Impact of *Emblica Officinalis* Pulp Aqueous Extract on Hyperglycemia and Hyperlipidemia in Diabetic Rats

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ABSTRACT

Objective: To explore the hypo-glycemic and hypo-lipidemic potential of aqueous extract of *Emblica officinalis* (EO) pulp in streptozotocin (STZ)-induced diabetic rats with comparing to gliclazide as an anti-diabetic drug. Method: Type-2 diabetes (T2D) was caused in adult male Wister rats (150-180g) by a sole intraperitoneal (i.p.) injection of STZ (40 mg/kg), 15 min after the injection (i.p.) of nicotinamide (230 mg/kg). After induction of T2D status, the rats were split into five groups (n=8) as follow: non-diabetic (ND), T2D (D), diabetic received gliclazide (10 mg/kg bw)(DG), diabetic received EO extract (200 mg/kg/d), diabetic received EO extract (400 mg/kg/d). Next 8 weeks, body weight, fasting serum glucose, serum insulin, and glycohemoglobin (HbA1c) quantities were estimated, with the calculation of insulin resistance (HOMA-IR). Moreover, the lipid profile and some biomarkers of oxidative stress were also measured. Results: Remarkable increases in serum blood glucose, HbA1c, serum insulin and total cholesterol (T-Ch), triglycerides (TG), and Low-density lipoprotein (LDL-C) levels, in addition to a significant decrease in weight gain %, were detected in T2D rats. Treatment with both doses of EO notably ($p \leq 0.001$) reduced the elevated levels of all previous parameters in T2D rats. Furthermore, the EO doses showed also considerable ($p \leq 0.001$) reduction in HOMA-IR values in T2D rats. The remarkable reduction was observed in the malondialdehyde (MDA) level with increment in reduced glutathione (GSH) values in treated groups by two EO extract doses. The EO both dose showed significant decreases (($p \leq 0.05$) in serum insulin, T-Ch, TG, LDL, and MDA concentrations compared to the gliclazide treated group, however, there was a remarkable increase in ($p \leq 0.01$) serum GSH level. Conclusion: The EO pulp aqueous extract is effective in ameliorating hyperglycemia, insulin resistance, and hyperlipidemia in T2D rats and it was dose-independent. However, both EO extract doses used confirmed better than the gliclazide drug. This recommends an obvious anti-diabetic capability of the EO pulp extracts refers to their polyphenolic constituents.

Key Words: *Emblica officinalis*, gliclazide, Diabetes, hyperglycemia, hyperlipidemia.

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INTRODUCTION

Diabetes mellitus (DM) is one of the most predominant constant illnesses around the world [1]. It is expected that the assessed number of diabetes will exceed 552 million people worldwide by 2030 [2, 3]. The World Health Organization (WHO) ranked the Kingdom of Saudi Arabia (KSA) as the second-highest country in the Middle East, while it ranked worldwide as the seventh in DM incidence [4-6]. Moreover, T2D has a higher occurrence rate in KSA than other types with approximately 32.1% of Saudi people suffering from it, bringing about an ever-expanding load on the medicinal services framework [2, 7, 8].

The T2D is described by hyperglycemia that outcomes from either a relative lack in insulin discharge or insulin resistance [9, 10]. The progression of T2D starts with the debilitation of glucose tolerance that is frequently connected with a condition of insulin resistance. Insulin resistance is defined as an impaired sensitivity to insulin, which means insulin that is released by the β-cells and

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bound to muscle, liver, and fat cells become inefficacious in doing its metabolic activities [9]. The β-cells ordinarily remunerate insulin resistance by releasing more portions of insulin to keep up glucose homeostasis [11]. Insulin resistance considers as a center pathophysiological aspect of other wellbeing issues, for example, dyslipidemia, hypertension, and obesity clustering in the alleged metabolic disorder [12].

There are several different types of T2D drugs. The Diamicron MR (gliclazide modified release) is one of the common oral hypoglycemic drugs for non-insulin-dependent T2DM in adults, which its efficacy has proven in monotherapy or combination therapy in many clinical studies [13]. The plant-based drugs are advised as complementary medicine by the World Health Organization (WHO) [14]. The researchers' interest regenerated for classical herbal medicine usage as natural therapies. The worldwide community is genuinely looking for a medication that is modest and together powerful against T2DM to chop down the number of death cases yearly [15]. In KSA, the use of plant medicine by diabetes patients seems to be high [16, 17]. Thus, research is needed to identify and investigate plant medicines that may have an effective and important effect in treating DM and prevent its subsequent complications.

Emblica Officinalis (EO), is one of the popular important herbs in the Indian Ayurveda and is commonly called amla [18]. The most important part of this plant is a fruit, which is widely used for their protective, remedial, and health curative properties [18]. Previous animal and human studies conducted that EO fruit had many beneficial effects including hypoglycemic, *anti*-hyperlipidemic, and antioxidant characteristics [19-21]. These quality attributed to the high amounts of vitamin C, polyphenols, tannins, minerals, certain amino acids, and fiber that existed in EO fruit [22, 23].

The current study assumed that feeding of EO aqueous extracts in two doses would prompt to adjust glucose and serum lipids on STZ-induced diabetic male rats. In addition to a comparative assessment of EO extract doses with gliclazide, an antidiabetic drug.

