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Research Article

Antidiabetic efficiency of *Trigonella foenum-graecum* in combination with sitagliptin on streptozotocin induced diabetic rats.

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Abstract

A combination of a plant extract used in conventional diabetes treatment and an oral hypoglycemic medication has been studied to see if it has an anti-diabetic effect as the anti-diabetic medications available on the market today have side effects, toxicity, and even treatment resistance. To investigate the effect, oral hypoglycemic drug sitagliptin, seed extract of *Trigonella foenum-graecum* and both of them combined were studied on glycemic control parameters, lipid profile and hepatoprotective effects. The blood glucose level, TC, TG, HDL, LDL, serum ALT (SGPT) and AST (SGOT) were measured using diagnostic kits.Remarkable ($p<0.05$) drop in blood sugar level had been detected for sitagliptin (19.63 ± 0.204 mmol/L to 5.86 ± 0.310mmol/L) and combination therapy treated groups (18.086 ± 0.215 mmol/L to 9.92 ± 0.658mmol/L) in STZIDRs. After three weeks treatment the discovery was that sitagliptin, *Trigonella foenum-graecum* and both of them combined reduced ($p<0.05$) total cholesterol (TC) level 28.57%, 0.45% and 16.24% respectively. In case of LDL cholesterol, the combination treatment lessened LDL cholesterol level 26.71% and improved HDL cholesterol level 23.37%. After the mentioned duration it was shown that sitagliptin *Trigonella foenum-graecum* combination decreased ($p<0.05$) SGPT 70.44% and SGOT level 20.42% in comparison to those of Streptozotocin Induced Diabetic Rats (STZIDRs).Combining Sitagliptin with *Trigonella foenum-graecum* may be a better option for treating hyperglycemia than taking either one alone. This combination had strong hepatoprotective activity at a lower dose and demonstrated significant management of hyperlipidemia, which may be advantageous for diabetic patients seeking to avoid the negative effects of synthetic oral hypoglycemic medications.

Key words: Sitagliptin, *Trigonella foenum-graecum*, Diabetes, hypoglycemic, Combination therapy.

1. Introduction

Diabetes is a prolonged metabolic condition defined by persistent hyperglycemia caused by either insufficient insulin production by the pancreas or peripheral target tissues are unable of responding to regular insulin concentrations (Bekele *et al.*, 2008). 422 million people worldwide have diabetes which was108 million in 1980. Low- and middle-income countries have seen a faster increase in prevalence than high-income countries. Diabetes was one of the top most causes of mortality in 2019, projected to be responsible for 1.5 million deaths (WHO, 2021).

Diabetes with uncontrolled blood sugar levels can lead to a variety of long-term problems, such as blindness, heart disease, and kidney failure (Mamun-or-Rashid *et al.*, 2014). Despite significant advancements in diabetes therapy over the past three decades, patient outcomes are still far from perfect. Some disadvantages of these therapies include side effects, toxicity, and drug resistance (reduced efficacy). Sulfonylureas, for example, decrease their effectiveness in 44 percent of patients after 6 years of treatment. It's also claimed that glucose-lowering medications can't regulate hyperlipidemia (Dey *et al.*, 2002).

The fenugreek (*Trigonella foenum-graecum* Linn. Fabaceae family), which is an annual plant with a brief lifespan. It is cultivated as food, condiment, spice, and native medicine in various countries of Asia, Africa, and Europe (Sinskaja, 1962). Furthermore, fenugreek seeds are high in numerous vital chemicals, which play a crucial part in the treatment and health care management by regulating various biological functions. Fenugreek seed contains a high amount of antioxidants that can help prevent pathogenesis by scavenging free radicals (Almatroodi *et al.*, 2021). *Trigonella foenum-graecum* seeds have demonstrated to produce hypoglycemic and hypocholesterolemic effects in type 1 and type 2 diabetes patients as well as laboratory animals with diabetes in previous investigations. (Xue *et al*., 2007; Renuka *et al*., 2009).

Trigonella foenum-graecum supplementation brought about a large reduction in the glucose levels of rabbits with diabetes and a minor effect in diabetic-free rabbits. Furthermore, fenugreek supplementation reduced cholesterol levels in diabetics by a small amount. The results showed that *Trigonella foenum-graecum* seeds have anti-diabetic and insulin-mimetic properties in rabbits (Abdelatif *et al.*, 2012).

