Original Research Article

Linagliptin in combination with *Emblica officinalis* improves glycemic control through alleviating dyslipidemia and oxidative stress on streptozotocin induced diabetic rats

Abstract:

Objectives: Diabetes is a complex chronic metabolic disorder. Now a day, many diabetic patients are known to use herbal medicines with antidiabetic properties in addition to their mainstream treatments. The study was designed to investigate the hypoglycemic, hypolipidemic and hepatoprotective activity of the fixed dose combination of linagliptin and *Emblica officinalis* (aq FE).

Methods: Diabetes was induced in Wister albino rats through the intraperitoneal administration of streptozotocin (45 mg/kg b.w.). Linagliptin (5 mg/ 70kg b.w), aqueous fruit extract of *Emblica officinalis (*200 mg/kg b.w.) and fixed dose combination therapy of linagliptin (2.5 mg /70kg b.w) with aqueous fruit extract of *Emblica officinalis* (100 mg/kg b.w) were administered orally once daily for four weeks. After that fasting blood glucose level (FBG), low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol (TC) and triglycerides (TG) were measured in serum with streptozotocin (STZ)-induced diabetes. Standard protocols were followed to determine the antioxidant activity by the estimation of catalase (CAT) and superoxide dismutase (SOD) activity. The possible side

effects of the combined therapy, the survival rate and the weight of different groups of rats were also measured.

Results: The combination therapy significantly ($p<0.05$) decreased the FBG, TC, TG, LDL level in compared to the diabetic control group ($p<0.05$). Significant ($p<0.05$) increased of HDL was also observed. The antioxidant activity significantly increased after the administration of fixed dose combination therapy in compared to diabetic control group. These changes were significantly better than those of linagliptin with *Emblica officinalis* (aqFE) monotherapy.

Conclusion: This study suggests that the fixed dose combination therapy of linagliptin and *Emblica officinalis (*aq FE*)* might be potent on antihyperglycemic antidyslipidemic and antioxidative effect.

Key words: *Emblica officinalis, linagliptin*, combination therapy, antihyperglycemic, antidyslipidemic, hepatoprotective, aqueous fruit extract (aqFE).

1. Introduction:

Diabetes mellitus (DM) is a major and growing health problem throughout the world. It is metabolic disorder of carbohydrate, fat, and protein resulting in hyperglycemia due to defects in insulin secretion, insulin action, or both [1]. According to the latest World Health Organization (WHO) data, DM-induced death reached to 28,065 or 3.61% of total deaths in Bangladesh [2] and it increases to 642 million by 2040 [1] in all over the world. Hyperglycemia promotes auto-oxidation of glucose to form free radicals. The production of excess free radicals beyond the scavenging abilities of endogenous antioxidant results in macro- and microvascular dysfunction [3] that were associated with Coronary Heart Disease (CHD) or stroke (by a factor of two to three compared with non-diabetic patients) and cardiovascular disease (CVD) [4-5].

The reduction of antioxidant is another cause to increase the burden of oxidative stress in diabetic patients. Streptozotocin (STZ) increases oxygen free radicals that would causes the necrosis of beta cell on pancreas leading to cause hypoinsulinimia and hyperglycemia. Diabetogenic activity of Streptozotocin can stimulate lipoprotein specifically unsaturated fatty acid containing lipoprotein which in turn stimulates glycation of protein, enzyme inactivation, the alteration in the structure and function of collagen, basement and other membranes and play a role in the long term complications of diabetes [6-7].

Antioxident protect our cell by scavenging free radicals. It was reported that there is an inverse relationship between the dietary intake of antioxidant-rich foods and live style diseases such as obesity, hypertension, diabetes and cardiovascular diseases [8-9]. *Emblica officinalis* also known as amla is one of the most important medicinal plants in various traditional and folk systems of medicine in Southeast Asia [10]. The fruits of *Emblica officinalis* are rich source of vitamin C, minerals, different tannins, amino acids, fixed oils, rutin and quercetin. In the traditional system of medicine it is used for the treatments of antibacterial, antiulcerogenic, antioxidant, antimutagenic, anti-inflammatory, immunomodulatory, antipyretic, analgesic etc [11-14]. Because the high source of vitamin C and act as potential antioxidant, we investigated the fixed dose combination therapy of linagliptin with *Emblica officinalis* (aqFE) potentiates the anti-hyperglycemic, antidyslipidemic and antioxidant activity against monotherapy.

