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Linagliptin in Combination with *Emblica officinalis* Gaertn Improves Glycemic Control through Alleviating Dyslipidemia and Oxidative Stress on Streptozotocin Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: Diabetes is a complex chronic metabolic disorder. Today, many diabetes patients are known to supplement their standard therapies with herbal medications that have antidiabetic characteristics. The purpose of the study was to examine the fixed dose combination of linagliptin and Emblica officinalis Gaertn (aq FE) for its hypoglycemic, hypolipidemic, and antioxidant properties.

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Methods: Streptozotocin (45 mg/kg b.w.) was administered intraperitoneally to Wister albino rats to cause diabetes. Linagliptin (5 mg/ 70kg b.w), aqueous fruit extract of *Emblica officinalis* Gaertn *(*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* Gaertn (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. Typical procedures were used to measure the antioxidant activity by the estimation of catalase (CAT) and superoxide dismutase (SOD) activity.
Results: The combination therapy significantly ($p<0.05$) reduced 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 alterations were vastly superior to those of linagliptin with *Emblica officinalis* Gaertn (aqFE) monotherapy.
Conclusion: This study suggests that the fixed dose combination therapy of linagliptin and *Emblica officinalis* Gaertn *(*aq FE*)* might be potent on antihyperglycemic antidyslipidemic and antioxidative effect.

Keywords: Emblica officinalis gaertn; linagliptin; combinatiotherapy; antihyperglycemic; antidyslipidemic; antioxidant; aqueous fruit extract (aqFE).

1. INTRODUCTION

"Globally, diabetes mellitus (DM) is a serious and spreading health issue. It is a protein, lipid, and carbohydrate metabolic disease that causes hyperglycemia as a result of abnormalities in insulin secretion, insulin action, or both" [1]. "In Bangladesh, DM-induced death reached 28,065 people, or 3.61% of all deaths, according to the most recent World Health Organization (WHO) data, [2] and by 2040, that number will rise to 642 million" [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 by which it contributes to the long-term consequences of diabetes by promoting protein glycation, enzyme inactivation, changes in the structure and function of collagen, basement membranes, and other membranes. [6-7].

Antioxident protect our cell by scavenging free radicals. It was reported that lifestyle disorders including obesity, hypertension, diabetes, and cardiovascular diseases have an adverse correlation with dietary intake of foods high in antioxidants [8-9]. One of the most significant medicinal plants in many traditional and folk systems of medicine in Southeast Asia is *Emblica officinalis* Gaertn, a member of the phyllanthaceae family popularly known as amla [10]. Vitamin C, minerals, various tannins, amino acids, fixed oils, rutin, and quercetin are abundant in the fruits of the *Emblica officinalis* plant. Antibacterial, antiulcerogenic, antioxidant, antimutagenic, anti-inflammatory, immunomodulatory, antipyretic, and analgesic treatments are among its uses in traditional medicine [11-14]. We looked at whether Emblica officinalis Gaertn (aqFE), a high source of vitamin C, may act as a possible antioxidant and enhance the anti-hyperglycemic, anti-dyslipidemic, and antioxidant action of linagliptin in comparison to monotherapy.

2. MATERIALS AND METHODS

2.1 Chemicals

The study's source of streptozotocin was the Saint Louis, Missouri, USA-based Sigma-Aldrich Chemical Company. We used RANDOX commercial kits for biochemical analyses. The remaining compounds used were all of standardl analytical grade.

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

2.2 Preparation of Aqueous Fruit Extract of *Emblica officinalis* Gaertn

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. The mixture was then filtered through a fine sieve, and the crude extract was allowed to air-dry for three days [15,16].

2.3 Experimental Animals

Twenty five male Wister rats (150-200 gm) were brought from Pharmacology Research Laboratory, Department of Pharmacy, Jahangirnagar University. Over the course of a week, all the rats were adjusted to their new habitat. The rats were housed in an animal facility with good ventilation and were kept in a roomy 25°C environment while receiving normal ICDDRB pellets and clean drinking water. All of the rats were housed in cages with a 12-hour natural light and dark cycle. Ethical clearance was obtained from the institutional ethical committee of Varendra University, Bangladesh.To study the effects of linagliptin, aqueous fruit extract of *Emblica officinalis* Gaertn and their combination on blood glucose levels, lipid profiles, and antioxidant capabilities in streptozotocin-induced diabetic rats, 25 Wister rats were randomly assigned into 5 groups, A, B, C, D, and E, with 5 rats in each group for dosage treatment for four weeks.