The mechanism of *Trigonella foenum-graecum* seed as a significant part in diabetes management was explored in alloxan-induced subdiabetic mice. The drug caused a considerable flattening of the glucose tolerance curve as well as an improvement in the insulin response to glucose, suggesting that the Langerhans beta pancreatic cells produce and/or secrete more insulin, which mediates the hypoglycaemic effect. (Puri *et al.*, 2002).

Sitagliptin (Figure 1) is an anti-diabetic drug that is an oral dipeptidyl peptidase-4 inhibitor that works by reducing the inactivation of incretin hormones in type 2 diabetes patients (Sushma and Mukthinuthalapati, 2020).

As *Trigonella foenum-graecum* has acquired popularity for its antidiabetic characteristics, so the goal of this research is to give convincing evidence for the most efficient treatments based on experimental investigations and widely used hypoglycemic medication (Sitagliptin) and the traditional medicinal plant *Trigonella foenum-graecum* seed alone and in combination of both on glycaemic control parameters, lipid profile and hepatoprotective effect.

2. Materials and methods

2.1 Collection of plant material

Trigonella foenum-graecum seeds were purchased from the local market of Enayetpur, Sirajganj. For a few days, the plant material was dried in the sun. After that, the dried plant was ground into a coarse powder in the phytochemical research laboratory, Department of Pharmacy, Khwaja Yunus Ali University, using a high-capacity grinding machine.

2.2 Plant Extract preparations

Seeds powder of *Trigonella foenum-graecum* was soaked into ethanol and kept in an air tight glass container for 10-12 days. After that, the materials were filtered and dried by air to get the extract. (Chowdhury *et al.*, 2017).

2.3. Animal Studies

Male Swiss Albino rats weighing between 110 and 1 150 g, aged 1.5 months, were procured from a local rat seller.

2.4 Measurement of Glucose Level

Trigonella foenum-graecum, Sitagliptin and the combination of both the plant and the drug was given with daily dosages over the course of three weeks and using a Glucometer, blood glucose levels were assessed two hours following the previous dose. (VivaChek Biotech (Hangzhou) Co., Ltd, China).

2.5 Measurement of Lipid Profile

Chloroform was used to make the rats unconscious when the treatment was completed. After that the abdomen was cut with the scissor and thoracic artery was opened. Then, 3 - 5 ml of blood was collected by a syringe directly from the thoracic artery. The serum was obtained after centrifuging the blood for 10 minutes at 4000 rpm. Serum lipid profile, such as total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C), was evaluated utilizing diagnostic tools (Human, Germany).

2.6. Measurement of SGPT and SGOT Level

Serum glutamic oxaloacetate transaminase (SGPT) and serum glutamic oxaloacetate transaminase (SGOT) levels were tested in serum obtained from the blood after the sacrifice of rats using diagnostic kits (Human, Germany). The prevalence of liver impairment is assessed using these assays.

2.7. Statistical Analysis

Using the Graph Pad Prism (version 5.0) software (Graph pad Software San Diego, CA, USA), the data were analyzed and reported as mean± SEM. Dunnett's post-hoc test was employed after a one-way analysis of variance (ANOVA). Each figure had a description of the statistical methodology that was used in the analysis. The results were deemed significant when p values were less than 0.05 ($p < 0.05$).

3. Results

3.1 The Effect of combination therapy on the blood the glucose level in STZIDRs

Injection of streptozotocin intraperitoneally in rats considerably raised blood sugar levels (18.18 ± 0.95 mmol/L) compared to normal rats (5.38 ± 0.143 mmol/L). Sitagliptin caused a remarkable (p0.05) drop in blood glucose levels (from 19.63 ± 0.204 mmol/L to 5.86 ± 0.310 mmol/L), Whereas, *Trigonella foenum-graecum* seed extract treated group showed a notable diminution of glucose level in blood (from 18.63 ± 0.215 mmol/L to 10.36 ± 0.498 mmol/L) and the combination therapy treated group (from18.086 ± 0.128 to 9.92 ± 0.658) in STZIDRs also.

