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Differential and Combined Effects of Simvastatin and Vildagliptin on Angiogenic Markers and Oxidative Stress in Hind Limb Model of Ischemia in Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Background: This study investigated the potential therapeutic effect of simvastatin and vildagliptin and their combination on angiogenesis in diabetic hind-limb ischemia.
Methods: 60 Sprague-dawely rats were divided into 5 groups, group A (normal control), group B (diabetic ischemic control), group C (simvastatin treated diabetic ischemic group), group D (vildagliptin treated diabetic ischemic group) and group E (combined sim. + vild. diabetic ischemic group). Parameters of angiogenesis as well as oxidative stress markers were evaluated.
Results: Increased capillary density in ischemic gastrocnemius tissue of diabetic rats treated with either simvastatin or vildagliptin with marked increase in its combination. This effect was accompanied by up-regulated plasma levels of HO-1, nitric oxide, vascular endothelial growth factor (VEGF) and expression of HIF-1 alpha levels. Tissue SOD and Catalase enzymes activities were normalized in groups treated with simvastatin or their combination with vildagliptin with concomitant decrease of lipid peroxidation.
Conclusion: Both vildagliptin and simvastatin has antioxidant and angiogenic effects and their combination could be a promising strategy in the management of diabetes associated peripheral arterial disease.

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Keywords: Simvastatin; vildagliptin; oxidative stress; diabetes; angiogenesis.

1. INTRODUCTION

Peripheral arterial disease is one of the major macro vascular complications of diabetes where the incidence and severity of limb ischemia increases in diabetic patients [1].

Treatment strategies for intensive blood-glucose control substantially decrease the risk of micro vascular complications but not macro vascular ones [2] hence other strategies rather thanglucose controlare therefore necessary to reduce the risk of macro vascular complications in diabetes.

Therapeutic angiogenesis, a strategy to cure tissue ischemia by promoting collateral growth, has emerged as one of the most promising therapies for ischemic diseases [3].

Large numbers of molecules are involved in the angiogenic cascade [4] including vascular endothelial growth factor (VEGF), nitric oxide, hemeoxygenase-1(HO-1) and hypoxia inducible factor-1 α (HIF-1α).

VEGF is the main factor responsible for angiogenesis and vascular permeability. Its expression is regulated mainly by oxidative stress [5], hypoxia [6], and nitric oxide [7]. The regulation of capillary growth by nitric oxide is complex due to both angiogenic and antiangiogenic effects of nitric oxide [8,9]. Moreover, the role of nitric oxide synthase isoforms in angiogenesis is controversial.

HO-1has been suggested to have a role in angiogenesis in vitro, however, the relevance of this in vivo remains unknown. HO-1 is induced during hypoxia, ischemia/reperfusion, and inflammation, providing cytoprotection and inhibiting leukocyte migration to inflammatory sites.

A mutual relationship between VEGF and HO-1 has been reported [10]. It has been reported clinically and experimentally that elevated doses of VEGF may cause fragile neovascularization, which in turn would lead to bleeding and micro vascular leakage [11,12,13].

HIF-1α is a transcriptional activator of vascular endothelial growth factor (VEGF) and inducible nitric oxide (NO) synthase (iNOS) and its Peak of expression may contribute to limitation of hypoxic injury by promoting angiogenesis and wound healing [14]. It has been shown that diabetes impairs HIF-1α and VEGF expressions [15,16], as well as low levels of HIF-1α expression have been evidenced in foot ulcer biopsies in patients with diabetes [17]. Finally, the oxidative stress activity has a role in its degradation by the proteasome pathway [18].

Thus, therapeutic angiogenesis that does not involve the administration of excessive amounts of angiogenic growth factors is mandatory.

The incidence of vascular complications in patients with vascular disease are found to be reduced by statins [19]. Statins have antioxidant, antithrombotic, anti-inflammatory, fibrinolytic, and angiogenic effects and also increase nitric oxide production [20,21]. Some beneficial effects of statins that evidenced in several clinical [22,23] and experimental

studies [24,25] are independent of their lipid-lowering properties and are due to their pleiotropic effects.

