 3). Histopathological study strongly supported these biochemical estimations (Fig. 1-2).

Table 1: Effect of LOVA, MET, GLIC and their combinations on serum glucose level and pancreatic contents of TBARS, GSH, NO\textsuperscript{2-}, GST, SOD AND CAT in STZ-treated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment Regimen</th>
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<tbody>
<tr>
<td></td>
<td>Control\textsuperscript{1}</td>
</tr>
<tr>
<td>Serum Glucose (mg/dl)</td>
<td>95.58 ± 2.355</td>
</tr>
<tr>
<td>TBARS content (µmol/g)</td>
<td>5.90 ± 0.051</td>
</tr>
<tr>
<td>GSH content (µmol/g)</td>
<td>4.43 ± 0.041</td>
</tr>
<tr>
<td>GST activity (µmol/g)</td>
<td>2.52 ± 0.019</td>
</tr>
<tr>
<td>Total NO\textsuperscript{2-} content (µmol/g)</td>
<td>18.00 ± 0.683</td>
</tr>
<tr>
<td>SOD activity (U/g)</td>
<td>0.42 ± 0.027</td>
</tr>
<tr>
<td>CAT activity (U/g)</td>
<td>0.00021 ± 0.00012</td>
</tr>
</tbody>
</table>

Data are presented as mean of 6-7 rats ± SEM. *Control group, received only vehicles; STZ control group, administered a single i.p. dose of STZ (50 mg/kg); *LOVA group, administered LOVA (15 mg/kg/day, p.o.) daily for 30 days after STZ; *MET group, administered MET (100 mg/kg/day, p.o.) daily for 30 days after STZ; *GLIC group, administered GLIC (20 mg/kg/day, p.o.) daily for 30 days after STZ; *LOVA plus MET group, administered LOVA and MET daily for 30 days after STZ; *LOVA plus GLIC group, administered LOVA and GLIC daily for 30 days after STZ. Statistical analysis was performed by using one way analysis of variance (ANOVA) with turkey's post hoc comparison test. Significantly different from normal control group at p < 0.05. Significantly different from STZ control group at p < 0.05.

The diabetogenic agent STZ is a glucopyranose derivative of 1-methyl-1-nitrosourea, and the existence of 2-deoxy-d-glucose in its structure expedites the preferential uptake of STZ through Glucose transporter 2 (GLUT2) into the pancreatic β-cells\textsuperscript{27}. In a previous work, the diabetogenic action of STZ has been explained to cause alkylation of DNA together with production of nitric oxide and free radicals, leading to pancreatic damage and decreased insulin biosynthesis\textsuperscript{28}. This is in harmony with our work results showing hyperglycemia coupled with oxidative damage of pancreatic tissue caused by STZ administration.

Dyslipidemia is common in patients with DM and is considered as a risk factor for the progression of other cardiovascular disease (CVD) like atherosclerosis\textsuperscript{29}. Generally, lipoprotein abnormalities, presented as increased plasma levels of very low density lipoproteins (VLDL), LDL and TGs, are common in diabetics. In agreement with our results, previous investigators showed similar dyslipidemia in rats with experimental diabetes\textsuperscript{30}. Deficiency of insulin in diabetic rats is believed to be associated with increased HMG-CoA reductase activity, mediating many steps in the process of dyslipidemia\textsuperscript{31}.

Serum level of CRP appears to predict future CVD events, among apparently healthy individuals, such as stroke, myocardial infarction.
and perivascular disease. Release of proinflammatory cytokines as IL-6 is considered a key feature in the process of atherogenesis in patients with DM. Additionally, the increased plasma concentrations of IL-6 is associated with the development of insulin resistance and type 2 DM, since these cytokines are potential to suppress the action of insulin through interfering with insulin receptor-mediated signal transduction. In agreement with our work results, previous investigators reported elevated serum levels of such inflammatory mediators in experimentally-induced diabetes.

