ANTIDIABETIC ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *ALLIUM CEPA*

Aimun Shakir[1], Syeda Afroz[2], Syed Muhammad Masood Ali[3], Abdullah[4], Mahwish Fatima[5] Azmat Ara[6]

[1]PhD Scholar, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

.

[2]Associate Professor, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

[3]Associate Professor, Department of Pharmacology, Al-Tibri Medical College, Isra University, Karachi, Pakistan.

[4]Lecturer, University of Balochistan, Quetta, Pakistan.

[5] Research Co-ordinator, The Aga Khan University, Karachi, Pakistan.

[6] Department of Pharmaceutics, University of Karachi, Karachi, Pakistan.

Corresponding Author: Syeda Afroz, Associate Professor, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

ABSTRACT:

The ethanolic leaves extract of *Allium Cepa* with its increasing dosages, that is, 200 mg/kg, 250 mg/kg and 300 mg/kg were used to determine the antidiabetic activity in rats in which diabetes was induced by using alloxan monohydrate for 2 weeks at the dose of 150 mg/kg. The results were compared to the increasing dosages of standard antidiabetic drug glibenclamide at the dose of 2.5 mg/kg, 3.8 mg/kg and 5 mg/kg respectively and also against the control, that is, normal saline. It was observed that with the increasing dosages (200 mg/kg, 250 mg/kg and 300 mg/kg) of ethanolic leaves extract of ALLI given to rats, it produced dose dependent significant (that is, $p < 0.05$) reductions in the blood glucose levels of diabetic rats after a treatment of 6 weeks in comparison to the control rats that received normal saline. The most effective concentration at which there was significant blood glucose level reduction seen was at the dose of 300 mg/kg. Hence, from this

experiment, it is evident to conclude that *Allium Cepa* has the ability to exhibit promising hypoglycemic activity.

Keywords: *Allium cepa*, hypoglycemia, antidiabetic activity, alloxan, ethanolic leaves extract.

INTRODUCTION

The term "diabetes mellitus" actually refers to a set of diseases that share the characteristic of hyperglycemia and which can be caused by a variety of factors, including genetics, the immune system, and metabolism (Egan and Dinneen, 2019). Aging, obesity, poor energy consumption, alcohol consumption, smoking, and other variables all have a role in aetiology (Raman, 2016). The United States, India, and China are predicted to have the largest diabetes populations by the year 2030 (Wild et al., 2004).

Allium cepa L., belongs to the family Alliaceae. It has been demonstrated that onions contain antibacterial (Santas et al., 2010) and antifungal effects (Kyung, 2012). Extracts of onions prevent dental cavities caused by oral microorganisms (Kim, 1997). The pungent flavour of onions comes from allyl-propyl disulfide which is a volatile oil and which may have anti-diabetic benefits (Andallu et al., 2001). The enzymes alpha-glucosidase, alpha-amylase, lipase and lipoxygenase are all known to be inhibited by the red onion skin extract (Stoica et al., 2021). Onions can stop bleeding caused by piles (Zahid et al., 2008). They can also treat asthma, cancer, hypocholestremia, as well as osteoporosis (Marrelli et al., 2018). Effects such as analgesic, anti-inflammatory, anti-hypertensive, as well as immune-protective actions are just some of the pharmacological benefits associated with the bioactive compounds found in *Allium Cepa* (Teshika et al., 2018). Onions are well-known for their high iron content. As a result, they are useful in the treatment of anaemia. They are also used as a good cholera treatment and also useful in the treatment of urinary tract infections and bleeding piles as well (Zahid et al., 2008).

Since A. cepa contains flavonoids, phenolic acids, thiosulfinates and organosulfur compounds, it is an extremely abundant source of antioxidants (Tedesco et al., 2015). The hypoglycemic characteristics come from the presence of S-methylcysteine and flavonoids like quercetin. These compounds increase insulin secretion overall antioxidant enzyme activity while decreasing serum lipids, oxidative stress, as well as lipid peroxidation. Onion extracts have been shown to have

hypolipidemic effects, specifically through their ability to normalise liver hexokinase, glucose 6-phosphatase as well as HMG coenzyme-A reductase activity (Akash et al., 2014).

Four diagnostic tests for diabetes are currently recommended which includes fasting plasma glucose where a 10 hour to 14 hour fast is observed (Clinical Methods: The History, Physical, and Laboratory Examinations, 1990). Other methods include oral glucose tolerance test (OGTT) that provides a more complete and sensitive assessment of glucose metabolism (Stolk et al., 1995), HbA1c represents the average plasma glucose during the past eight to twelve weeks (Nathan et al., 2007) and random blood glucose where a person's blood glucose levels are measured randomly during the course of the day and does not need fasting or ongoing monitoring, unlike other blood tests for diabetes (Bowen et al., 2015).