MATERIAL AND METHODS

Chemicals and reagents

Chemicals were in analytical grade and the drugs were gotten commercially. Streptozotocin and Nicotinamide (Sigma-Aldrich Co., St. Louis, MO, USA), Gliclazide (Merck Serono Middle East). Commercial kits for followed determinations: serum glucose, total cholesterol, triglycerides, LDL-C, and HDL-C (Bio STC, Egypt.). Insulin enzyme-linked immunosorbent assay kit (SinoGeneClon Biotech Co., Ltd, China). Glycosylated hemoglobin (HbA1c) (Teco Diagnostics, USA), Reduced Glutathione (GSH) (SinoGeneClon Biotech Co., Ltd), and Malondialdehyde (MDA) (Bio-Diagnostic, Egypt).

Plant materials and pulp aqueous extract preparation

The *Emblica officinalis* fresh fruits were procured from Local hypermarket store in Jeddah, Saudi Arabia. The fruits were authenticated and identified by the Department of Biological Sciences, Faculty of Science, King Abdulla Aziz University (KAU), Jeddah.

The EO extract preparation: Fresh fruits of EO were cleaned, seeds were removed, and the pulp was cut into small pieces. Then, 100 g of amla pulps were weighed, blended with 150 ml distilled water in the electric mixer [24]. The mixture was left-over night (18 hrs) in cold and dark conditions with a magnetic stirrer. The mixture was filtered through a clean muslin cloth to collect EO juice [25]. After that the residue (solid part) was boiled with an equal volume of distilled water under refluxing for 2 hours [26]. After cooling the mixture was filtered and combined with all filtrates for lyophilizing by Freeze Dryer (ilShinBioBase, Korea). The EO extract lyophilized powder was ground and keep at -20C to use in biological study. The 100 g of fresh pulp yielded 10.6 g freeze-dried extract.

Experimental animals

Adult male Wister rats were acquired from King Fahd Medical Research Center, KAU, Saudi Arabia with mean body weight 150-180 g (7-8 weeks old). Throughout the experiment, rats were houses at moderate temperature (24°C ± 1°C), and humidity (55% ± 5%), with a daily round of 12-hour light-dark. The animals fed and drink was ad libitum during the entire experimental period. The protocol was ethically approved by the Unit of Biomedical Ethics, Research Ethics Committee, Faculty of Medicine, KAU, Saudi Arabia (Ethical approval number: Reference No 101-19).

Inductionof type 2 diabetes

The overnight fasted rats were injected with nicotinamide (230 mg/kg, i.p.) dissolved in 0.9% sodium chloride solution. After 15 minutes rats were re-injected with a sole dose of STZ (40 mg/kg, i.p.) [27, 28]. Notably, the STZ should be dissolved in 50 mM sodium citrate buffer (pH 4.5) and adjusted to reach 40 mg/ml concentration, immediately before the injection [27]. At the sixth hour after STZ-injection, each rat was injected with 1 ml of glucose (5%) to prevent fatal hypoglycemia. After the fifth day of injection, the fasting blood glucose was detected to identify the diabetic rats that had blood glucose more than 200 mg/dl for starting the experiment [29].

Gliclazide preparation

The solution of Diamicron MR (gliclazide) drug will be dissolved in limited drops of NaOH (0.1N), followed by

adjustment to accurate volume using water, then will be administered daily by gavage to rats as 10 mg/kg b.w. daily [30].

Experimental design

The rats were split into five groups (one non-diabetic group and four T2D groups) each group embracing eight animals as follows:

1- ND: Non-Diabetic + saline (1 ml/kg b.w).
2- D: T2D (STZ-injected) + saline (1 ml/kg b.w) .
3- DG: T2D rats (STZ-injected) + Gliclazide (10 mg/Kg b.w)
4- DLEO: T2D rats (STZ-injected) + EO extract (200 mg/kg b.w) low dose[31]
5- DHEO: T2D rats (STZ-injected) + EO extract (400 mg/kg b.w) high dose[31]

The solutions of saline, gliclazide and both EO extract doses were daily administered by cavage for 8 weeks (experimental period).

Serum sampling and biochemical analyses

At the experimental period ending, each rat was left fasted 13-16 hrs and body weight recorded. Rats were anesthetized using ether. Blood samples were withdrawn from Venous retro-orbital [11]. Samples underwent centrifugation at 3000 rpm for 10 min for serum separation, then samples stored at -20C until assayed the followed parameters:

- The diabetic parameters: fasting glucose [32], HbA1c [33], Insulin [34], and calculation of HOMA-IR scores [35].
- Lipid profile indices: Total cholesterol (TC) [36], Triglycerides (TG) [37], and High dentistry Lipoprotein (HDL) [38]. While LDL calculated using Friedewald formula [39].
- The Antioxidant markers: Malondialdehyde (MDA) [40], and reduced glutathione (GSH) [41].

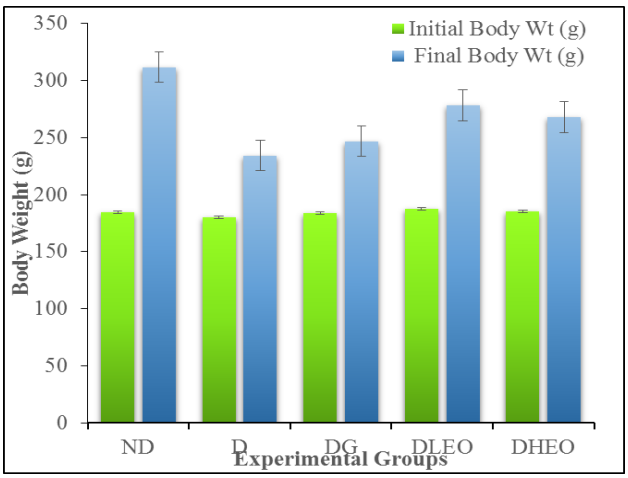
Statistical analysis

The data were statistically analyzed by SPSS program Version 24. Values will be expressed as average ± SE, with the usage of one-way variance (ANOVA) followed by (t-test). The results will be considered as statistical significance at $P \leq 0.05$.