2. Materials and Methods

Chemicals: Streptozotocin used in this study was sourced from Sigma-Aldrich Chemical Company of Saint Loius, Missouri, USA. RANDOX commercial kits were employed for biochemical analysis. All other chemicals employed were of standard analytical grade.

The antidiabetic drug, linagliptin was collected from Square Pharmaceuticals Ltd, Bangladesh.

Preparation of aqueous fruit extract of *Emblica officinalis:*

The fruits were parched from local market of Rajshahi city, Bangladesh, dried under the sunlight and cruse by electric grinder into coarse powder. The coarse powders dissolved in distilled water for 24 h. After that the mixture was strained out using a fine sieve and the crude extract air- evaporated for 3 days [15-16]

Experimental Animals:

Twenty five male Wister rats (150-200 gm) were purchased from Pharmacology Research Laboratory, Department of Pharmacy, Jahangirnagar University. All the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period the rats were kept in a well-ventilated animal house at room temperature of (25°C and were supplied with standard pellets from ICDDRB and fresh drinking water. All the rats were kept in cages and maintained with natural 12 hour light and dark cycle. Ethical clearance was obtained from the institutional ethical committee of Varendra University, Bangladesh. 25 male Wister rats were randomly assigned into 5 groups A, B, C, D and E, 5 rats in each group for dose treatment for four weeks to observe the effects of drugs on blood glucose level, lipid profile and antioxidant properties in streptozotocin induced diabetic rats.

Group A (Normal)	: Normal Control group (received 0.5 mL of distilled water)
Group B (STZIDRs)	: Diabetic Control group (received 0.5 mL of distilled water)
Group C (STZ+ Linagliptin)	: Diabetic group treating with linagliptin (received 1 mL of 5 mg/ 70kg b.w of linagliptin)
Group D (STZ+ *Emblica officinalis)*	: Diabetic group treating with aqueous fruit extract of *Emblica officinalis* (received 1 mL of 200 mg/ kg b.w of aqueous fruit extract of *Emblica officinalis*)
Group E (STZ+ combination)	: Diabetic group treating with combination of linagliptin and aqueous fruit extract of *Emblica officinalis* (received 1 mL of 2.5 mg/ 70kg b.w and 100 mg/ kg b.w of aqueous fruit extract of *Emblica officinalis*)

Experimental induction of diabetes:

Except Group A, all other animals were not fed 16 h before injection and were rendered diabetic by injecting intraperitoneally with a freshly prepared single dose of streptozotocin (45mg/kg BW). The streptozotocin (STZ) solution was prepared by dissolving in 0.01 M citrate buffer which were freshly made and adjusted to pH 4.5. Following the STZ injection, rats were given drinking water supplemented with sucrose (15 g/L) for 48 h, to limit early

mortality as stores of insulin are released from damaged pancreatic islets. Diabetes was confirmed 3 days later by measuring blood glucose level by using glucose test meter (Bioland G-423S Test Strip, Germany) using blood sample collected from the tail vein of the rats. When the condition of diabetes was established animals with blood glucose levels above 11.1 mmol/L was selected for the study.

At the end of the treatment period, all the animals were fasted for at least 16 hours and tested for baseline glucose level. After sacrificing the animals, blood samples were collected directly from thoracic artery by heparinized syringe and centrifuged at 4000 rpm for 30 minutes. Serum was separated and then quickly stored at refrigerator for biochemical analysis. Biochemical analysis: Blood glucose level of serum from each rat was determined by the glucose oxidase method using glucose test meter (Bioland G423S Test Strip, Germany). Serum total cholesterol (TC), Triglycerides (TGs), low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol levels were measured by the UV spectrophotometric method using diagnostic kits (Human, Germany). Liver was isolated from the sacrificed animals and homogenized in chilled Tris buffer (10% w/v). After then, the homogenate was centrifuged at 4000 rpm for 15 min in cold centrifuge and the supernatant was analyzed for SOD and CAT activity. The SOD activity was determined by the method of Kakkar et al. [17] and CAT activity was assayed by the method of Sinha. [18]

Statistical analysis:

The results are expressed as mean ± SEM using Graph Pad Prism (version 4.0) computer program (Graph pad Software San Diego, CA, USA). We used a one-way analysis of variance (ANOVA), followed by Dunnett's post-hoc test or students paired or unpaired t-test where appropriate. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when p values were less than 0.05 ($p<0.05$ and $P<0.01$).