Serious cardiovascular complications of diabetes, including peripheral arterial disease (PAD) and coronary artery disease (CAD), are strongly associated with hyperfibrinogenemia. Fibrinogen mediates all steps of atherosclerosis, starting from plaque formation through thromboembolism over a ruptured plaque, leading to myocardial infarction and other complications. A significant elevation of plasma fibrinogen was evident in the present investigation, which is in agreement with previous investigations. In addition, leucocytosis was previously reported to predict progression of diabetes as well as future CVD. Our results showing elevated leucocyte count in diabetic rats come in accordance with previous investigations.

Results of the present study showed that combination of LOVA with either MET or GLIC to rats significantly reduced STZ-induced pancreatic injury compared to rats receiving either drug alone, evidenced by suppression of blood glucose level coupled with improvements of oxidative markers of pancreatic injury including TBARS, GSH, GST, NO2- and SOD (Table 1). These results suggest that the beneficial action of LOVA may be related to its antioxidant power, ameliorating diabetes-induced oxidative and nitrosative stress. In harmony with our findings, previous investigators reported protective potential for statins against oxidative and nitrosative stress. This is particularly important as oxidative stress associated with decreased glycemic control has key effects in the pathogenesis of diabetic complications. Nitrosative stress, associated with increased nitric oxide (NO) production, is also an important event in the pathogenesis of diabetic complications. It was previously reported that NO production increases in early stages of diabetes progression, and that inhibition of NO production may be beneficial in antagonizing STZ-induced diabetes.

According to our work results, combination of LOVA with MET or GLIC revealed better improvements of parameters of dyslipidemia, including serum TGs, TC, LDL and HDL, as compared to LOVA, MET or GLIC monotherapies (Table 2).

These effects are related to cholesterol-lowering effect of LOVA, which is an inhibitor of HMG-CoA reductase enzyme that converts HMG-CoA reductase to mevalonate, the building unit of cholesterol biosynthesis. This hypocholesterolemic effect of LOVA is particularly beneficial in diabetes as it is known to cause dyslipidemia. Dyslipidemia associated with diabetes results from the decreased inhibitory effect of insulin on lipolysis caused by decreased insulin production or increased insulin resistance. Stimulated lipolysis in adipocytes causes release of free fatty acids, the latter being transported to liver, re-esterified to TGs and used for assembly of VLDL. Thereafter, serum LDL level increases and HDL level decreases. This diabetic dyslipidemia, known as atherogenic dyslipidemia, is a risk factor for atherosclerosis and consequently many cardiovascular complications. This strongly supports the idea of adding hypocholesterolemic agents with antidiabetics concerned in the present study.

Results of the present work showed a significant modulatory potential of LOVA on MET and GLIC effects on serum CRP and IL-6 levels, apparent from significant differences between LOVA plus MET or LOVA plus GLIC groups in comparison with corresponding groups receiving monotherapies (Table 3). In agreement, previous investigators reported similar effect of statins on both CRP and IL-6, mostly attributed to beneficial LOVA effect on dyslipidemia. Effect of LOVA on CRP and IL-6 levels is important in diabetes management as serum CRP and IL-6 levels are indices of acute phase inflammation and early markers of diabetes incidence. CRP is synthesized in the liver under the influence of factors released from adipocytes in dyslipidemic conditions and can exacerbate complement-dependent ischemic necrosis leading to myocardial infarcts. Moreover, the pro-inflammatory cytokine IL-6 is an important mediator in the pathogenesis of diabetes and diabetic complications. It increases peripheral insulin resistance and increases atherosclerotic risk in diabetics. Recently, cytokines were reported not only to be released in response to diabetic inflammatory state, but also to have a mechanistic role in beta islet destruction in diabetes progression, and it was also reported that therapeutic manipulation of cytokines leads to improved glycemic control in diabetics. These findings magnify the beneficial role of LOVA on vascular and even non-vascular diabetic complications.

Results of the present work showed that LOVA combination with either MET or GLIC significantly improved their suppressant effect on blood FBG level (Table 3). The antithrombogenic effect of statins is attributed to lipid-lowering potential, correcting hemostatic abnormalities resulting from diabetic dyslipidemia. The effect of LOVA on blood FBG level is particularly important in diabetes as hyperfibrinogenemia is a key factor in atherosclerotic and inflammatory complications. FBG mediates platelet cohesion and reversible RBCs aggregation, enhancing atherosclerosis, plaque formation and formation of thrombus over ruptured plaques. Additionally, FBG degradation products bind LDL, thus enhancing
dyslipidemia, and stimulate cell proliferation and migration, thus enhancing inflammatory cascades.