RESULTS

Impact of EO pulp aqueous extract on body and pancreatic weight

The initial weight of all rat groups was almost similar with less or more 10 g differences. The administration of STZ resulted in significant ($p \leq 0.01$ and $p \leq 0.05$) declines in body weight in diabetic groups compared to non-diabetic and treated rats. The body weight measurement significantly ($p < 0.01$) increased in rats received EO extract (200 mg/kg), it, however, significantly higher than that of the gliclazide treated group, the EO dose (400 mg) showed less significant ($p<0.05$) difference in body weight. However, the bodyweight of rats in EO extract-treated groups was significantly different from that of diabetic control. There was likewise no remarkable difference in body weight between the two extract concentration treated groups (Figure 1 A).



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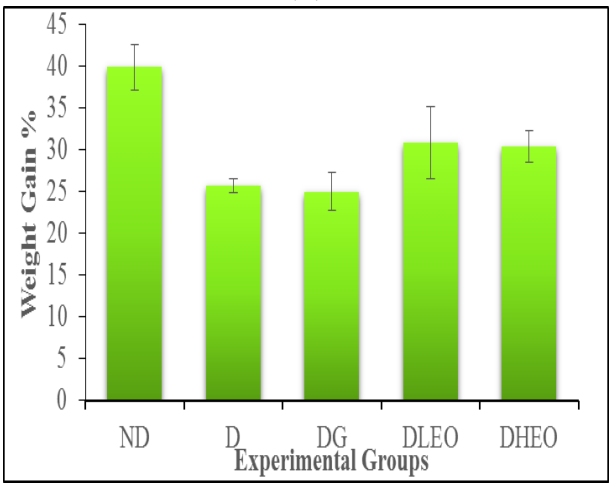

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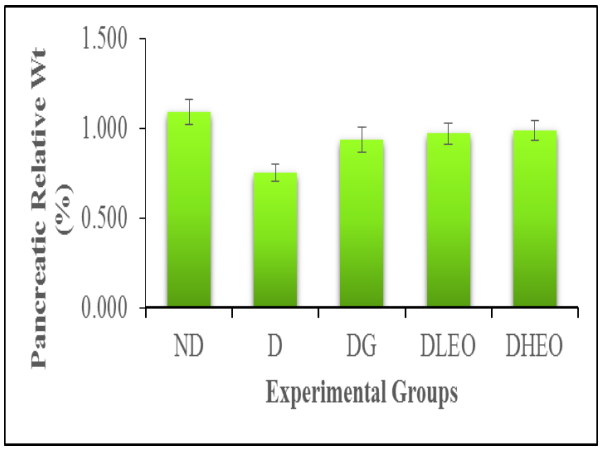


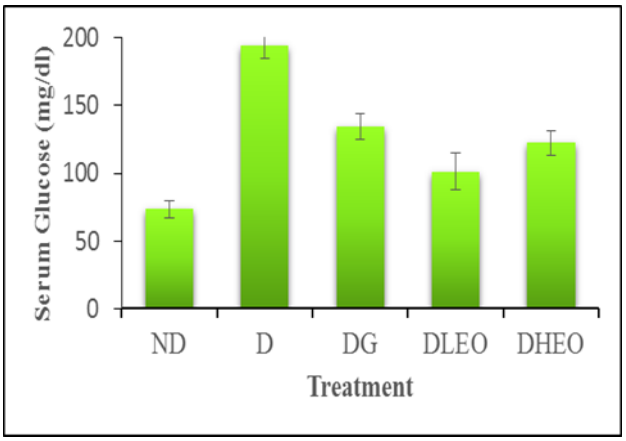
(C)

Figure 1. Effect of EO pulp aqueous extract on **(A)** initial and final body weight, **(B)** weight gain percentage, and **(C)** pancreatic relative weight % in all experimental rat groups

Impact of EO pulp aqueous extract on serum glucose, insulin, insulin resistance, and glycosylated hemoglobin HbA1c

The fasting blood glucose levels were increased in the T2D group almost 2.6 fold 194.2± 9.72 versus 73.37 ± 6.23 in the non-diabetic group. The treated groups with two dosages of EO extract displayed no remarkable differences from the gliclazide treated group. The glucose level of all the cured groups indicated significant ($p \leq 0.001$) decline in contrast with the T2D group; while, there was no respectable difference observed between the two EO extract cured groups (Figure 2, A). In the diabetic group, the serum insulin and HbA1c levels were raised significantly ($p \leq 0.001$) as contrasted to non-diabetic rats. While the same parameters displayed remarkable ($p \leq 0.01$) reduction in diabetic rats treated using gliclazide or both doses of EO pulp extract (200mg/kg and 400mg/kg). There were no remarkable differences observed between the EO extract-treated groups and gliclazide treated group in both parameters (Figure 2 B and C). On the other hand the HOMA-IR index showed significant differences ($p \leq 0.001$, $p \leq 0.05$) in the diabetic group contrasted to all treated groups (Figure 2D).

The blood glucose level of rodents from all the treated gatherings indicated a huge ($p \leq 0.001$) decline in contrasted with diabetic control; be that as it may, there was no huge distinction seen between the EO remove treated gatherings (Figure 2, A).