3. Results and Discussion:

Results:

The effect of the monotherapy of linagliptin, *Emblica officinalis* (aqFE) and their fixed dose combination therapy of linagliptin with *Emblica officinalis* (aqFE) on the different biochemical parameters such as BGL, lipid profile such as TC, TG, LDL and HDL-cholesterol levels and endogenous antioxidant enzyme CAT and SOD were investigated in this research.

Effects on blood glucose level:

The actions of repeated dosing for four weeks of linagliptin, *Emblica officinalis* (aqFE) and their fixed dose combination therapy on FBGL in normal and STZIDRs are shown in the Figure 1. The single dose of streptozotocin was injected intraperitoneally in animal models, it selectively destroyed pancreatic β cells and significantly elevated blood glucose level (BGL) (20.38 ± 0.308 mmol/l) with respect to normal control group (5.6 ± 0.130 mmol/l). Long-term daily dose therapy of linagliptin, *Emblica officinalis* (aqFE) were decrease the BGL on (11.34 ± 0.121 mmol/l) and (12.92 ± 0.12) respectively but the fixed dose combination therapy of linagliptin with *Emblica officinalis* (aqFE) remarkably decrease BGL (7.74 ± 0.250 mmol/l) with respect to the treatment STZIDRs. Our results suggested that the fixed dose combination of linagliptin with *Emblica officinalis* (aqFE) gave more significantly (**$p<0.01$) effective result on blood glucose level than mono-therapy in STZIDRs.

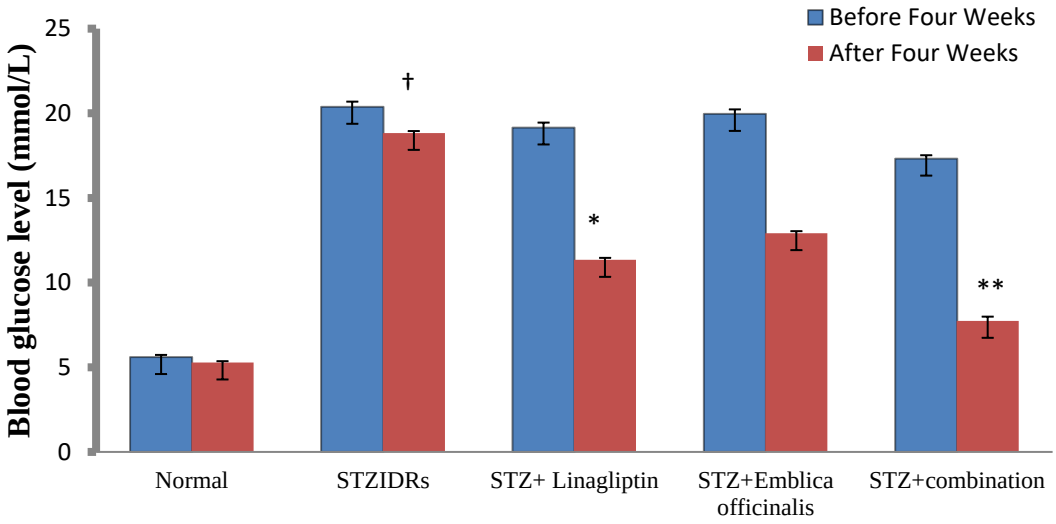

Figure.1: Before and after four weeks treatment effects of blood glucose level in STZIDRs (n = 5 in each group and mean ± SEM). *Where significant value is *p<0.05 and **p<0.01 compared to diabetic control group (ANOVA followed by Dunnett's test).†: Significantly different (p<0.05) from normal group. STZIDRs=Streptozotocin-induced diabetic rats*

Effects of the fixed dose combination therapy on lipid profile in STZIDRs:

Hyperlipidemia is a common complication for diabetic patients. Hypertriglyceridemia and reduced LDL levels should be aggressively managed in these patients. The effect of the mono therapy of linagliptin, *Emblica officinalis* (aqFE) and the fixed dose combination therapy of linagliptin with *Emblica officinalis* (aqFE) were tested on the serum level of TC, TG, LDL and HDL in STZIDRs (Figure 2 : A, B, C and D).