(a) (b)

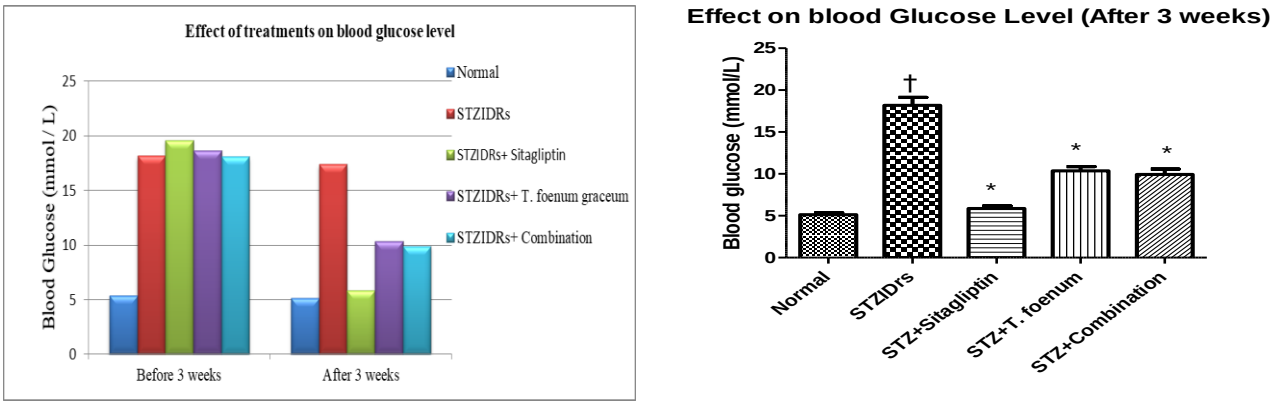


Figure 1: *The effects of sitagliptin and Trigonella foenum-graecum therapy at repeated doses for three weeks on blood sugar levels in STZIDRs. (a)The data showed a comparison between the before and after effect of sitagliptin and Trigonella foenum-graecum alone and in combination on STZIDRs. (b) Effects of repeated dose treatment of sitagliptin and Trigonella foenum-graecum for three weeks on the blood glucose level in STZIDRs.*

*One way ANOVA followed by Dunnett's test was performed to analyze the data. The data were shown as mean ± SEM; n = 5 in each group, *p<0.05 in contrast to the diabetic control control group. †: Significantly different (p<0.05) from normal group.*

3.2. The Effect of *Trigonella foenum-graecum* and Sitagliptin on Lipid Profile in STZIDRs

i) The Effect on TC level

After three weeks treatment it was discovered that sitagliptin, *Trigonella foenum-graecum* and their combined effect decreased total cholesterol (TC) level 28.57%, 0.45% and 16.24% respectively.

ii) The Effect on TG level

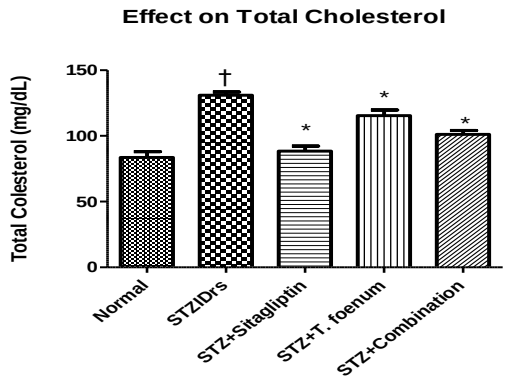
Following three weeks of therapy, sitagliptin, *Trigonella foenum-graecum* and the two together reduced triglycerides level 28.80%, 24.56% and 20.15% respectively.

iii) The Effect on LDL level

After three weeks treatment, according to research, sitagliptin, *Trigonella foenum-graecum* and their combination decreased LDL cholesterol level 22.19%, 15.28% and 26.71% respectively.

iv) The Effect on HDL level

After three weeks treatment, there was evidence that sitagliptin, *Trigonella foenum-graecum* and their combination increased HDL cholesterol level 47.28%, 22.65% and 23.37% respectively.