Vildagliptin is a selective and potent DPP-4 inhibitor that inhibits rapid degradation of endogenous GLP-1 and GIP, and increases α- and β-cell responsiveness to glucose, thereby improving glycemic control in T2DM [26].

Vildagliptin has a strong binding ability to DPP-4 and a long half-life, so it is more potent than other DPP-4 inhibitors such as sitagliptin in suppressing glucagon, and causes less glycemic variation [27].

Experimental and pathological studies suggest that incretin hormone glucagon-like peptide-1 (GLP-1) may improve VEGF generation, [28] and promote the upregulation of hypoxia inducible factor-1(HIF-1α) through a reduction of oxidative stress [29].

on the other hand, the effect of the augmentation of GLP-1, by inhibitors of the dipeptidyl peptidase-4 (DPP-4), such as vildagliptin, on oxidative stress and angoigenic markers have been evaluated in wound healing in diabetic chronic ulcers [30].

Therefore, this current study aimed to compare the angiogenic and antioxidant effects of simvastatin and vildagliptin in hind-limb ischemia model in diabetic rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Streptozotocin was purchased from (Sigma Pharmaceutical Co., Quesna, Egypt), Simvastatin was purchased from (Sigma Pharmaceutical Co., Quesna, Egypt). Vildagliptin was purchased from (Santa Cruz Biotechnology, Santa Cruz, CA, USA). VEGF Immunoassay kit [Quantikine, ELISA] was purchased from R&D systems (Minneapolis, USA). Plasma HO-1 levels were determined using Rat HO-1Enzyme Linked Immunosorbent Assay (ELISA) Kit (Stressgens, MI, USA). Commercially available kits (Bio-Diagnostic, Egypt) were used for determining nitric oxide, the enzymes superoxide-dismutase and catalase and the lipid-peroxide marker malondialdehyde. All other chemicals were obtained from Sigma (St. Louis, MO, USA).

2.2 Animals

All animal procedures and the experimental protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals. Male Sprague dawely rats weighing 200–220 g were obtained from the research center of our university (MERC) and housed under controlled temperature (25±1ºC) on a 12h light/dark cycle. Food and water were allowed ad libitum during the study period.

2.3 Induction of Type II Diabetes

The animals were acclimatized for one week before initiation of the experiment. After overnight fasting, rats were injected intraperitoneally with freshly prepared streptozotocin (60 mg/kg) or vehicle (0.130 mol/L citrate-phosphate buffer, pH 4.5) according to the references [31,32]. Blood samples were obtained by tail prick, and blood glucose concentration

measured using a blood glucose meter (B. Braun, Germany), 72 h later, only rats with fasting glucose concentrations (≥ 300mg/dL) were considered diabetic and assigned for different treatment regimens.

2.4 Pharmacological Treatment

Sixty Sprague-dawely male rats (220-240 g) were randomly divided into 5 groups, 12 rats each, and were treated as follows. The first group received vehicle injection and served as normal control. A second group received vehicle injection and served as diabetic control. A third group received simvastatin (1mg/kg, ip) [33] It was freshly dissolved in a solution of 30% DMSO and 70% normal saline and given daily until the day of sacrifice [34]. Fourth group were treated with vildagliptin [10 mg/kg] [35]. It was freshly dissolved in a solution of 30% DMSO and 70% normal saline and given daily until the day of sacrifice. Fifth group were treated ip with combination of simvastatin (1mg/ kg,ip) and vildagliptin (10mg/kg/ day, ip). All treatments started after the onset of diabetes and continued daily for six weeks. At the end of experiment, six surviving rats from each group were randomly selected for further analysis.

2.5 Induction of Hind Limb Ischemia (Tourniquet Method)

All the rats in each group underwent 4h of lower extremity ischemia and 24h of reperfusion. Rats were anesthetized with a mixture of ketamine (50mg/kg,ip)/xylazine (10mg/kg,ip). Occlusion of the lower extremity was performed by the tourniquet method. The tourniquet (rubber band)was looped six times briefly as proximally as possible on the thigh. After 4h of ischemia, reperfusion was initiated by releasing the tourniquet. The rats were hydrated by an intraperitoneal injection of 40mg/kg normal saline every 2h for 8 h and allowed free access to water [33].