It was found that the STZ induced diabetic rats significantly enhanced serum level of TC (*p<0.05), TG (*p<0.05) and LDL (*p<0.05) level, but significantly reduce serum HDL (*p<0.05) level as that were compared with normal control group in Figure. 2 (A, B, C and D). After four weeks treatment with the mono therapy of linagliptin, *Emblica officinalis* (aqFE) reduced TC level 15.45%, 19.26% ,TG level 26.23%, 26.75% and serum LDL level 23.00%, 25.62% with respect to STZIDRs, but TC, TG and serum LDL level was significantly (**p<0.01) reduced by fixed dose combination therapy of 25.00%, 37.58% and 37.87% as compared with STZIDRs. Serum HDL level elevated by the monotherapy of linagliptin, *Emblica officinalis* (aqFE) of 19.48%, 28.08% respectively but it was significantly (**p<0.01) raised 42.43% when tested mice were treated with the fixed dose combination therapy of linagliptin with *Emblica officinalis* (aqFE). As the combination therapy showed higher efficacy than that of monotherapy alone, so it might be more effective in lipid profile management than monotherapy alone with STZIDRs.

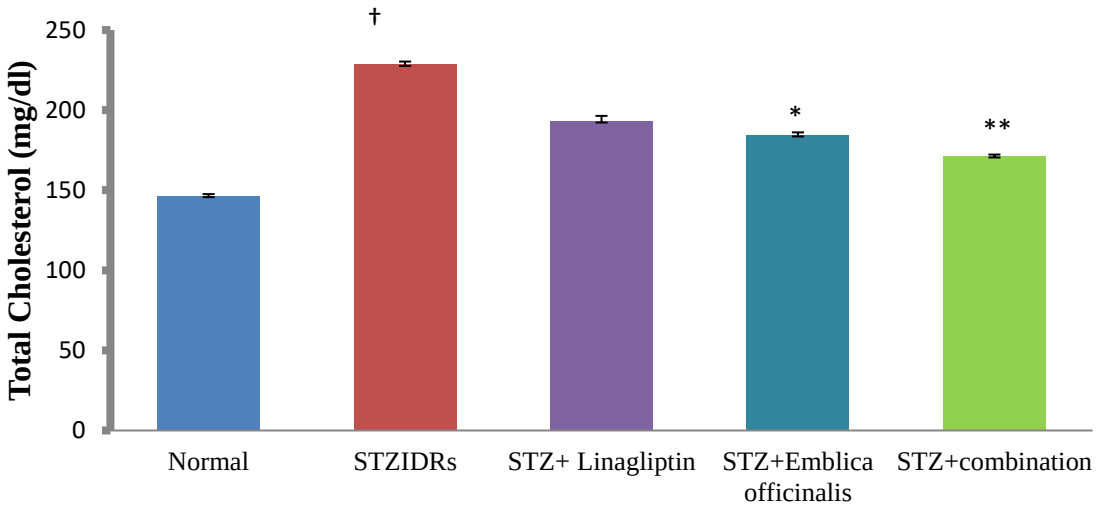

Figure 2: A

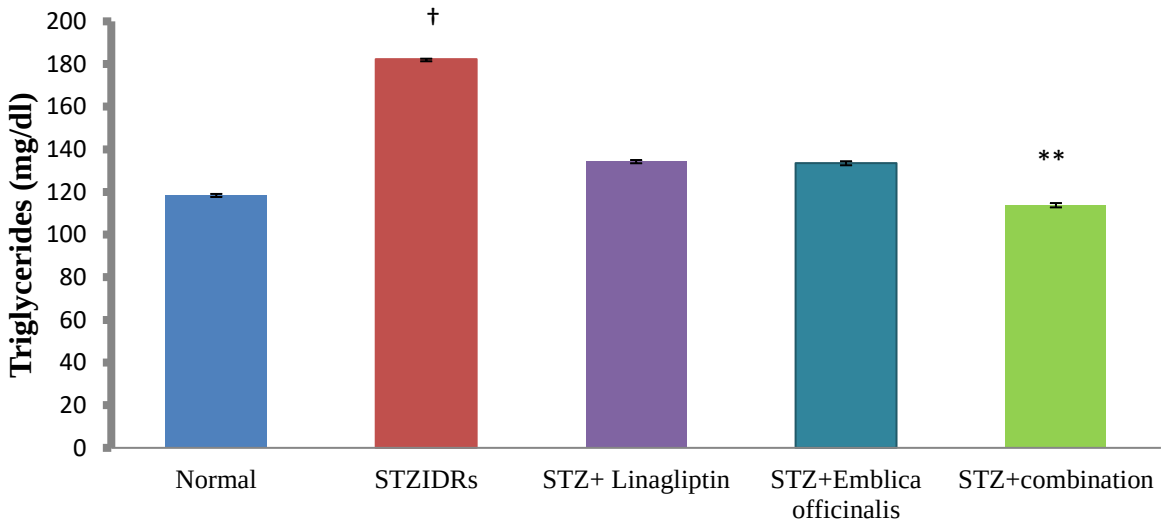

Figure 2: B

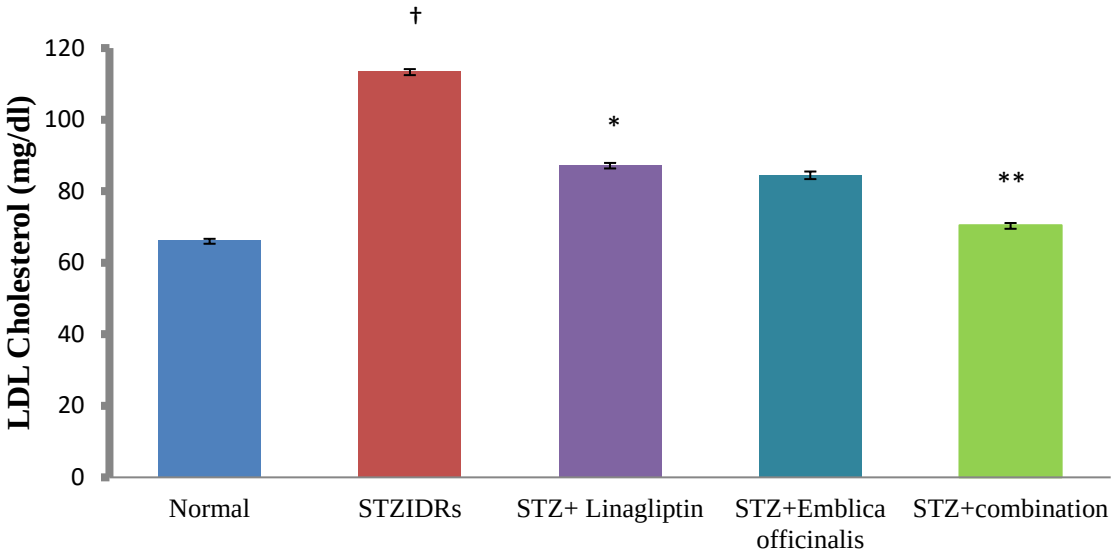

Figure 2: C

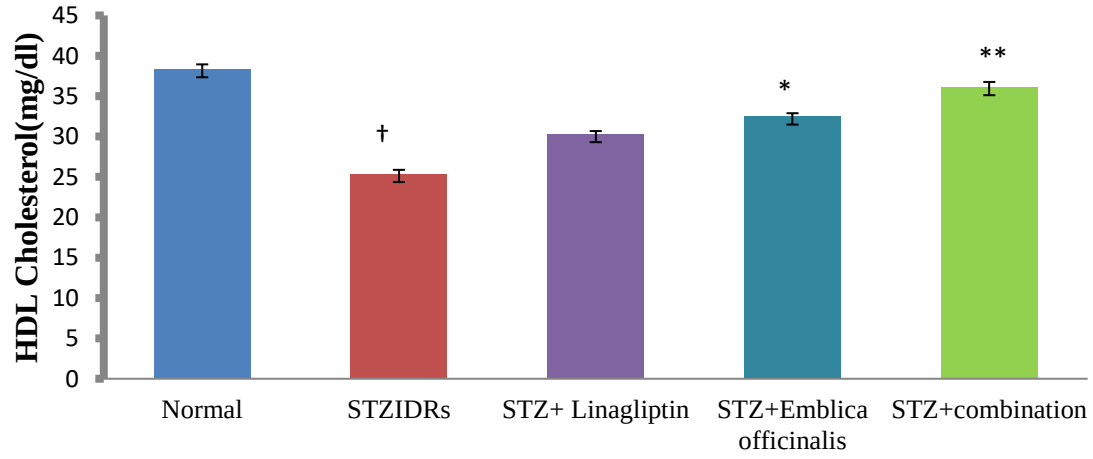

Figure 2: D