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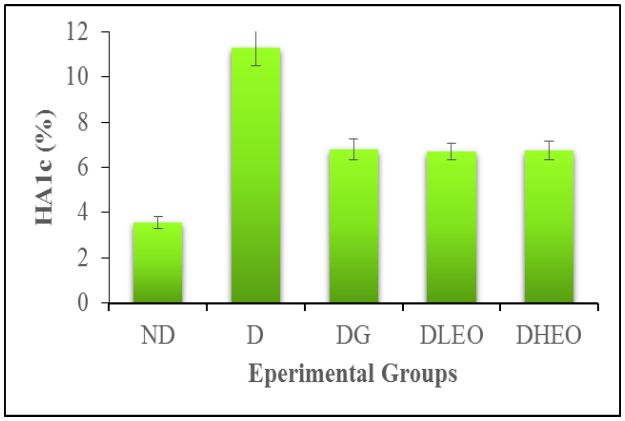

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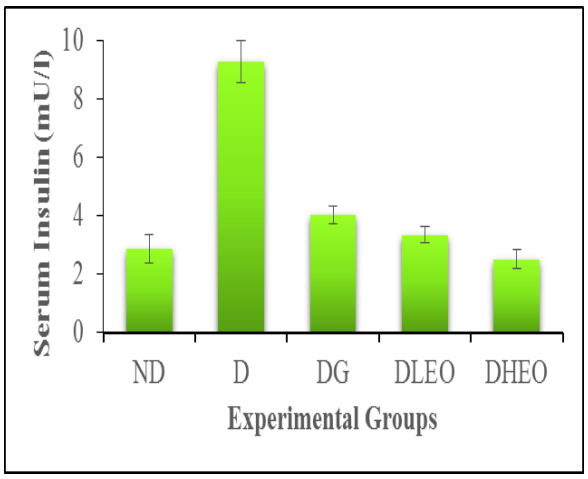

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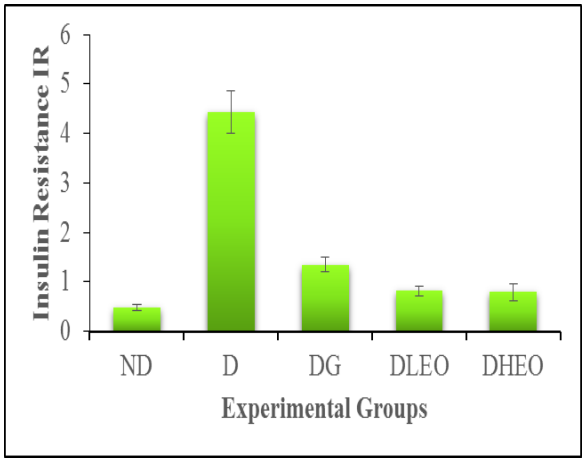


(D)

Figure 2. Effect of EO pulp aqueous extract on **(A)** Blood fasting glucose, **(B)** glycosylated hemoglobin % (HbA1c), **(C)** serum insulin, and **(D)** insulin resistance (IR) in all experimental rat groups

Impact of EO pulp aqueous extract on serum lipids (mg/dl) in all experimental rat groups

Diabetic rats (D) showed significant ($P \leq 0.001$) rises in the serum levels of TC, TG, and LDL. Differently, HDL was remarkably ($P \leq 0.05$) lowered in comparison with non-diabetic rats (ND). Regular feeding of both EO pulp extract dosages for 8 weeks nearly normalized the TC, LDL, and HDL levels but TG in T2D rats. Oral receiving of Gliclazide significantly ($P \leq 0.001$) lowered the serum TC, TG, and LDL levels but not normalized them. In CT2D rats cured with a high dosage of EO pulp extract, the amount of TC (48%↓), LDL (52.3%↓), and TG (45.8%↓) decreased significantly ($P \leq 0.05$) by the study end; conversely, the serum HDL amount was remarkably raised in comparing to T2D rats (14.4%↑).

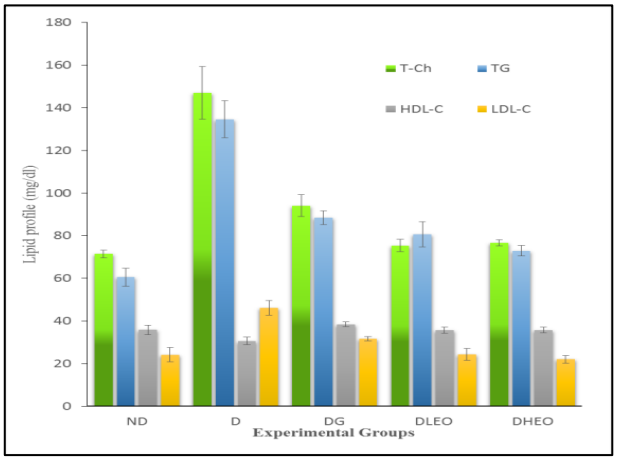
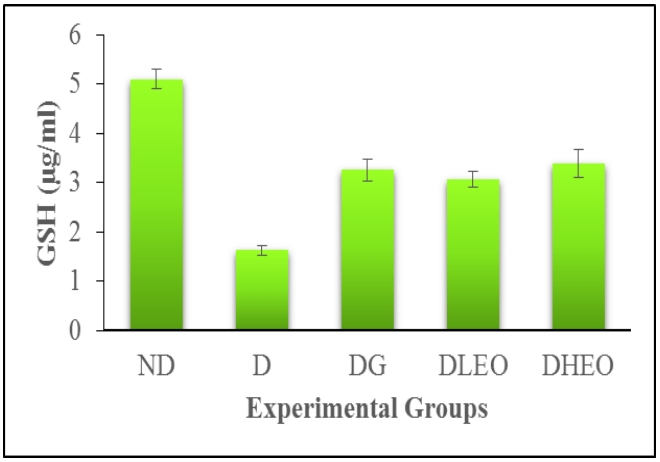