In this research method, we will observe fasting blood sugar levels to assess the antidiabetic levels of our test extracts.

Phytotherapeutics are derived from medicinal plants and their active compounds have the potential to cure illnesses, while they occasionally have negative side effects (Trojan-Rodrigues et al., 2012). Also, because of the expensive cost of modern anti-diabetic drugs and other pharmaceuticals, many low-income and rural people turn to herbal remedies instead (KUSANO & ABE, 2000). So the ultimate goal of using *Allium cepa* as a trusted source of herb is to treat diabetes affordably and effectively with minimal side effects.

MATERIALS AND METHODS

Plant material

The *A. cepa* utilised in the study was bought from the University of Karachi's Agriculture Department. The plant's species names were preserved at the University of Karachi's Herbarium unit, located in the Department of Botany.

Animal model

63 adult white wistar strain albino rats which weighed around 200 to 250 g were bred in the animal house of the Faculty of Pharmacy. They were given time to acclimatize in a controlled environment

with a constant temperature of 26±2°C, humidity of 50-60%, and an equal exposure of light and dark, that is, a 12-hour ON and a 12-hour OFF light cycle with free access to water and food.

Preparation of plant extract

Fresh *Allium Cepa* with leaves attached therein, was purchased from a grocery store in order to make an ethanolic leaf extract. Those leaves were picked off and cleaned. 500 grams of fresh leaves were combined with 1000 mL of ethanol followed by a 15-minute blending session at room temperature. The resulting mixture was filtered using analytical filtrate (da Silva et al., 2008). Airtight bottles were used to store the extract at 4 °C in the fridge until it was time to use it. The extracted substance was administered orally to the rats via a feeding syringe.

Induction of diabetes mellitus

After acclimatization period, the rats were subjected to a 12-hour overnight fast during which the rats had access to just water after which alloxan was introduced intraperitoneally at the dose of 150 mg/kg. The animals did not receive food or water until 30 minutes after receiving alloxan (Sheriff et al., 2020). This was done for a period of 2 weeks and then rats with serum glucose levels between 250 and 400 mg/dl were utilized in the experiment (Ozougwu, Jevas, 2011).

Experimental design

A total of 63 rats were split up into three categories:

Group I-Control: Nine non-diabetic rats (non-diabetic control) which included 9 rats given 1.0 ml of normal saline given orally daily.

Group II-Test: Twenty-seven alloxan induced diabetic rats were split into three groups of nine (IIa, IIb, and IIc). The test subjects in this group were given ethanolic leaves test extracts orally.

IIa includes 9 rats that were given 200 mg/kg of the test extract.

IIb includes 9 rats that were given 250 mg/kg of the test extract.

IIc includes 9 rats that were given 300 mg/kg of the test extract.

Group III-Standard: Twenty-seven alloxan induced diabetic rats were split into three groups of nine (IIIa, IIIb, and IIIc). They were assigned to the "control" group and were given glibenclamide orally.

IIIa included 9 rats that were given 2.5 mg/kg of glibenclamide.

IIIb included 9 rats that were given 3.8 mg/kg of glibenclamide.

IIIc included 9 rats that were given 5.0 mg/kg of glibenclamide.

The experiment was conducted for six weeks (Ozougwu, Jevas, 2011).

Prior to the animals receiving extract treatments, all measurements were recorded and then monitored over the course of six weeks. After fasting for 16 hours, tails of rats were pricked to collect blood sample. The rats were given doses of glibenclamide, test extracts, and normal saline orally.

Blood glucose level determination

Blood glucose levels were assessed by using a glucometer and a test strip by pricking the tail of rats and drawing blood to check the blood glucose levels.

Data analysis

The data was collected, pooled and analysed for statistic values indicated by mean ± standard deviation. Level of significance was calculated and ANOVA was applied.