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* Gaertn (received 1 mL of 200 mg/ kg b.w of aqueous fruit extract of *Emblica officinalis* Gaertn)
Group E (STZ+ combination)	: Diabetic group treating with combination of linagliptin and aqueous fruit extract of *Emblica officinalis* Gaertn (received 1 mL of 2.5 mg/ 70kg b.w and 100 mg/ kg b.w of aqueous fruit extract of *Emblica officinalis* Gaertn)

2.4 Experimental Induction of Diabetes

With the exception of Group A, all other animals were not fed 16 h before injection , received a freshly prepared intraperitoneal injection of streptozotocin (45 mg/kg BW) to induce diabetes. A solution of streptozotocin (STZ) was made by dissolving in 0.01 M citrate buffer which were freshly made and adjusted to pH 4.5. To prevent early mortality as insulin reserves are released from injured pancreatic islets, rats were given drinking water laced with sugar (15 g/L) after receiving an injection of STZ for 48 hours. Three days later, diabetes was identified by measuring blood glucose levels using a glucose test meter (Bioland G-423S Test Strip, Germany) using rat tail vein blood samples. When the condition of diabetes was established animals with blood glucose levels above 11.1 mmol/L was selected for the study.

All of the animals underwent baseline glucose testing after having been fasted for at least 16 hours at the conclusion of the treatment session. After sacrificing the animals, blood samples were taken straight from the thoracic artery using a heparinized syringe and centrifuged for 30 minutes at 4000 rpm. 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). The animals' livers were separated from them and homogenized in chilled Tris buffer (10% w/v). The homogenate was then centrifuged at 4000 rpm in a cold centrifuge for 15 min, and the supernatant was examined for SOD and CAT activity. The SOD activity was assessed using the Kakkar et al. [17] method, while the CAT activity was assessed using the Sinha method [18].

2.5 Statistical Analysis

Using IBM SPSS statistics 23 and Microsoft Office Excel 2007, the data are presented as

mean SEM. One-way analysis of variance (ANOVA) was employed, and when necessary, Dunnett's post-hoc test or students' paired or unpaired t-test was utilized. Each picture included a description of the statistical technique used in each analysis. When p values were less than 0.05 ($p < 0.05$ and $P < 0.01$), the results were deemed significant.

3. RESULTS AND DISCUSSION

3.1 Results

In this study, the effects of linagliptin, *Emblica officinalis* Gaertn (aqFE), and their fixed dose combination therapy on various biochemical parameters, including BGL, lipid profile, including levels of TC, TG, LDL, and HDL cholesterol, and endogenous antioxidant enzymes CAT and SOD, were examined.

3.2 Effects on Blood Glucose Level

The effects of repeated dose for four weeks, of linagliptin, *Emblica officinalis* Gaertn (aqFE) and their fixed dose combination therapy on FBGL in normal and STZIDRs are shown in the Fig. 1. In animal models, a single dosage of streptozotocin was administered intraperitoneally; it selectively destroyed pancreatic β cells and markedly increased blood glucose levels (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* Gaertn (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* Gaertn (aqFE) gave more significantly (**p<0.01) effective result on blood glucose level than mono-therapy in STZIDRs.

3.3 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* Gaertn (aqFE) and the fixed dose combination therapy of linagliptin with *Emblica officinalis* Gaertn (aqFE) were tested on the serum level of TC, TG, LDL and HDL in STZIDRs (Fig. 2. A, B, C and D).