Figure 3. Effect of EO pulp aqueous extract on lipid parameters TC, TG, LDL and HDL in all experimental rat groups



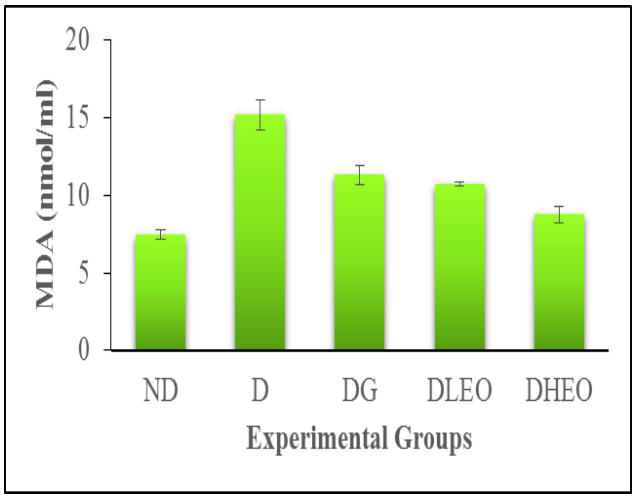
(B)

Figure 4. Effect of EO pulp aqueous extract on antioxidant parameters **(A)** Malondialdehyde (MDA) and **(B)** reduced Glutathione (GSH) levels in all experimental rat groups

Impact of EO pulp aqueous extract upon antioxidant parameters

As assumed, T2D rats displayed the greatest level of fat peroxidation as serum MDA amount was 15.2± 1.0 nmol/ml whereas non-diabetic rats had 7.43± 0.3 nmol/ml. The levels of serum MDA were significantly (P = 0.001) lowered in the gliclazide treated group in comparison to the T2D group. The EO pulp aqueous extract (400 mg/kg) administration to diabetic rats tends to normalize the serum MDA level (8.7± 0.5 nmol/ml), while the low dose of EO extract (200 mg/kg) reduces the MDA level but just outside the normalization (Figure 4 A).

The amount of the main antioxidant (GSH) was dropped in T2D rats (1.63±0.1 µg/ml) compared to (5.1±0.2 µg/ml) in non-diabetic rats. Diminishing of GSH in serum is sponsors of pathogenic complications associated with chronic T2D state. The GSH content raised significantly (P=0.001) in both doses of EO pulp extract-treated rats, but outside the normalization. The same result also conducted in Gliclazie treated rats (Figure 4 B).



(A)

DISCUSSION

The current study demonstrated a remarkable reduction in body weight, weight gain percentage, and pancreatic relative weight in T2D rats compared to non-diabetic rats. In diabetes, the body unable to break down carbs as fuel sources, which leads to an alteration of independence to fatty fuels and protein, weakening of muscles, and declines in weight gain rate [42]. Moreover, exaggerated secretion of insulin, due to insulin resistance, leads to the gradual malfunction of β-cells, cells endure apoptosis and β-cell function and mass are lowered [43, 44]. However, the EO pulp extract-treated groups had a weight gain percentage better than diabetic groups compared to the non-diabetic group. Possibly, the phenolic rich content of EO extracts such as gallic acid and quercetin were able to defend the pancreatic β cells [22, 23]. Similar outcomes were achieved by other researches, and together, indicate that EO extract intake may deal with weight loss in T2D status [31, 45, 46]. The T2D is a heterogeneous illness distinguished by increasing insulin resistance, blood glucose, and malfunction of β cells [9]. In the current study, the induction of T2D in rats was made by the administration of STZ after the injection of nicotinamide. The induced diabetic rats showed increases in fasting glucose, serum insulin, and HbA1c amounts, in addition to calculated HOMA-IR. To induce T2D with insulin resistance in rats, nicotinamide affords protection to the β-cell versus free radicals, that may be produced from cytotoxic compounds like streptozotocin [28, 47]. The coincidence of remarkable elevation in blood glucose and serum insulin amounts in diabetic rats indicated that insulin resistance and T2D have been created in these rats [9]. The insulin resistance was then confirmed by HOMA-IR indexes, an applicable measurement for insulin resistance (IR) in rats [48]. The IR is described as weakening sensitivity to insulin led to an increase in insulin secretion(hyperinsulinemia) [49], which

could be due to impaired signaling of post-receptor (main cause) or mutation in insulin receptor [9]. On the other hand, the study indicated that hyperinsulinemia could be due to the reduction of insulin hepatic clearance [43].

However, the administration of EO pulp aqueous extract (200 and 400 mg/kg) effectively restored the alterations in levels of plasma glucose, insulin, HbA1C and subsequently decreasing IR index in T2D rats. It has been proven that EO aqueous extract, which contains phytochemicals such as gallic acid, exerts antihyperglycemic effect by upregulation of GLUT4 receptor which improves glucose transporters and insulin sensitivity [31, 46, 50, 51]. It was also indicated that EO may enhance the metabolism and uptake of glucose by lowering the glucose-6-phosphatase activity, which resulting in glucose homeostasis [52]. In T2D rats, the excess of glucose in the blood causes increasing in HbA1c levels compared to non-diabetic rats due to increasing of oxidative reaction that associated with protein glycation [53]. The detected reduction of HbA1c in treated groups with EO extract, related to its high content of polyphenolic compound that reducing the glycosylation of hemoglobin and thus decreased the level of HbA1c [54].