Table 2: Effect of LOVA, MET, GLIC AND their combinations on serum HMG-COA reductase, TGS, TC, LDL-C and HDL-C in STZ-treated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control 1</th>
<th>STZ 2</th>
<th>STZ 2 + LOVA</th>
<th>STZ 2 + MET</th>
<th>STZ 2 + GLIC</th>
<th>STZ 2 + (LOVA plus MET)</th>
<th>STZ 2 + (LOVA plus GLIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum HMG-CoA reductase (ng/ml)</td>
<td>0.54</td>
<td>1.22a</td>
<td>0.33ab</td>
<td>0.63b</td>
<td>0.82ab</td>
<td>0.44b</td>
<td>0.55b</td>
</tr>
<tr>
<td>TGs (mg/dl)</td>
<td>94.50</td>
<td>151.16a</td>
<td>40.00ab</td>
<td>67.33b</td>
<td>70.55ab</td>
<td>31.16ab</td>
<td>35.33ab</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>70.83</td>
<td>112.33a</td>
<td>64.16ab</td>
<td>81.16b</td>
<td>90.83ab</td>
<td>58.83ab</td>
<td>62.16ab</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>20.60</td>
<td>68.10a</td>
<td>15.00ab</td>
<td>32.36b</td>
<td>48.16ab</td>
<td>8.46b</td>
<td>12.93ab</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>30.83</td>
<td>14.00a</td>
<td>41.16ab</td>
<td>35.33b</td>
<td>28.66b</td>
<td>44.83b</td>
<td>42.16ab</td>
</tr>
</tbody>
</table>

Data are presented as mean of 6-7 rats ± SEM.  1Control group, received only vehicles.  2STZ control group, administered a single i.p. dose of STZ (50 mg/kg).  3LOVA group, administered LOVA (15 mg/kg/day, p.o.) daily for 30 days after STZ.  4MET group, administered MET (100 mg/kg/day, p.o.) daily for 30 days after STZ.  5GLIC group, administered GLIC (20 mg/kg/day, p.o.) daily for 30 days after STZ.  6LOVA plus MET group, administered LOVA and MET daily for 30 days after STZ.  7LOVA plus GLIC group, administered LOVA and GLIC daily for 30 days after STZ.  8Statistical analysis was performed by using one way analysis of variance (ANOVA) with turkey's post hoc comparison test.  9Significantly different from normal control group at p < 0.05.  10Significantly different from STZ control group at p < 0.05.

Table 3: Effect of LOVA, MET, GLIC and their combinations on serum levels of CRP AND IL-6, as well as blood FBG level and WBCS count in STZ-treated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control 1</th>
<th>STZ 2</th>
<th>STZ 2 + LOVA</th>
<th>STZ 2 + MET</th>
<th>STZ 2 + GLIC</th>
<th>STZ 2 + (LOVA plus MET)</th>
<th>STZ 2 + (LOVA plus GLIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum CRP (ng/ml)</td>
<td>0.60</td>
<td>3.36a</td>
<td>2.19ab</td>
<td>1.45ab</td>
<td>1.86ab</td>
<td>0.94ab</td>
<td>1.16ab</td>
</tr>
<tr>
<td>Serum IL-6 (pg/ml)</td>
<td>5.70</td>
<td>10.51a</td>
<td>8.43ab</td>
<td>7.40ab</td>
<td>8.81ab</td>
<td>6.46ab</td>
<td>7.36ab</td>
</tr>
<tr>
<td>Blood FBG (mg/dl)</td>
<td>173.00</td>
<td>567.85a</td>
<td>211.17ab</td>
<td>220.50ab</td>
<td>199.00ab</td>
<td>181.75ab</td>
<td>176.00b</td>
</tr>
<tr>
<td>Blood WBCs (count/mm³)</td>
<td>7498.30</td>
<td>19295.00a</td>
<td>8315.00ab</td>
<td>8388.30ab</td>
<td>9293.30ab</td>
<td>6998.30ab</td>
<td>7802.50b</td>
</tr>
</tbody>
</table>