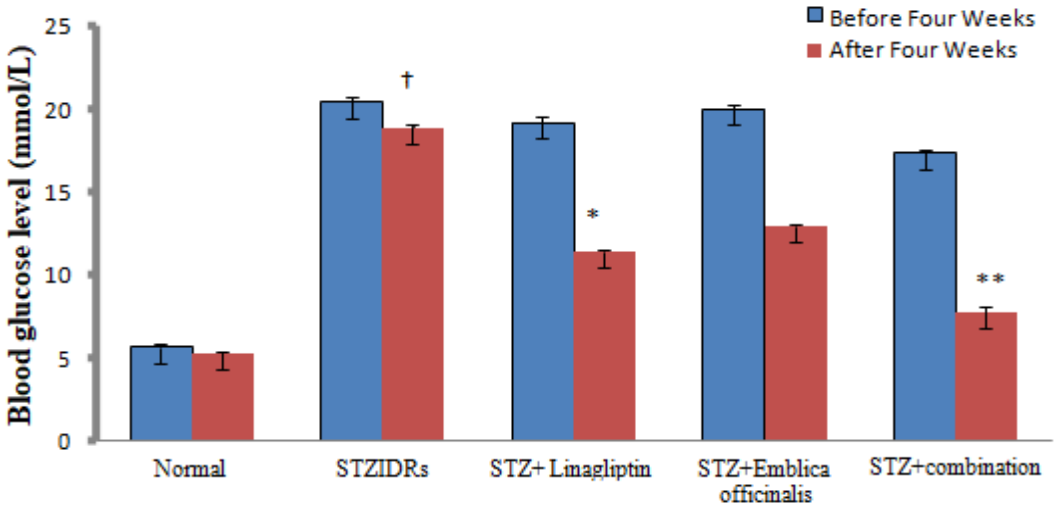

Fig. 1. Before and after four weeks treatment effects of blood glucose level inSTZIDRs (n = 5 in each group and mean ± SEM)

*ANOVA followed by Dunnett's test, where significant values are *p <0.05 and **p <0.01 when compared to the diabetes control group..†: Significantlydifferent (p<0.05) from normal group. STZIDRs=Streptozotocin-induced diabetic rats*

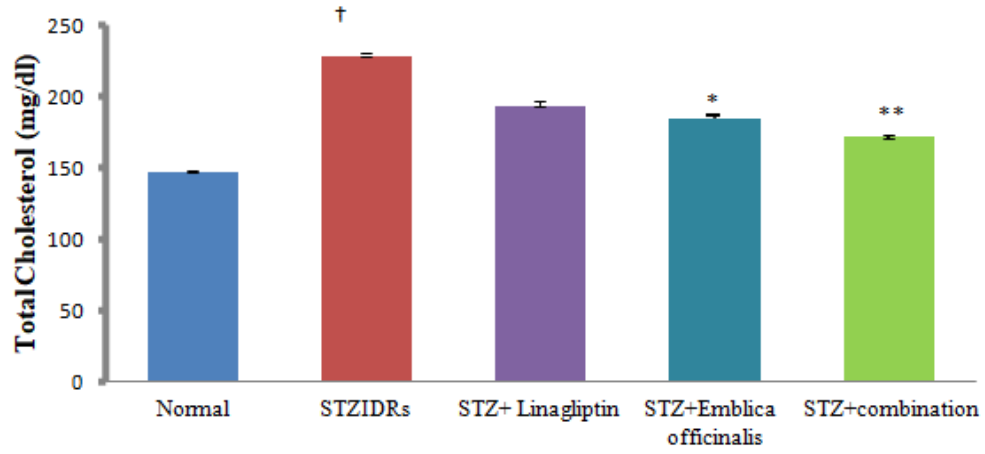

Fig. 2A.

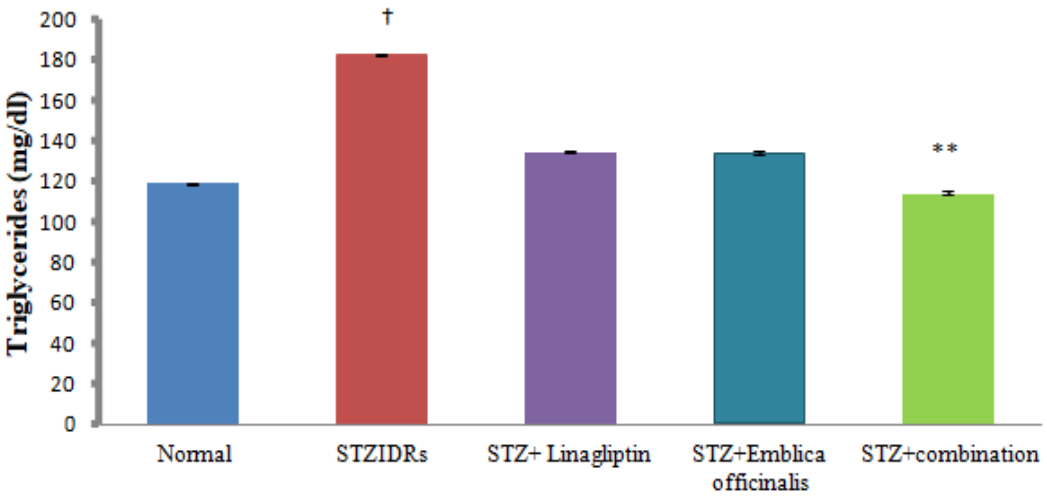

Fig. 2B.