The connection of dyslipidemia and insulin resistance had been demonstrated in various studies including T2D [35, 55, 56]. The induced dyslipidemia by T2D and insulin resistance in this study is distinguished by raised serum levels of TC, TG, and LDL, with a concomitantly reduced concentration of HDL, which were in agreement with other studies [12, 55]. The IR causes impairment in the synthesis of glycogen and catabolism of protein in muscles with lipoprotein lipase inhibition in adipose cells; leading to more releasing of fatty acids (FFA) results in hypertriglyceridemia [56]. The impairment of glucose output and fatty acid metabolism by IR, subsequently leading to a rise in the liver TG content and VLDL secretion [12]. Moreover, the increment of cholesterol level could be due to the diminution of HDL-C, whereas HDL is involved in cholesterol transportation from tissue to liver for subsequent catabolism [57]. Additionally, plasma and tissue cholesterol levels were elevated in diabetes due to the activity of enzyme HMG CoA reductase, the cholesterol biosynthesis rate-limiting step enzyme [58]. Previous studies agreed that EO aqueous extract has antihypercholesterolemic and antihypertriacylglycerolaemic effect in T2D rats [31, 59]. The outcomes in the current study demonstrate that treatment with EO pulp extracts and Gliclazide produced similar highly significantly decreased in T-Ch, TG, and LDL-C amounts in T2D rats. The remarkable impact of EO pulp aqueous extract on T2D hypertriglyceridemia, may be related to its effects on glycemic control, which subsequently improved the lipid profile [60]. This improvement in lipid profile occurs via numerous mechanisms, involving inhibition of HMG-CoA reductase, cholesterol absorption restriction, and upgrading of lecithin cholesterol acyltransferase [61].

The EO is considered one of the most important herbs in Ayurveda because of its extremely high content of phytochemicals that could prevent many health disorders [23]. Many studies attributed the EO controlling effect on hyperglycemia and hyperlipidemia to its content of potent antioxidants, which may lower the risk of T2D incidence[15, 39]. The EO aqueous solvent extract possessed the highest polyphenol content when compared to lemon and cucumber peels aqueous extracts [62]. In T2D, the hyperglycemia caused an elevation in oxidative stress resulted in intensive lipid peroxidation (expressed by MDA amounts) [63]. Based on these, the MDA amount elevation in the current study T2D group was significantly reduced by the administration of EO extract. The tannins present in EO extract may interfere with oxidative stress induced by the polyol pathway, where there has been a reflection of changes conerning lipid peroxide, carbonyl protein content, and antioxidant enzymes activity [46]. Raising of oxidative stress caused by a significant increase in aldehydic products of lipid peroxidation may reduce glutathione content [64]. Glutathione is one of the most abundant tripeptides, act as cellular antioxidants to protect the cell from harmful action of lipid peroxidation. Thus, the T2D group in the current study exhibited a high remarkable decrease in serum GSH amounts, which improved by EO extract treatment. The EO aqueous extracts improve endogenous antioxidant defenses in HepG2 cells, through reducing creation of ROS and improvement of GSH amounts [65]. The increase in GSH concentration supported the EO strength to rise the antioxidant particle and the efficacy of extract to scavenge the ROS overproduction during diabetes [65].

CONCLUSION

The *Emblica officinalis* pulp aqueous extract exerts hypoglycemic activity through the action of decreasing blood glucose and insulin resistance, ameliorating hyperinsulinemia, and increasing antioxidant status in T2D rats. The anti-diabetic mechanism of EO extract seems to involve the improvement of insulin resistance. Moreover, EO aqueous extract exhibited hypolipidemia activity by lowering the T-Ch, TG, and LDL-C and slightly increasing HDL-C. The polyphenols content appears to be the active principle in EO aqueous extract responsible for its potent inhibitor of oxidative damage. Because of EO plant availability, low price, and safety profile, all make it considered as a low-risk alternative to commonly used glucose-lowering medication. In addition to considered as a strong applicant for future diabetic drug research, traditional glucose-lowering medication, and a strong candidate for diabetic drug research.

REFERENCES

[1] AL-Asiri IS, Alotaibi F. Predictors of Depression among Diabetes Mellitus Outpatient Attending King Abdul-Aziz Specialist Hospital in Taif, Saudi Arabia (2018). Int. J. Pharm. Res. Allied Sci. 2018;7(4):97-103.

[2] Alotaibi, A, Perry L, et al. Incidence and prevalence rates of diabetes mellitus in Saudi Arabia: An overview. Journal of Epidemiology and Global Health, 2017; 7(4): 211-218.

[3] Almoraie NM. The Role of Ipomoea Batatas Leaves Extract on the Treatment of Diabetes Induced by Streptozotocin. Pharmacophores. 2019;10(3):14-20.

[4] World Health Organization, Noncommunicable diseases: progress monitor 2017; 2017.

[5] Robert AA, Al Dawish MA, et al. Type 2 diabetes mellitus in Saudi Arabia: major challenges and possible solutions.Current Diabetes Reviews, 2017; 13(1): 59-64.

[6] Priyadi A, Muhtadi A, Suwantika AA, Sumiwi SA. An economic evaluation of diabetes mellitus management in South East Asia. J Adv Pharm Educ Res. 2019;9(2):53-74.

[7] Alqurashi KA, Aljabri KS, and Bokhari SA Prevalence of diabetes mellitus in a Saudi community.Annals of Saudi medicine, 2011; 31(1): 19-23.

[8] Alharbi NS, Towards the holistic management of diabetes in Saudi Arabia: A multi-method study. Ph.D. Thesis, 2018, University of Surrey (United Kingdom).

[9] Petersen MC and Shulman GI. Mechanisms of insulin action and insulin resistance.Physiological Reviews, 2018; 98(4): 2133-2223.