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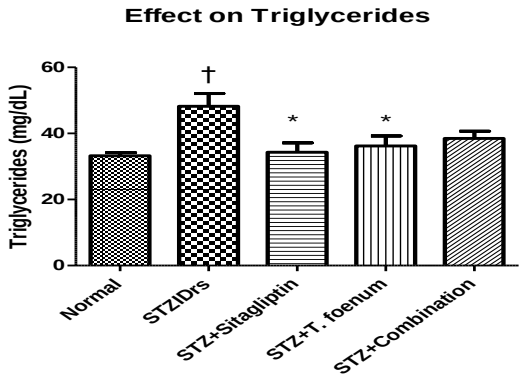

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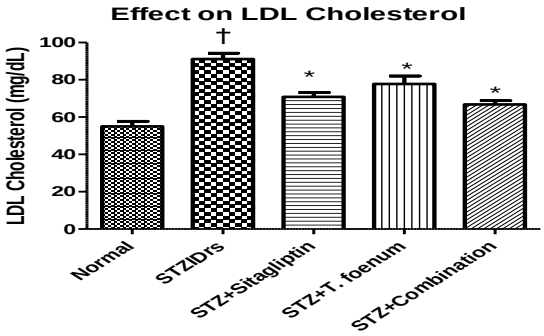

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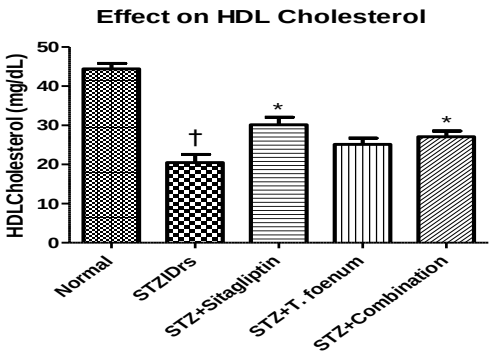

(d)

Figure 2 : *The effects of sitagliptin and Trigonella foenum-graecum therapy at repeated doses for three weeks on (a) TC, (b) TG, (c) LDL and (d) HDL) level in STZIDRs. One way ANOVA followed by Dunnett's test was performed to analyze the data. The data were presented as mean ± SEM; n = 5 in each group, *p<0.05 compared to diabetic control group. †: Significantly different (p<0.05) from normal group.*

3.6. The Effect of combination therapy on liver dysfunction indicators on STZIDRs

i) The Effect on SGPT (ALAT) level

Following the three-week treatment program it was observed that sitagliptin, *Trigonella foenum-graecum* and their combination decreased SGPT level 51.89%, 20.66% and 70.44% respectively.

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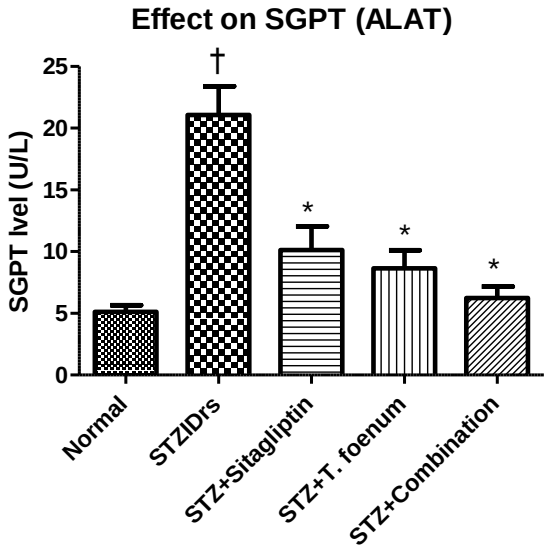
ii) The Effect on SGOT (ASAT) level

After three weeks treatment, as per study, sitagliptin, *Trigonella foenum-graecum* and their combination decreased SGOT level 24.57%, 13.77%and 20.42% respectively



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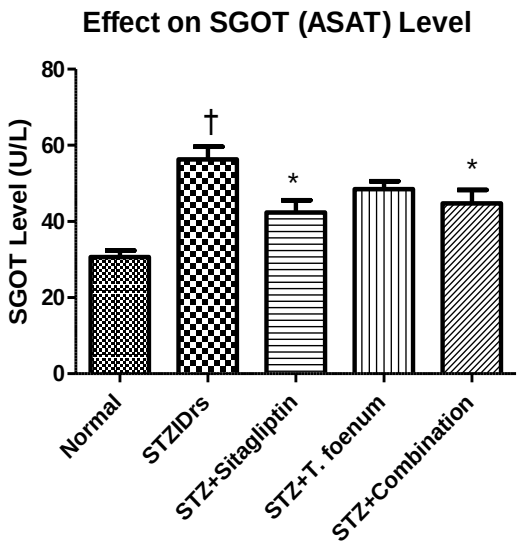