2.6 Collection of Serum and Tissue Samples

After animal scarification blood samples were collected on EDTA and centrifuged at 1000xg for 15 min, plasma samples were separated and stored at 4ºC for the determination of VEGF and HO-1. Gastrocnemius muscle was excised and divided into 2 portions. One portion was rinsed in cold saline, plotted dry, weighted, and frozen immediately at -80ºC for homogenization. The other portion was fixed in 10% neutral buffered formalin for immunohistochemical determination of capillary density.

2.7 Analysis of Capillary Density

To assess angiogenic response, capillary density counts were evaluated in tissue sections incubated with monoclonal anti- PECAM-1 after being diluted to 1:50 with PBS. Ten fields from each slide were examined at 400X and capillary density (mean number of capillaries/mm^2) was determined.

2.8 Determination of Plasma VEGF

Plasma VEGF levels were determined using enzyme linked immunosorbent assay (ELISA). Plasma samples were subjected to mouse VEGF ELISA in duplicate following the manufacturer protocol (R&D systems).

2.9 Determination of Plasma HO-1

Plasma HO-1 levels were determined using ELISA following the manufacturer protocol.

2.10 Measurement of RNA (RT-PCR) For HIF-1 A

Each sample was analyzed for the presence of transcripts encoding HIF-1α as already published [36]. Total RNA was extracted using RNAzol reagent (Biotecx Laboratories) according to the manufacturer's protocol. Levels of HIF-1α RT-PCR amplification by using the following primer sequences:

HIF-1α 5'3' = CTGCTTGGTGCTGATTTGTGA and 3'5' = TCCTGTACTGTCCTGTGGTGA
complementary to the rat HIF-1α gene

2.11 Determination of Tissue Nitric Oxide

Nitrite concentration was measured in tissue samples as indicator of nitric oxide production using Griess reaction [37].

2.12 Lipid Peroxidation Level in Gastrocnemius Muscle

Levels of malondialdehyde as thiobarbituric acid-reactive substances were measured in tissue homogenate by the method of Ohkawa [38].

2.13 Antioxidant Enzyme Activities in Gastrocnemius Muscle

The frozen individual tissue samples were homogenized in 50 mM phosphate buffer (10%W/V), pH7.4, using Glass-Col tissue homogenizing system(Cole-Parmer, VernonHills, USA). Supernatants obtained upon centrifugation at 8000xg at 4ºC were used for measuring the activity of the antioxidant enzymes superoxide-dismutase and catalase using standard spectrophotometric assays. Briefly, superoxide-dismutase activity in the tissue homogenate was determined by generating superoxide radicals by the photochemical reduction of phenazine methosulphate, which reduces nitro blue tetrazolium into a blue-colored compound, formazane. Superoxide-dismutase quenches free oxygen radicals and inhibits reduction of nitro blue tetrazolium, which was measured at 560nm [39]. Catalase assay was carried out by checking the rate of hydrogen peroxide degradation at 510nm in presence of the homogenate [40].

2.14 Statistical Analysis

The data were expressed as mean±S.D. Statistical significance was tested by one way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis. A pvalue $\leq$0.05 was considered statistically significant.

3. RESULTS

3.1 Effect on Capillary Density

In the current study, diabetic control rats with hindlimb ischemia showed a significant increase ($p<0.05$) in capillary density of the gastrocnemius muscle. The treatment with either

simvastatin or vildagliptin significantly increased the capillary density compared to the diabetic control ($p<0.05$). However, its combination with simvastatin significantly increased the capillary density compared to the diabetic control (Figs. 1, 2).