Data are presented as mean of 6-7 rats ± SEM.  1Control group, received only vehicles.  2STZ control group, administered a single i.p. dose of STZ (50 mg/kg).  3LOVA group, administered LOVA (15 mg/kg/day, p.o.) daily for 30 days after STZ.  4MET group, administered MET (100 mg/kg/day, p.o.) daily for 30 days after STZ.  5GLIC group, administered GLIC (20 mg/kg/day, p.o.) daily for 30 days after STZ.  6LOVA plus MET group, administered LOVA and MET daily for 30 days after STZ.  7LOVA plus GLIC group, administered LOVA and GLIC daily for 30 days after STZ.  8Statistical analysis was performed by using one way analysis of variance (ANOVA) with turkey's post hoc comparison test.  9Significantly different from normal control group at p < 0.05.  10Significantly different from STZ control group at p < 0.05.

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Fig 1: A photomicrograph of pancreas section obtained from normal adult male albino rat, showing normal histological structure of the islands of Langerhans cells (s) as endocrine portion as well as the acini (a) as exocrine portion.

Fig 2a: A photomicrograph of pancreas section obtained from adult male albino rats treated with a single dose of streptozotocin (STZ, 50 mg/kg, i.p.), showing atrophy of islands of Langerhans cells (s).

Fig 2b: A photomicrograph of pancreas section obtained from adult male albino rats treated with a single dose of streptozotocin (STZ, 50 mg/kg, i.p.), showing dilatation of blood vessels (V) with perivascular edema and inflammatory cells infiltration (m).

Fig 3a: A photomicrograph of pancreas section obtained from adult male albino rats treated with a single dose of streptozotocin (STZ, 50 mg/kg, i.p.) followed by 30 consecutive days of oral daily administration of lovastatin (LOVA; 15 mg/kg/day), showing atrophy of islands of Langerhans cells (s).

Fig 3b: A photomicrograph of pancreas section obtained from adult male albino rats treated with a single dose of streptozotocin (STZ, 50 mg/kg, i.p.) followed by 30 consecutive days of oral daily administration of lovastatin (LOVA; 15 mg/kg/day), showing multiple number of newly formed pancreatic duct (d).

Fig 4a: A photomicrograph of pancreas section obtained from adult male albino rats treated with a single dose of streptozotocin (STZ, 50 mg/kg, i.p.) followed by 30 consecutive days of oral daily administration of metformin (MET; 100 mg/kg/day), showing atrophy of islands of Langerhans cells (s).
Finally, results of the present work revealed that STZ-induced leucocytosis was improved by LOVA addition to MET or GLIC to degrees significantly better than either drug alone (Table 3). In agreement, previous authors reported similar effect of statins on leucocyte count and activation\textsuperscript{56}. This effect may be attributed to the fact that statins have the selective ability to inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site\textsuperscript{57}. Additionally, the anti-inflammatory and cytokine-lowering effect of statins logically decreases leucocyte activation and proliferation\textsuperscript{49,58}.

Results of histopathological examination were in complete harmony with biochemical findings, where rats co-treated with LOVA plus MET or with LOVA plus GLIC after STZ showed almost normalized pancreatic histology, which is not the case in rats treated with either drug alone (Fig. 3-7).

5 Conclusion

Results of the present study conclude that combination of LOVA with conventional antidiabetics has great beneficial effects on diabetes-induced pancreatic and cardiovascular complications, including hyperglycemia, oxidative and nitrosative stress, dyslipidemia, inflammatory disorders, hyperfibrinogenemia and leucocytosis. These results are promising for further clinical trials and strongly suggest combining LOVA with antidiabetics in clinical cases of DM.

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7 Competing interests

No conflicts of interest are evident.
8 Author’s contributions

Ehab A.M. El-Shoura: Practical work
Basim A.S. Messiha: Manuscript preparation and submission
Amira M. Abo-Youssef: Data collection and resources
Ramadan A.M. Hemeida: Work idea and general supervision

9 References


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