(b)

Figure 3: *The effects of sitagliptin and Trigonella foenum-graecum therapy at repeated doses for three weeks on (a) SGPT and (b) SGOT level in STZIDRs. One way ANOVA followed by Dunnett's test was performed to analyze the data. The data were presented as mean ± SEM; n = 5 in each group, *p<0.05 compared to diabetic control group. †: Significantly different (p<0.05) from normal group.*

4. Discussion

The prevalence, morbidity, and mortality rates of diabetes mellitus are high worldwide. Synthetic medications have restrictions and significant adverse effects, making it challenging to manage type2 diabetes with them. Owing to the presence of crude fiber and saponin of *Trigonella foenum-graecum* seed as well as an estrogenic component that indirectly increases thyroid hormone, a lot of people are interested in medicinal herbs as a substitute to control type 2 diabetes that are native, affordable, and food-based (Anwar *et al.*, 2011). Faecal matter biliary acid and cholesterol excretion can be increased by *Trigonella foenum-graecum* seed consumption which results in reaction between fenugreek containing saponin and the bile acid that causes the lower absorption of the formed micelles in the intestine (Sharma & Raghuram, 1990). Powdered *Trigonella foenum-graecum* seed may slow down the ingestion of fatty acids and glucose, reducing the amount of substrate available for the production of triglycerides (Renuka *et al*, 2009).

A study reported that fenugreek marginally reduced cholesterol levels. The findings showed that fenugreek seeds have anti-diabetic and insulin-mimetic actions in alloxan induced diabetic rabbits (Abdelatif *et al*., 2012). In the current research, Streptozotocin intraperitoneal injections were used to produce diabetes (Gupta & Gupta, 2009). Streptozotocin's methylnitrosourea moiety's ability to alkylate DNA dictates its toxicity [18].

DNA methylation ultimately causes beta cell death, even though streptozotocin also methylates proteins (LeDoux et al, 1986; Wilson *et al*., 1988).

This study showed the antihyperglycemic effect of alcoholic extract of *Trigonella foenum-graecum* seeds. Here, standard antidiabetic drug sitagliptin was used both separately and in combination in normal and STZ-induced rats. Compared to a standard medicine, the extract both by itself and in combination with sitagliptine significantly lowered blood sugar levels. (Fig1).

According to the results, serum total cholesterol levels had significantly increased as well as triacylglycerol, LDL, AST, and ALT levels in diabetic rats. The ingestion of fenugreek seed extract (1g/kg body weight) and Sitagliptine (100 mg/70kg) significantly decreased serum triacylglycerol and total cholesterol diabetic rats but not in control rats. The administration of the fenugreek seed extract (0.5 g/kg body weight) and Sitagliptine (50 mg/70kg) combination significantly decreased serum cholesterol (TC), LDL, AST, and ALT levels and improved HDL cholesterol level in STZIDRs (Fig 2 and Fig 3). The slowing of both fat and carbohydrate absorption may be the cause of the soluble dietary fiber fraction's hypolipidemic effect due to the agent's inclusion of bioactive fibres. This might be caused by a metabolic imbalance in diabetes, as evidenced by excessive xanthine oxidase activity, lipid peroxidation, and elevated levels of triacylglycerol and cholesterol (Bennett et al, 1981).

In comparison to diabetic rats, the consequences of fenugreek seed extract on blood lipids in the current revealed a considerable decrease in triglyceride levels. In contrast to other treatment groups that did not demonstrate a significant rise in HDL levels, diabetic rats showed a significant increase in HDL levels following a daily administration of the described doses. The group treated with fenugreek showed very little effect on overall cholesterol levels. Findings on cholesterol differ somewhat from those of earlier reports (Sharma & Choudhary, 2014), wherein an improvement in lipid profile (HDL, cholesterol, and triglycerides) was achieved with a dosage of 500 mg/kg for four weeks. Possible causes for this disparity include duration of the protocol used in the current study (three weeks).