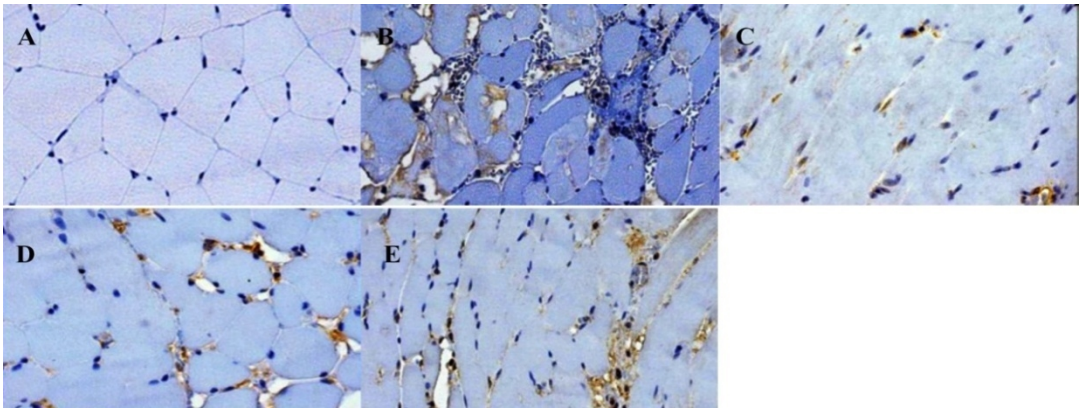

Fig. 1. Effects of simvastatin(1mg/kg, *ip*), and vildagliptin(10mg/kg, *ip*) on capillary density in gastrocnemius muscle of diabetic hind-limb ischemic rats. Capillary density was evaluated immunohistochemically using monoclonal mouse anti-CD31 (PECAM-1, M-20) the counted in 10 different fields and expressed as mean+S.D. Photomicrographs are representative to capillary density (brown) in different groups at 400X magnification. Fig. A (control), B (hind limb ischemia diabetic control), C (Simvastatin treated group), D(Vildagliptin treated), and E (Simvastatin + vildagliptin treated group)

3.2 Plasma Levels of VEGF

A significant increase in plasma VEGF levels was observed in the diabetic control group compared to the normal group. Individual as well as combined treatment with simvastatin and vildagliptin showed a further significant increase in plasma levels of VEGF compared to both normal and diabetic control (Table 1).

Table 1. Effect of simvastatin (1mg/kg, ip) and vildagliptin (10mg/kg, ip) on plasma VEGF (pg/ml) and Hemoxygenase (ng/ml) in hind limb ischemic model of streptozotocin induced type II diabetes in rats (Mean±SD) (n=6)

	VEGF (pg/ml)	Hemeoxygenase (ng/ml)
Group A (normal)	4.5±1.04	2±0.89
Group B (Diabetic)	11.83±1.16[a]	4±0.89[a]
Group C (Simvastatin treated)	17.5±1.04[ab]	14±0.89[ab]
Group D (vildagliptin treated)	14.66±1.03[ab]	5.00±1.41[ac]
Group E (Sim+Vild)	19±0.89[abcd]	16±1.78[abcd]

[a]p<0.001 vs. control group,[b]p< 0.001 vs. diabetic group ,[c]p<0.001 vs. Simvastatin treated group, [d]p<001 vs. vildagliptin treated group