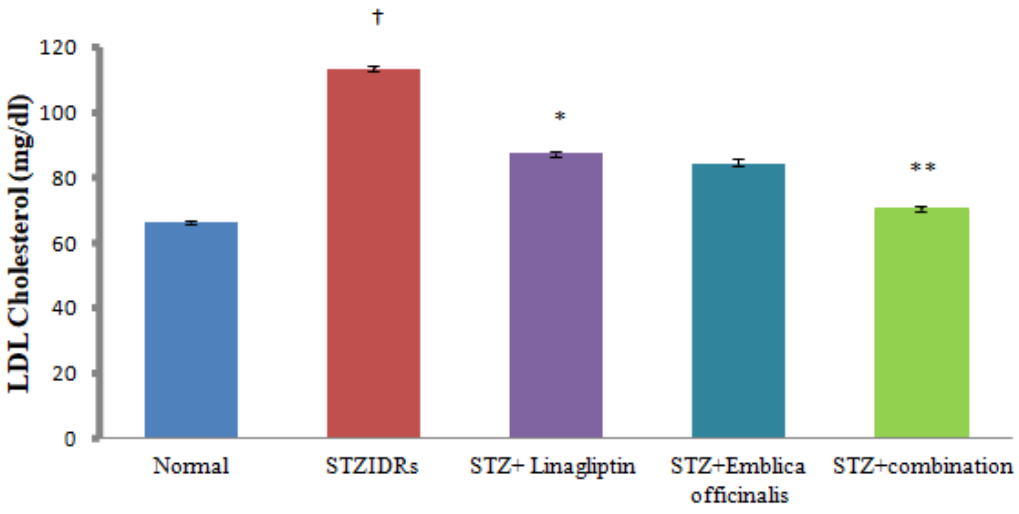

Fig. 2C.

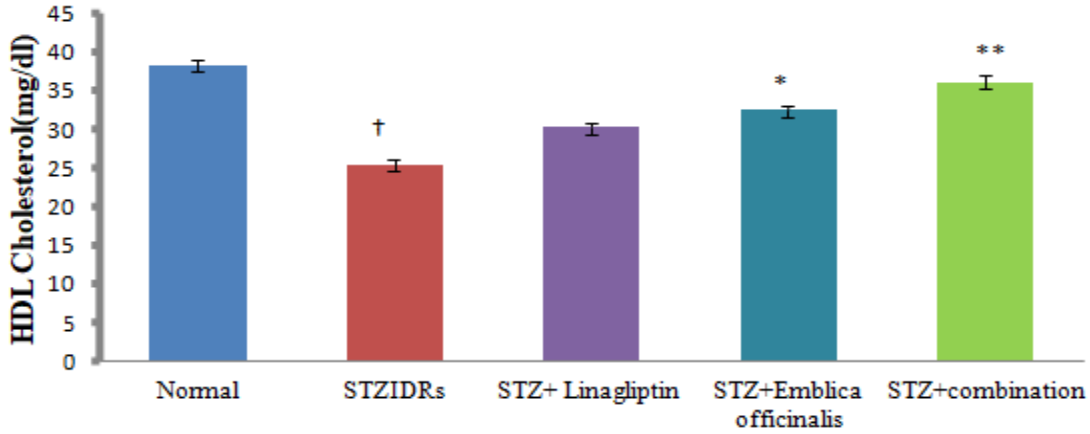

Fig. 2D.

Fig. 2. Effects of four weeks treatment with llinagliptin, aqueous fruit extract of *Emblica officinalis* Gaertn and their combination on lipid profile: A) TC, B) TG, C) LDL-C and D) HDL-C in STZIDRs

Data were shown as the 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*

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 Fig. 2 (A, B, C and D). After four weeks treatment with the mono therapy of linagliptin, *Emblica officinalis* Gaertn (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* Gaertn (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* Gaertn (aqFE). Combination therapy may be more effective in managing lipid profiles than monotherapy alone with STZIDRs because it shown more efficacy than monotherapy alone.