Being a drug with an innovative mode of action and few harmful side effects, sitagliptin is anticipated to aid in improving glycemic control. Nonclinical (animal) investigations carried out abroad have shown that inhibiting DPP-4 raises the GLP-1 level and thus alters the small intestine's production of cholesterol and apoB.(Hsieh *et al*., 2014) .

Clinical investigations have also shown that inhibiting DPP-4 reduces the high TG levels after meals, CMs, and apoB48 in people with type 2 diabetes (Matikainen *et al*., 2014; Kubota *et al*, 2012). What impact sitagliptin's typical clinical dose may have on lipid metabolism, nevertheless, is unknown. In general, there is disagreement over the lipid parameters that change after sitagliptin treatment, despite clinical studies reporting decreases in TC, TG, and non-HDL-C (Takihata *et al.*, 2012).

In this study, the combination of *Trigonella foenum-graecum* and sitagliptine at a 500 mg/kg and 50mg/70kg dosage respectively showed betterment in the declination of blood sugar, TG, LDL, SGOT and SGPT and a significant increment in HDL level. The proposed mechanism may be the due to the additive effect of combining the extract and the medication alone.

5. Conclusion

The goal of the current investigation was to explore the antidiabetic outcome of a combination of the *Trigonella foenum-graecum* extract from plant used traditionally in diabetes treatment and oral hypoglycemic drug sitagliptine both in their half doses. The observed hypoglycemic and hepatoprotective effects were found promising in this combination which can be alternative to both the plant and drug used individually. Though the plant extract and the combination therapy didn’t show any significant cholesterol lowering effect, but these significantly increased the HDL level in diabetic rats. Due to the ineffectiveness of anti-diabetic medicine monotherapy on glycemic control and rising side effects for getting better glycemic control when administered at high doses, the plant and drug combination can be a

better therapeutic alternative in the treatment of diabetes. Further research to be executed in order to discover the mechanism of action regarding this therapeutic effect.

6. Acknowledgements

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7. Ethics Approval

This study was approved by the Human Ethics Committee (HEC) from Khwaja Yunus Ali University (KYAU/DEAN/SBS/2023/001). The HEC also authorized access to participants who met the requirements for inclusion. Blood samples and all demographic data were de-identified from the study team.

8. Declarations

The author(s) state that they have no financial or other conflicts of interest to disclose. This research was done as a part of B. Pharm coursework with funding provided by the KYAU research grant, Khwaja Yunus Ali University, Bangladesh.

9. References

1. Abdelatif, A. M., Ibrahim, M. Y., & Mahmoud, A. S. (2012). Antidiabetic effects of fenugreek (*Trigonella foenum-graecum*) seeds in the domestic rabbit (*Oryctolagus cuniculus*). *Research Journal of Medicinal Plant*, *6*(6): 449-455
2. Almatroodi, S. A., Almatroudi, A., Alsahli, M. A., & Rahmani, A. H. (2021). Fenugreek (*Trigonella Foenum-graecum*) and its Active Compounds: A Review of its Effects on Human Health through Modulating Biological Activities. *Pharmacognosy Journal*, *13*(3).
3. Anwar, S., Desai, S., Eidi, M., & Eidi, A. (2011). Antidiabetic Activities of Fenugreek (*Trigonella Foenum-graecum*) Seeds. In *Nuts and Seeds in Health and Disease Prevention* (pp. 469-478). Academic Press.
4. Bekele, T., Hymete, A., Tadesse, M., & Mekonnen, Y. (2008). Antidiabetic activity and phytochemical screening of crude extracts of Stevia rebaudiana Bertoni and Ajuga remota Benth grown in Ethiopia on alloxan-induced diabetic mice. *Department of Pharmaceutical Chemistry, School of Pharmacy, Addis Ababa University April*.
5. Bennett, R. A., & Pegg, A. E. (1981). Alkylation of DNA in rat tissues following administration of streptozotocin. *Cancer research*, *41*(7): 2786-2790.
6. Chowdhury, M. A. R., Islam, M. R., & Muktadir, M. A. G. (2017). Thrombolytic activity of *Lagerstroemia speciosa* Leaves. *Discovery Phytomedicine*, *4*(4): 41-45.
7. Dey, L., Attele, A. S., & Yuan, C. S. (2002). Alternative therapies for type 2 diabetes. *Alternative medicine review*, *7*(1): 45-58.
8. Gupta, R., & Gupta, R. S. (2009). Protective role of *Pterocarpus marsupium* in diabetes-induced hyperlipidemic condition. *Journal of complementary and integrative medicine*, *6*(1).
9. Hsieh, J., Longuet, C., Baker, C. L., Qin, B., Federico, L. M., Drucker, D. J., & Adeli, K. (2010). The glucagon-like peptide 1 receptor is essential for postprandial lipoprotein synthesis and secretion in hamsters and mice. *Diabetologia*, *53*(3): 552-561.
10. Kubota, A., Maeda, H., Kanamori, A., Matoba, K., Jin, Y., Minagawa, F., ... & Matsuba, I. (2012). Pleiotropic effects of sitagliptin in the treatment of type 2 diabetes mellitus patients. *Journal of clinical medicine research*, *4*(5): 309.
11. LeDoux, S. P., Woodley, S. E., Patton, N. J., & Wilson, G. L. (1986). Mechanisms of nitrosourea-induced β-cell damage: alterations in DNA. *Diabetes*, *35*(8): 866-872.
12. Mamun-or-Rashid, A.N.M., Hossain, M.S., Hassan, N., Dash, B.K., Sapon, M.A. and Sen, M.K.(2014). A review on medicinal plants with antidiabetic