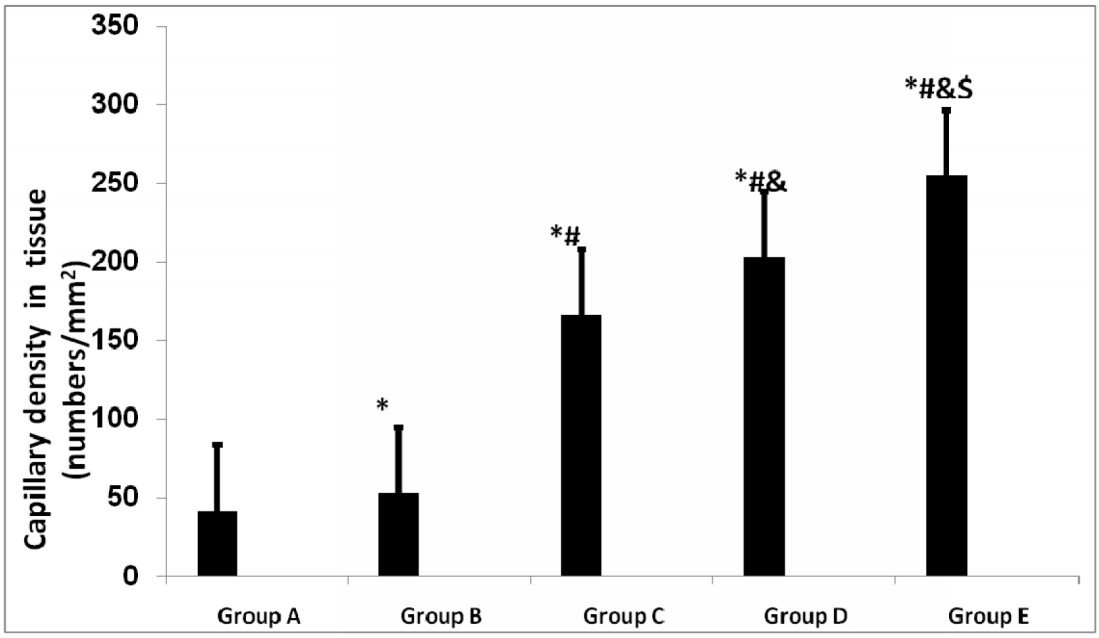

Fig. 2. Effect of simvastatin (1mg/kg*, ip*) and vildagliptin (10mg/kg, *ip*) on tissue capillary density (numbers/mm^2) in the gastrocnemius muscle of the control group (group A), hind limb ischemic diabetic control group (Group B), simvastatin treated group (Group C), vildagliptin treated group (group D) and simvastatin+vildagliptin treated group (Group E). (Mean±SD), n=6. *p<0.05, vs. corresponding control rats; $^{\#}p$< 0.05, vs. corresponding diabetic group, $^{\&}p$< 0.05, vs. simvastatin treated group, $^{\$}p$< 0.05, vs. vildagliptin treated group

3.3 Plasma Levels of HO-1

Hindlimb ischemia in diabetic rats increased plasma HO-1 level in comparison to normal control. Simvastatin significantly increases the level of HO-1 in comparison to diabetic control group. On the other hand, vildagliptin showed no significant effect on HO-1 level in comparison to diabetic group (Table 1).

3.4 Effect on HIF-1α Expression

The RNA expression of HIF-1α is increased in vildagliptin treated group in comparison of diabetic control group. Simvastatin treated group showed no significant effect on RNA expression of HIF-1α. Combined treatment was significantly increase expression of HIF-1α than the individual drugs again (Figs. 3 and 4).

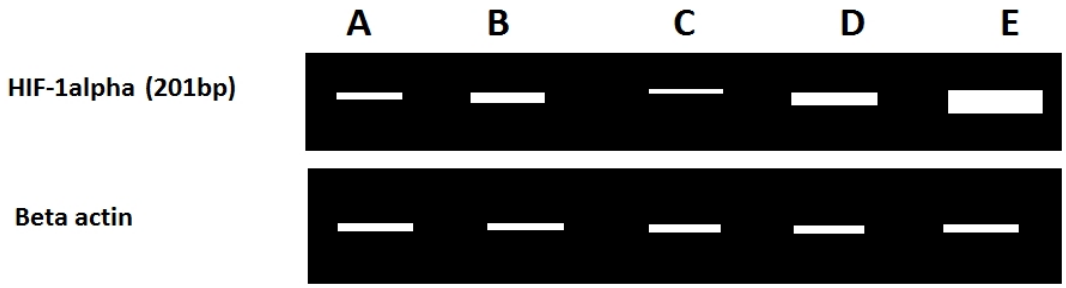

Fig. 3. Reverse-transcriptase polymerase chain reaction (RT-PCR) of HIF-1 alpha in gastrocnemius muscle of control (lane A), hind limb ischemic diabetic control (lane B), simvastatin treated (1mg/kg, ip)(lane C), vildagliptin treated (10 mg/kg, ip)(Lane D) and combination of both drugs (lane E). Ratio to B actin