[10] American Diabetes Association, 5.Prevention or Delay of Type 2 Diabetes: Standards of medical care in diabetes—2018. Diabetes Care 2018; 41(Suppl. 1): S51-S54.

[11] American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2018. Diabetes Care, 2018; 41(Suppl. 1): p. S13-S27.

[12] Ormazabal V, Nair S., et al. Association between insulin resistance and the development of cardiovascular disease.Cardiovascular Diabetology, 2018; 17(1): 122.

[13] Ruiz M. Diamicron (gliclazide) MR the secretagogue with clinical benefits beyond insulin secretion. Medicographia, 2013; 35: 81-89.

[14] International Diabetes Federation (IDF). IDF Diabetes Atlas. 7th edition, Brussels, Belgium: International Diabetes Federation; 2015.

[15] Brahmachari G. Bio-flavonoids with promising anti-diabetic potentials: a critical survey. In Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry, 1st edition, Editors, Tiwari VK, and Mishra BB, Trivandrum: Research Signpost, 2011; 2: 187–212.

[16] Alrowais NA, and Alyousefi NA, The prevalence extent of Complementary and Alternative Medicine (CAM) use among Saudis.Saudi Pharmaceutical Journal, 2017; 25(3): 306-318.

[17] Al-Ghamdi MA, Mohammed AA, et al. Herbal medicine use by Saudi patients with chronic diseases: A cross-sectional study (experience from Southern Region of Saudi Arabia), Journal of Health Specialties, 2018; 6(2): 77.

[18] Variya BC, Bakrania AK, and Patel SS. *Emblica officinalis* (Amla): a review for its phytochemistry, ethnomedicinal uses and medicinal potentials with respect to molecular mechanisms. Pharmacological Research, 2016; 111: 180-200

[19] Akhtar MS, Ramzan A, et al. Effect of Amla fruit (*Emblica officinalis* Gaertn.) on blood glucose and lipid profile of normal subjects and type 2 diabetic patients. International Journal of Food Sciences and Nutrition, 2011; 62 (6): 609-616

[20] Pareek, S., et al., Indian Gooseberry (*Emblica officinalis* Gaertn.), in Fruit and Vegetable Phytochemicals. In Chemistry and Human Health,2nd edition. 2017, p. 1077-1106.

[21] Rai N, Tiwari L, et al. Pharmaco-botanical Profile on *Emblica officinalis* Gaertn.–A Pharmacopoeial Herbal Drug.Research & Reviews: Journal of Botany, 2018; 1(2): 38-50.

[22] Dasaroju S, and Gottumukkala KM, Current trends in the research of *Emblica officinalis* (Amla): a pharmacological perspective, Int. J. Pharm. Sci. Rev. Res., 2014; 24 (2): 150-159.

[23] Kulkarni KV, and Ghurghure SM, Indian gooseberry (*Emblica officinalis*): Complete pharmacognosy review.International Journal of Chemistry Studies, 2018; 2(2): 05-11.

[24] Mathur R, Sharma A, et al., Hypolipidaemic effect of fruit juice of *Emblica officinalis* in cholesterol-fed rabbits, Journal of Ethnopharmacology, 1996; 50(2): 61-68.

[25] Yadav M and Agarwal A, Effect of *Emblica officinalis* against SO 2 induced Hepatotoxicity in Wistar rats.Int J Pharm Bio Sci, 2016; 7(4-B): 130-136.

[26] Antony B, Benny M, and Kaimal T, A Pilot clinical study to evaluate the effect of *Emblica officinalis* extract (Amlamax™) on markers of systemic inflammation and dyslipidemia.Indian Journal of Clinical Biochemistry, 2008; 23(4): 378-381.

[27] Donovan J, and Brown P, Parenteral injections.Current protocols in immunology, 2006; 73(1): 1.6. 1-1.6. 10.

[28] Furman BL, Streptozotocin-induced diabetic models in mice and rats. Current Protocols in Pharmacology, 2015; 70(1): 5.47. 1-5.47. 20.

[29] Ghasemi A, Khalifi S, and Jedi S, Streptozotocin-nicotinamide-induced rat model of type 2 diabetes.Acta Physiologica Hungarica, 2014; 101(4): 408-420.

[30] Mastan S, and Kumar KE, Effect of antiretroviral drugs on the pharmacodynamics of gliclazide with respect to glucose-insulin homeostasis in animal models.Journal of experimental pharmacology, 2010; 2: 1-11.

[31] Elobeid MA, Virk P, et al., Antihyperglycemic activity and body weight effects of extracts of *Emblica officinalis*, Tamarix nilotica and cinnamon plant in diabetic male rats.Wulfenia Journal, 2013; 20(11): 18-31.

[32] Braham D, and Trinder P, An improved color reaction for the determination of blood glucose by oxidase system. Analyst, 1972; 142-144.

[33] Bannon P, Effect of pH on the elimination of labile fraction of glycosylated hemoglobin. Clin Chem. 1982; 28: 2183.

[34] Morgan CR, and Lazarow A. Immunoassay of insulin: two antibody system, plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes 1963; 12: 115-126.

[35] Bonora E, Targher G, et al., Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care, 2000. 23(1): 57-63.

[36] Allain CC, Poon LS, et al. Enzymatic determination of total serum cholesterol, Clinical Chemistry, 1974; 20(4): 470-475.

[37] Young, DS, Effects of Drugs on Clinical Laboratory Tests, 5th edition, AACC Press, Washington, D.C. 2000

[38] Young DS, Pestaner L, and Gibberman V, Effects of drugs on clinical laboratory tests, Clinical Chemistry, 1975; 21(5), 1D-432D.