activity. *Journal of Pharmacognosy and Phytochemistry*, *3*(4): 149-159.

13. Matikainen, N., Mänttäri, S., Schweizer, A., Ulvestad, A., Mills, D., Dunning, B. E., & Taskinen, M. R. (2006). Vildagliptin therapy reduces postprandial intestinal triglyceride-rich lipoprotein particles in patients with type 2 diabetes. *Diabetologia*, *49*(9): 2049-2057.
14. Puri, D., Prabhu, K. M., & Murthy, P. S. (2002). Mechanism of action of a hypoglycemic principle isolated from fenugreek seeds. Indian Journal of Physiology and Pharmacology, 46(4): 457-462.
15. Renuka, C., Ramesh, N., & Saravanan, K. (2009). Evaluation of the antidiabetic effect of *Trigonella foenum-graecum* seed powder on alloxaninduced diabetic albino rats. *International Journal of PharmTech Research*, *1*(4): 1580-1584.
16. Sinskaja, E. N. (1962). Flora of cultivated plants of the USSR. XIII. Perennial leguminous plants. Part I. Lucerne, sweet clover and fenugreek. *Flora of cultivated plants of the USSR. XIII. Perennial leguminous plants. Part I. Lucerne, sweet clover and fenugreek.*
17. Sharma, R. D., & Raghuram, T. C. (1990). Hypoglycaemic effect of fenugreek seeds in non-insulin dependent diabetic subjects. *Nutrition research*, *10*(7): 731-739.
18. Sharma, M. S., & Choudhary, P. R. (2014). Hypolipidemic effect of fenugreek seeds and its comparison with atorvastatin on experimentally induced hyperlipidemia. *J Coll Physicians Surg Pak*, *24*(8): 539-42.
19. Sushma, G., & Mukthinuthalapati, M. A. (2020). New first derivative spectrophotometric methods for the determination of Sitagliptin-An antidiabetic agent. *Research Journal of Pharmacy and Technology*, *13*(6): 2838-2842.
20. World Health Organization, *WHO Diabetes Fact Sheet on diabetes,* 10 November (2021). https://www.who.int/news-room/fact-sheets/detail/diabetes
21. Wilson, G. L., Hartig, P. C., Patton, N. J., & LeDoux, S. P. (1988). Mechanisms of nitrosourea-induced β-cell damage: activation of poly (ADP-ribose) synthetase and cellular distribution. *Diabetes*, *37*(2): 213-216.
22. Xue, W. L., Li, X. S., Zhang, J., Liu, Y. H., Wang, Z. L., & Zhang, R. J. (2007). Effect of Trigonella foenum-graecum (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats. *Asia Pac J Clin Nutr*, *16* (Suppl 1): 422-426.

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