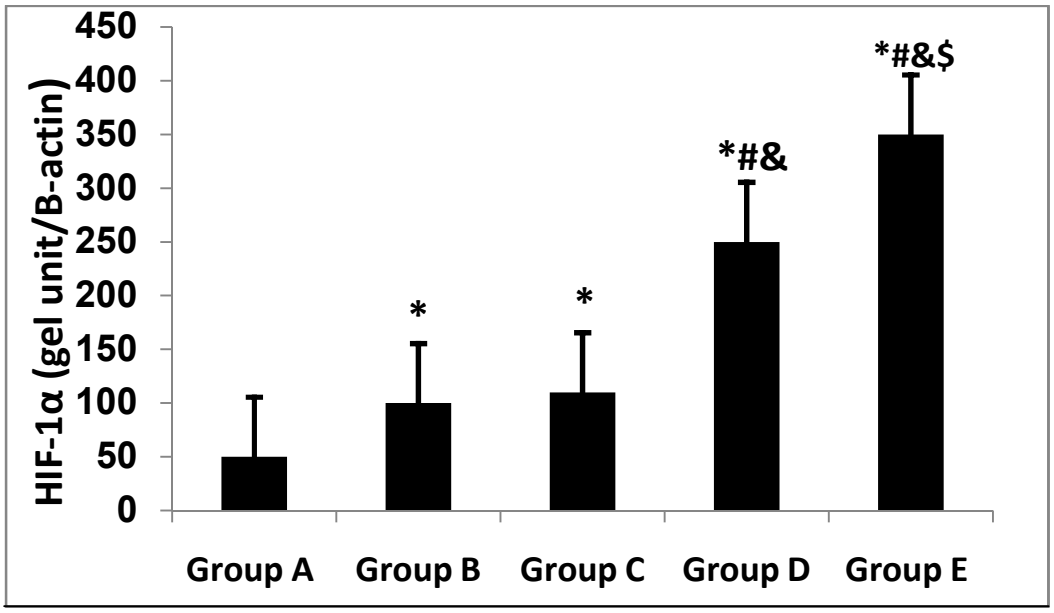

Fig. 4. Densitometry and statistics of HIF-1α mRNA (ratio to-B-actin) in the gastrocnemius muscle of the control group (group A), hind limb ischemic diabetic control group (group B), simvastatin treated group (group C), vildagliptin treated group (group D) and simvastatin+vildagliptin treated group (group E). Mean±SD, n=6 *p<0.05, vs. corresponding control rats; #p< 0.05, vs. corresponding diabetic group, &p<0.05, vs. simvastatin treated group, $$p$< 0.05, vs. vildagliptin treated group

3.5 Tissue Nitric Oxide

The induction of diabetic hind limb ischemia significantly (p<0.05) reduced tissue nitric oxide levels; with over production of reactive oxygen species. Interestingly, the treatment with simvastatin either alone or combined with vildagliptin showed the highest significant induction in nitric oxide levels compared to normal as well as diabetic control (Table 2).

Table 2. Effect of simvastatin (1mg/kg, *ip*) and vildagliptin (10mg/kg, *ip*) on tissue NO (Umol/g tissue), SOD (U/g tissue), CAT (U/g tissue) and MDA (nmol/g tissue) in hind limb ischemic model of streptozotocin induced type II diabetes in rats (Mean±SD) (n=6)

	NO (Umol/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)	MDA (nmol/g tissue)
Group A (normal)	23+0.89	252.5±1.64	442.8±2.31	3.5±1.04
Group B (Diabetic)	13+0.89[a]	122.5±1.87[a]	145.3±2.8[a]	7.8±1.16[a]
Group C (Simvastatin treated)	41+0.89[ab]	183.3±2.16[ab]	282.83±2.48[ab]	5.3±1.03[ab]
Group D (vildagliptin treated)	42.16+0.75[ab]	254.8±2.6[bc]	282.33±1.63[ab]	4±1.41[b]
Group E (Sim+Vild)	63+1.78[abcd]	306.16±3.71[abcd]	503.16±2.63[abcd]	3.16±1.16[bcd]

[a]p<0.001 vs. control group,[b]p< 0.001 vs. diabetic group ,[c]p<0.001 vs. Simvastatin treated group,[d]p<001 vs. vildagliptin treated group.NO: nitric oxide, SOD: superoxide dismutase, CAT: catalase, MDA: malondialdehyde

3.6 Lipid Peroxidation and Antioxidants in Gastrocnemius Muscle (Table 2)

Both simvastatin and vildagliptin significantly reduced the increased levels of MDA in comparison to control and diabetic groups (p<0.05). The combination of both drugs significantly reduced the levels more than the individual drugs alone.