3.4 Effects on Antioxidant Enzyme Activities

3.4.1 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* Gaertn (aqFE) and the fixed dose combination therapy of linagliptin with *Emblica officinalis* Gaertn (aqFE) in normal and STZIDRs were shown in Table 1. The STZIDRs exhibited significantly lower levels of SOD enzyme activity than the normal control group (p 0.05), which was observed. The treatment with linagliptin and *Emblica officinalis* Gaertn (aqFE) increased SOD level by 33.94% and 38.53% respectively as compared with STZIDRs. The fixed dose combination therapy of linagliptin with *Emblica officinalis* Gaertn (aqFE) increased significantly (p<0.01) 67.89% with STZIDRs the SOD activity, which is greater than either drug alone (p>0.05).

3.4.2 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. Linagliptin and *Emblica officinalis* Gaertn (aqFE) each enhanced the value of CAT as much as 23.26% and 35.42%, respectively, as compared to STZIDRs, after four weeks of treatment with the monotherapy regimen. The fixed dose combination therapy increased CAT enzyme activity by 57.46% in comparison to the diabetic control group (STZIDRs), as shown in the Table 1.

When it came to boosting the CAT enzyme activity in STZIDRs, combination therapy was significantly more effective (p < 0.01) than monotherapy alone.

Table 1. After four weeks of treatment, information of data on the SOD and catalase enzyme activity in normal and SIDRs groups

Groups	Super OxideDismutase (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 information of data was reported 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 for normal group*

4. 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* Gaertn showed the more effective result on BGL then monotheray of linagliptin and *Emblica officinalis* Gaertn fruit extract. *E. officinalis* fruits are well known for their pharmacological activities [19-24] as well as antidiabetic properties [25,26] 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. In addition to metformin, sulfonylureas, pioglitazone and insulin, linagliptin can be taken alone or in combination with these medications [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 whereas decreasing HbA_{1c} [31] with linagliptin is important because in our study, it was found after day 30 treatments of the monotherapy of linagliptin and the monotherapy therapy of *Emblica officinalis* Gaertn aqueous fruit extract degrease BGL were (11.34 ± 0.121) and (12.92 ± 0.12) mmole/L respectively that were compared as control rats group (18.84 ± 0.112) mmole/L. But the combination therapy of linagliptin and *Emblica officinalis* Gaertn showed significantly higher on BGL (7.74 ± 0.250) mmole/L than the mono therapy of linagliptin and *Emblica officinalis* Gaertn 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* Gaertn fruit extracts and the combination therapy of linagliptin with *Emblica officinalis* Gaertn 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* Gaertn 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, lowering the level of metabolic indicators assessed in diabetic condition, which demonstrates the synergistic nature of their interaction. However, the combination therapy of linagliptin and *Emblica officinalis* Gaertn fruit extracts slightly increase the HDL comparsion to monotherapy treatment.

Oxidative stress, which is a result of hyperglycemia, leads in the creation of free radicals through several metabolic processes. During autooxidation, glucose produces hydroxyl free radicals (OH) [32], which once more results in the autooxidation of unsaturated lipids in the cell membrane and plasma. Additionally, the polyol (sorbitol) pathway depletes glutathione stores due to increased glucose metabolism and free radical generation [33]. While endogenous antioxidant enzymes like SOD and CAT are in charge of detoxifying harmful oxygen radicals, these effects ultimately result in the

pathophysiology of numerous diseases. Due to enzyme glycation or H_2O_2 inactivation, SOD and CAT activity declines. SOD is essential for the enzymatic antioxidant defense system [34]. In our study, treatment with combination therapy of linagliptin and *Emblica officinalis* Gaertn fruit extracts increased the activity of these antioxidant enzymes in comparison to their diabetic control rats. On the other hand, compared to monotherapy, combination therapy significantly ($p<0.05$) boosted SOD and CAT activity [35]. Additionally, it might lessen the possibility of these enzymes being glycated, as well as lower reactive oxygen free radicals and enhance the functions of other antioxidant enzymes.

5. CONCLUSION

For the first time, it was discovered that the fixed dose combination therapy combining linagliptin and *Emblica officinalis* Gaertn can have a notable impact on the antihyperglycemic, antihyperlipidemic, and antioxidant properties in STZIDRs. Additionally, the combination therapy raised HDL levels in diabetes patients, which is an antioxidant and protective lipid component. Therefore, although more molecular research is needed to elucidate the mechanism underlying the antihyperglycemic and antihyperlipidemic effects of *Emblica officinalis* Gaertn, our results provide scientific support for the use of this combination therapy in traditional medicine for the management of diabetes and its associated complications.

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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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