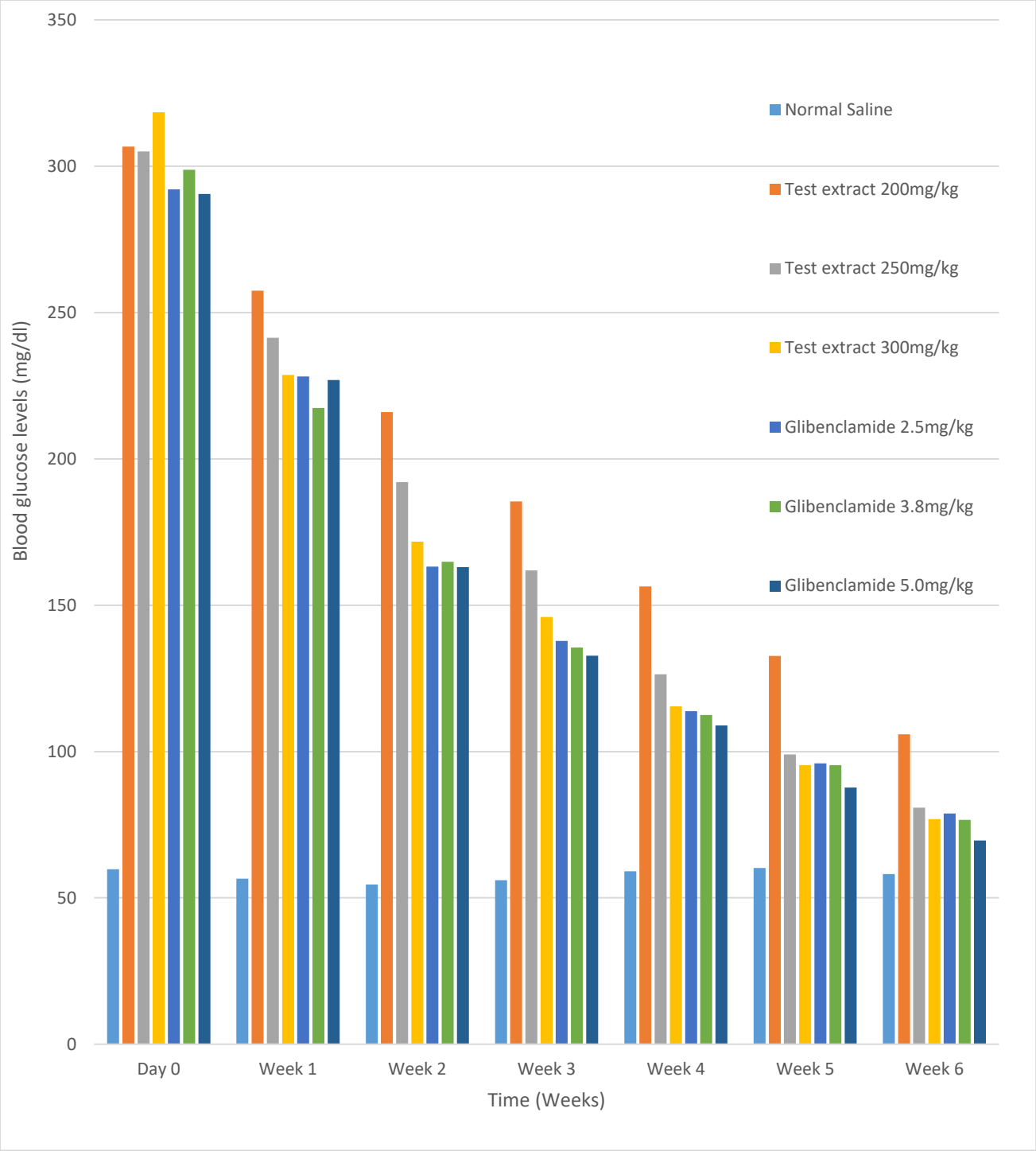

Figure 1: Effects of ethanolic leaves extract of *Allium cepa* on blood glucose levels of alloxan induced diabetic rats. The values represent mean of 9 observations. Normal saline represents non-diabetic control. Test extract represents ethanolic leaves extract of *Allium Cepa* in diabetic rats while Glibenclamide represents diabetic control and standard. P<0.05.

RESULTS

Blood glucose levels

The increasing dosages of ethanolic extracts of *Allium cepa* were applied to the rats at the concentrations of 200mg/kg, 250mg/kg and 300mg/kg and they brought about a dose dependent significant response ($P<0.05$) by lowering the blood glucose levels of diabetic rats as shown in Figure 1.

Allium cepa test extracts when given to rats at 200 mg/kg helped decreasing blood glucose levels from 306.67 mg/dl ± 54.67 to 105.85 mg/dl ± 27.73 with a decrease of 65.48%. *Allium cepa* test extracts when given to rats at 250 mg/kg helped decreasing blood glucose levels from 305 mg /dl ± 45.21 to 80.84 mg/dl ± 29.54 with a decrease of 73.49%. *Allium cepa* test extracts when given to rats at 300 mg/kg helped decreasing blood glucose levels from 318.44 mg /dl ± 40.48 to 76.95 mg/dl ± 25.48 with a decrease of 75.83%.