Superoxide dismutase level was significantly increased in simvastatin treated group in comparison to diabetic group however, vildagliptin showed no significant increase in SOD level. The combination of simvastatin and vildagliptin significantly increase the levels more than the individual drugs alone.

Catalase level was significantly increased in both simvastatin and vildagliptin treated group in comparison to diabetic group. The combination of simvastatin and vildagliptin significantly increase the levels more than the individual drugs alone.

4. DISCUSSION

The present study investigated the effectiveness of both simvastatin and vildagliptin treatment either alone or in combination in diabetic hindlimb ischemia model.

In the current study, pretreatment with simvastatin significantly enhanced angiogenesis as was evident by increased capillary density in the ischemic hind limb of treated diabetic rats. Similarly, vildagliptin, either alone or combined with simvastatin, gave a comparable effect.

An induction of ischemia was associated with increased MDA and reduced tissue nitric oxide. These are good indicators of oxidative stress (OS) as well as endothelial dysfunction. High MDA is a reflection of insufficient antioxidant defenses in combating ROS-mediated damage that consistent with decreased SOD and catalase level in diabetic rate group. Superoxide (O_2^-) is reported to inhibit catalase directly [41].

Pancreatic B-cells are highly prone to oxidative stress and damage because they have low expression and activity of antioxidant enzymes, which are the first line of defense against oxidative insult [42].

When the burst of reactive oxygen species (ROS) reaches threshold, it can induce mitochondrial swelling [43] which subsequently lead to an activation of the apoptotic pathway and eventually cell death [44].

However, animals treated with 10 mg/kg vildagliptin showed significantly increase in catalase activity which could minimize the deleterious effects of hydrogen peroxide with subsequent decrease in MDA as reported by Avila et al. [41].

A number of mechanisms may underlie improvement of oxidative stress marker by vildagliptin. First, Vildagliptin could decrease the expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits resulting in decreased ROS production with GLP-1 up-regulation [45].

Second, vildagliptin reduces expression of genes related to oxidative stress and ER stress-related cell apoptosis. This suppressive effect could be mediated secondary to improvement in glycaemic and lipid metabolism as reported by [46].

Whiting et al. [47] have observed that chronic hyperglycemia lead to excess free radical generation which may eventually lead to increased lipid peroxidation and depletion of antioxidants, and thereby enhanced OS. This in turn may contribute to the development of microvascularandmacrovascular complications [48].

Moreover, Lamers et al. [49] have been reported that DPP-4 impairs insulin signalingintype 2 diabetes mellitus (T2DM). Insulin resistance with a concomitant hyperinsulinemia may increase ROS generation and contribute to oxidative stress [50]. Inhibiting DPP-4 by vildagliptin may directly influence the insulin resistant effect of circulating DPP-4 itself [51].

However, OS has been associated with glycemic variability over a daily period as assessed from the mean amplitude of glycemic excursions (MAGE) [52].

Therefore, Vildagliptin might reduce the markers of oxidative stress as well as systemic inflammation by controlling of MAGE as reported by [51].

Also, decreased OS could be attributed to suppression of leucocyte activation and recruitment to the vessel wall as reported by [52].

An important source of ROS is activated leucocytes (most probably myelomonocytic cells) [53]. These cells adhere to and infiltrate the vascular wall leading to increased production of ROS primarily by the phagocytic NADPH oxidase within the vasculature and scavenging of nitric oxide; this diffuses freely from the endothelium to the smooth muscle cell layer. The reaction of endogenous nitric oxide with superoxide has the potential to decrease the bioavailability of this important vasodilator and promote formation of the highly toxic intermediate peroxynitrite that damage endothelial cells.

Heat shock protein (HSP)-72 is a major inducible HSP against several stresses as heat, ischemia or hypoxia, which protects cells through c-jun N-terminal kinase (JNK) inhibition [54]. HSP72 level is decreased in type 2 diabetes and restoration of HSP72 improves insulin resistance, glucose homeostasis in mice and attenuate NF-κB nuclear translocation [55,56]. Thus, Vildagliptin might up-regulate HSP72 ion as reported by [51].