Figure 2. Effects of four weeks repeated dose treatment of linagliptin, aqueous fruit extract of *Emblica officinalis* and their combination on lipid profile: A) TC, B) TG, C) LDL-C and D) HDL-C in STZIDRs. *Data were presented as mean ± SEM; n = 5 in each group. *p<0.05 and **p<0.01 compared to STZIDRs and † (p<0.05) compared to normal group. STZIDRs=Streptozotocin-induced diabetic rats*

Effects on antioxidant enzyme activities:

Effect on SOD activity:

Hyperglycemia can increase oxidative stress. The SOD is an impotent antioxidant defense against oxidative stress. At the end of four weeks treatment of the monotherapy of linagliptin, *Emblica officinalis* (aqFE) and the fixed dose combination therapy of linagliptin with *Emblica officinalis* (aqFE) in normal and STZIDRs were depicted in Table (1). The STZIDRs had shown significant decreased level of SOD enzyme activity ($p<0.05$) in comparison with normal control group. The treatment with linagliptin and *Emblica officinalis* (aqFE) enhanced SOD level by 33.94% and 38.53% respectively as compared with STZIDRs. The fixed dose combination therapy of linagliptin with *Emblica officinalis* (aqFE) increased significantly ($p<0.01$) 67.89% with STZIDRs the SOD activity which is higher than either drug alone ($p>0.05$).

Effect on CAT activity:

Similar to SOD activity, the CAT of STZIDRs group showed significantly decrease its activity ($p<0.05$) in compared with their normal control groups. After four weeks treatment with the monotherapy by linagliptin and *Emblica officinalis* (aqFE) separately increased the value of CAT as 23.26% and 35.42% respectively as compared with STZIDRs. The percentage of increment of CAT enzyme activity by the treatment of fixed dose combination was 57.46% as compared to diabetic control group (STZIDRs) are shown in table.