[39] Friedewald WT, Levy RI, and Fredrickson DS, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clinical Chemistry, 1972; 18(6): 499-502.

[40] Cross CE, Halliwell BH, et al. Oxygen radicals and human disease". Annals of Internal Medicine, 1987; 107: 526-545.

[41] Nair S, Singh SV, and Krishan A, Flow cytometric monitoring of glutathione content and anthracycline retention in tumor-cells. Cytometry, 1994;12(4) :336–342

[42] Sundaram R, Naresh R, et al.Modulatory effect of green tea extract on hepatic key enzymes of glucose metabolism in streptozotocin and high fat diet-induced diabetic rats, Phytomedicine, 2013; 20(7): 577-584.

[43] DeFronzo RA, and Tripathy D, Skeletal muscle insulin resistance is the primary defect in type 2 diabetes.Diabetes Care, 2009; 32(suppl 2): S157-S163.

[44] Szkudelski T, Streptozotocin–nicotinamide-induced diabetes in the rat. Characteristics of the experimental model.Experimental Biology and Medicine, 2012; 237(5): 481-490.

[45] Fatima N, Hafizur RM, et al. Ellagic acid in *Emblica officinalis* exerts anti-diabetic activity through the action on β-cells of pancreas.European journal of nutrition, 2017; 56(2): 591-601.

[46] Bashir A, Mushtaq A, and Mehboob T,Evaluation of antioxidant and Antidiabetic activity of Phyllanthus Emblica (fruit).Biologia Pakistan, 2018; 64(1): 85-91.

[47] Anwer T, Melatonin ameliorates hyperinsulinemia, glucose intolerance and insulin resistance in STZ-Nicotinamide induced type 2 diabetic rats.International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(2): 133-136.

[48] Antunes LC, Elkfury JL, et al. Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats.Archives of Endocrinology and Metabolism, 2016; 60(2): 138-142.

[49] Wilcox G, Insulin and insulin resistance.Clinical biochemist reviews, 2005; 26(2): 19.

[50] Fang, J-Y, Lin C-H, et al. In vivo rodent models of type 2 diabetes and their usefulness for evaluating flavonoid bioactivity.Nutrients, 2019; 11(3): 530.

[51] Variya BC, Bakrania AK, and Patel SS, Antidiabetic potential of gallic acid from *Emblica officinalis*: Improved glucose transporters and insulin sensitivity through PPAR-γ and Akt signaling.Phytomedicine, 2019; 152906.

[52] Vasant RA, and Narasimhacharya A, Amla as an antihyperglycemic and hepato-renal protective agent in fluoride-induced toxicity.Journal of Pharmacy &Bioallied Sciences, 2012. 4(3): 250.

[53] Sherwani SI, Khan HA, et al. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. Biomarker Insights, 2016;11: 95-104.

[54] Younus H, and Anwar S, Prevention of non-enzymatic glycosylation (glycation): Implication in the treatment of diabetic complication. International Journal of Health Sciences, 2016; 10(2): 261.

[55] Sparks JD, Sparks CE, and Adeli K, Selective hepatic insulin resistance, VLDL overproduction, and hypertriglyceridemia.Arteriosclerosis, thrombosis, and vascular biology, 2012; 32(9): 2104-2112.

[56] Dimitriadis G, Mitrou P, et al. Insulin effects in muscle and adipose tissue. Diabetes Research and Clinical Practice, 2011; 93(Suppl 1): S52-S59.

[57] Krishnaveni M, Mirunalini S, et al. Antidiabetic and antihyperlipidemic properties of Phyllanthus Emblica Linn. (Euphorbiaceae) on streptozotocin-induced diabetic rats.Pak J Nutr, 2010. 9(1): 43-51.

[58] Rodwell VW, Bender D, et al., Harper's illustrated biochemistry. 30th edition, McGraw-Hill Education, 2015.

[59] Elobeid MA, and Ahmed EA, Antidiabetic efficacy of aqueous fruit extract of Amla (*Emblica officinalis*, Gaertn) in streptozotocin-induced diabetes mellitus in male rats. Tropical Journal of Pharmaceutical Research, 2015; 14(5): 801-806.

[60] Mullugeta Y, Chawla R, et al. Dyslipidemia associated with poor glycemic control in type 2 diabetes mellitus and the protective effect of metformin supplementation. Indian Journal of Clinical Biochemistry, 2012; 27(4): 363-369.

[61] Usharani P, Fatima N, and Muralidhar N, Effects of Phyllanthus Emblica extract on endothelial dysfunction and biomarkers of oxidative stress in patients with type 2 diabetes mellitus: a randomized, double-blind, controlled study.Diabetes, metabolic syndrome, and obesity: targets and therapy, 2013; 6: 275.

[62] Agarwal M, Kumar A, et al. Extraction of polyphenol, flavonoid from *Emblica officinalis*, Citrus limon, Cucumis sativus and evaluation of their antioxidant activity. Oriental Journal of Chemistry, 2012; 28(2): 993-998.

[63] Mahreen R, Mohsin M, et al. Significantly increased levels of serum malonaldehyde in type 2 diabetics with myocardial infarction.International Journal of Diabetes in Developing Countries, 2010; 30(1): 49.

[64] Gaschler MM, and Stockwell BR, Lipid peroxidation in cell death.Biochemical and Biophysical Research Communications, 2017; 482(3): 419-425.

[65] Shivananjappa MM, and Joshi MK, Influence of *Emblica officinalis* aqueous extract on growth and antioxidant defense system of human hepatoma cell line (HepG2).Pharmaceutical Biology, 2012; 50(4): 497-505.