Moreover, Vildaglibtinthrough an increase circulating levels of GLP-1 could improve endothelium-dependent vascular responses while leaving endothelium-independent responses unaffected in healthy humans and patients with T2DM [57].

Rats treated with 1 mg/kg Simvastatin showed significantly increases the level of HO-1 in comparison to diabetic control group this is attributed to the induction of HO-1by Statin as reported by Mrad et al. [58]. Statin also increased the expression of HO-1 in human endothelial cells.

The statin-dependent upregulation of endothelial HO-1 is regulated mainly by the stabilization of HO-1 mRNA via phosphotidylinositol-3-kinase (PI3K)/Akt -dependent signaling pathway [59]. Also, the activation of MAPKs plays a key role in the induction of HO-1 gene expression [60].

An increase of the antioxidative defense protein, HO-1 level, might explain reduced level of MDA by Simvastatin as reported by Kim et al. [61].

HO-1 is an inducible enzyme that catalyzes the degradation of heme to biliverdin with the concomitant release of iron and carbon monoxide. Many studies have revealed the important function of HO-1 as a cytoprotective defense mechanism against oxidative insults through the antioxidant activities of biliverdin and its metabolite, bilirubin, as well as via the anti-inflammatory action of carbon monoxide [62].

We found significant induction in nitric oxide levels and improvement of endothelial function by either Simvastatin or vildaglibtin as well as in combination.

Statin directly enhance NO bioavailability by increasing Akt-dependent eNOS phosphorylation resulting in increased eNOS activity [63] and up-regulating eNOS expression *via* Rho inhibition [64].

In addition, statins decrease Endothelin-1 (ET-1) circulating levels and subsequent expression of adhesion molecules in endothelium of patients with diabetes [65]. ET-1 counteracts NO activities and impairs endothelial function.

[66,67] suggests that statin induce rapid, direct effects on arterial redox state and NO bioavailability in human atherosclerosis via tetrahydrobiopterin-mediated eNOS coupling.

However, an improved endothelial function by statin may also result from inhibition of Rac1-mediated NADPH oxidase activation and attenuated angiotensin II-induced ROS production. Finally statin improves endothelial function by lowering LDL levels [19].

ROS produced under hyperglycemia-mediated oxidative stress directly inhibit insulin-stimulated NO production by enhancing serine phosphorylation of insulin receptor substrate-1(IRS-1) resulting in impaired IRS-1-mediated activation of PI 3-kinase/Akt pathway [68]. Thus, vildagliptin could increase endothelial production of NO indirectly by influencing insulin levels as reported by [69].

In diabetic rats treated with vildagliptin, we detected increased tissue levels of HIF-1α and VEGF blood level. In control diabetic rats, the picture is quite different because both HIF- 1α and VEGF levels were significantly lower than those in vildagliptin-treated diabetic group. Thus, we can speculate that increased proteasome activity, as a consequence of increased

oxidative stress marker (MDA), and reduced tissue nitric oxide levels may enhance the degradation of HIF-1*α*, possibly representing a crucial step in the pathophysiology of ischemic injury [70].

Vildagliptin raised GLP-1 concentrations, which reduce the activation of proteasome by oxidative stress. In this context, the increase tissue HIF-1expression could be a consequence of the decreased HIF-1*α* degradation and hence increase VEGF activation as reported [71,72]. These events might explain an increased capillary density in vildagliptin treated rats than diabetic control. Also, increase VEGF plasma levels in Simvastatin treated group could result from increased VEGF mRNA and protein expressions [34].

However, Simvastatin do not increase RNA expression of HIF. This might be explained by other [73] finding. They reported that Simvastatin increased hypoxia-inducible factor-1alpha (HIF-1alpha) protein level without changing its mRNA expression. On other hand combined treatment by Simvastatin and Vildagliptin increase HIF as statin activate upstream regulators of HIF-1alpha in hypoxia as reported by Zhu et al. [74].

This study confirms that combined treatments which provide multiple targets at the molecular level is superior to single therapy as was evident in the current study by the joint treatment with simvastatin and vildagliptin.

5. CONCLUSION

Both vildagliptin and simvastatin has antioxidant and angiogenic effects and their combination could be a promising strategy in the management of diabetes associated peripheral arterial disease.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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