Combination therapy expressed high efficacy ($p<0.01$) than that of monotherapy alone in case of increasing CAT enzyme activity in STZIDRs.

Table 1. Data for the SOD and Catalase enzyme activities in normal and SIDRs after four weeks treatment.

Groups	Super Oxide Dismutase (SOD) (U / mg protein)		Catalase (CAT) (µ mol / min / mg protein)	
	Mean ± SEM	(%) Increased	Mean ± SEM	(%) Increased
Normal	8 ± 0.23		97.02 ± 1.06	
SIDRs	4.36 ± 0.37#		52.8 ± 0.66 #	
STZ+ Linagliptin	5.84 ± 0.32 *	33.94	65.08 ± 0.54*	23.26
STZ+*Emblica officinalis*	6.04 ± 0.19	38.53	71.5 ± 0.75	35.42
STZ+ combination	7.32 ± 0.28 **	67.89	83.14 ± 0.57**	57.46

*The data were presented as mean ± SEM; n = 5 in each group, *p<0.05, **p<0.01 to diabetic control group (One way ANOVA followed by Dunnett's test) and #p<0.05 vs. normal group*

Discussion:

STZ is an antibiotics, destructive to insulin producing beta cell. It causes hypoglycemia in experimental rat model for induction of diabetics. Combination therapy of linagliptin and aqueous fruit extract of *Emblica officinalis* showed the more effective result on BGL then monotheray of linagliptin and *Emblica officinalis* fruit extract. *E. officinalis* fruits are well known for their pharmacological activities [19-24] as well as antidiabetic properties [25-56] by relieving the oxidative stress and improving glucose metabolism in diabetes [27-29].

Linagliptin acts by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4) and help to increase insulin secretion and decrease of release of glucagone. Linagliptin can be used alone or in combination with metformin (Glucophage), sulfonylureas, pioglitazone (Actos), or insulin [28] Initial combination with linagliptin with pioglitazone in a single-pill formulation was an efficacious and well-tolerated therapeutic option [30]. Similar result was found in metformin and linagliptin combination therapy where as decreasing HbA_{1c} [31] with linagliptin is important because it In our study, it was found after day 30 treatments of the monotherapy of linagliptin and the monotherapy therapy of *Emblica officinalis* aqueous fruit extract degrease BGL were (11.34 $\pm$ 0.121) and (12.92 $\pm$ 0.12) mmole/L respectively that were compared as control rats group (18.84 $\pm$ 0.112) mmole/L. But the combination therapy of linagliptin and *Emblica officinalis* showed significantly higher on BGL (7.74 $\pm$ 0.250) mmole/L than the mono therapy of linagliptin and *Emblica officinalis* on day 30. The improvements in glycaemic control with this combination are likely due to the complementary mechanisms of action of the two drugs.

It was well-known that dyslipidimia was closely related with type 2 diabetes. The effect of diabetes complication was measured by the assessment of atherogenic lipids such as total cholesterol (TC) and triglyceride (TG). It was found that after 30 days administration of the monotherapy of linagliptin and *Emblica officinalis* fruit extracts and the combination therapy of linagliptin with *Emblica officinalis* fruit extracts significantly decreased serum total cholesterol (TC) and triglyceride (TG), LDL and increased HDL whereas the lipid profile remain unchanged in type 2 diabetes control group. The result showed that TC level was (197.9± 0.661), (186.16±0.333) and (171.86±0.496) mmole/L for the monotherapy of linagliptin , *Emblica officinalis* and the combination therapy of both of these .From the result of TC we found that it was significantly reduced (171.86±0.496) mmole/L than diabetic control group. Similar result was found from TG and LDL in STZ-induced diabetic rats, which indicates the synergistic nature of their interaction in lowering the level of metabolic parameters tested in diabetic condition. However, the combination therapy of linagliptin and *Emblica officinalis* fruit extracts slightly elevate the HDL comparsion to monotherapy treatment.

Hyperglycemia results oxidative stress, which causes free radical formation through various biochemical reactions. Glucose undergoes autooxidation and generates hydroxyl free radicals (OH·) [32], which again causes autooxidation of unsaturated lipids in plasma and cell membrane. Furthermore, glutathione reserves are depleted through polyol (sorbitol) pathway due to enhanced metabolism of glucose and enhanced production of free radicals [33]. These consequences ultimately lead to pathogenesis of many diseases while the endogenous antioxidant enzymes like SOD and CAT are responsible for the detoxification of deleterious oxygen radicals. The activities of SOD and CAT decrease due to inactivation by H_2O_2 or by glycation of enzymes. SOD plays a vital role in enzymatic antioxidant defense

system by catalyzing the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen, hence diminishing the toxic effects caused by their radical. Again, CAT protects the tissues from highly reactive OH· radicals by catalyzing the reduction of H_2O_2 [34]. In our study, treatment with combination therapy of linagliptin and *Emblica officinalis* fruit extracts increased the activity of these antioxidant enzymes in comparison to their diabetic control rats. However, combination therapy increased the SOD and CAT activity most significantly ($p<0.05$) than those of monotherapy [35]. It may also reduce the potential glycation of these enzymes or reduce reactive oxygen free radicals and improve the activities of other antioxidant enzymes.

4. Conclusion:

For the first time it was found that the fixed dose combination therapy of linagliptin with *Emblica officinalis* is capable of exhibiting significant effect on antihyperglycemic, antihyperlipidemic and antioxidant activities in STZIDRs. Moreover, the combination therapy increased the level of HDL, a protective lipid compound and antioxidative, in diabetic condition. Therefore, our results give scientific support for the use of this plant in traditional medicine for the management of diabetes and its associated complications though further molecular studies are required to investigate the mechanism underlying the antihyperglycemic and antihyperlipidemic effect of *Emblica officinalis*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance was obtained from the institutional ethical committee of Varendra University to carry out this study.

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