Glibenclamide as a standard when given at 2.5 mg/kg reduced blood glucose levels from 292.11 mg/dl ± 33.75 to 78.79 mg/dl ± 23.81 with a decrease of 73.02%. Glibenclamide as a standard when given at 3.8 mg/kg reduced blood glucose levels from 298.78 mg/dl ± 33.46 to 76.65 mg/dl ± 25.68 with a decrease of 74.34%. Glibenclamide as a standard when given at 5.0 mg/kg reduced blood glucose levels from 290.55 mg/dl ± 27.12 to 69.63 mg/dl ± 15.84 with a decrease of 76.03%.

Normal saline had no effect on the fasting blood glucose levels in rats and the levels remained between the normal ranges of blood glucose levels between 54.55 mg/dl to 60.19 mg/dl respectively.

DISCUSSION

Medicinal plants have a rich history as they have been used since the beginning of mankind. Also, they have been used quite extensively as each plant contains various phytochemicals that can treat not one but many diseases. Diabetes Mellitus is a pathological condition that increases blood glucose levels and its chronic effects lead to even more complex diseases. Chronic complications of DM include microvascular complications. Numerous organs can be affected by diabetes complications, including retinopathy, neuropathy, cardiovascular disease, and diabetic foot ulcers

(Tan et al., 2019). So many complications lead to one conclusion, which is to help decrease diabetic blood glucose levels to normal ranges or to prevent diabetes from occurring. Glycaemic homeostasis is the balance or control of glucose in living organism's circulation (Ighodaro et al., 2017), which is brought about by insulin and glucagon which are powerful glucose metabolism regulators. Other glucose-regulatory hormones include amylin, GLP-1, glucose-dependent insulinotropic peptide (GIP), adrenaline, cortisol, and growth harmones. Insulin and amylin come from the beta cells, glucagon comes from the pancreas alpha-cells, while GLP-1 and GIP come from the intestine's L-cells (Aronoff et al., 2004).

Alloxan has been widely used to cause diabetes mellitus in animals. It is renowned for its selective pancreatic islet beta cell cytotoxicity, which is why it is used in this experiment to induce diabetes (Nafisa et al., 2007).

The presence of high levels of phenolic content and antioxidants in onion extracts have proven to demonstrate inhibition of α–glucosidase activity resulting in normalising hyperglycemia to normoglycemia (Kwak et al., 2017). The presence of thiosulfinates and organosulphur compounds also prove to lower blood glucose levels (Tedesco et al., 2015). S-methylcysteine and flavonoids also aid in lowering serum lipids, oxidative stress, and lipid peroxidation levels while also boosting insulin secretion and antioxidant enzyme activity. It also restores normal liver hexokinase, glucose 6-phosphatase and HMG coenzyme-A reductase activity as well as showing hypolipidemic effects (Akash et al., 2014).

In general, by using these models of diabetes induced by chemical drugs, the majority of published studies report the amount of reduction of blood glucose that is always evaluated after a period of fasting following acute or chronic treatment with a specific natural product. Comparative studies are carried out with nondiabetic and diabetic animal groups treated with known antidiabetic drugs and researches are underway in assessing the mechanism of action in detail.

CONCLUSION

Allium cepa is beneficial to be used in diabetes to reduce hyperglycemia and can be used as an alternative to glibenclamide as it will impose less side effects. However, extensive researches can

now be done to detect antihyperglycemic effects of leaves of *Allium cepa* in humans. By 2025, it is the goal that has been universally agreed upon to stop the rise in diabetes and obesity.

The presence and association of antioxidants, quercetins, allyl propyl disulphide and S-methyl cysteine may provide a basis for dietary supplementation of onions compounds in diabetics to reduce over dependence on medicinal drugs.

Disclaimer: There are no disclaimer.

Conflict of interest: The authors have declared no conflict of interest

Funding disclosure: There are no financial conflicts of interest to disclose

REFERENCES

1. Akash, M. S. H., Rehman, K., & Chen, S. (2014, October). Spice plant Allium cepa: Dietary supplement for treatment of type 2 diabetes mellitus. Nutrition, 30(10), 1128–1137.
2. Andallu, B., Suryakantham, V., Lakshmi Srikanthi, B., and Kesava Reddy, G. (2001). Effect of mulberry (Morus indica L.) therapy on plasma and erythrocyte membrane lipids in patients with type 2 diabetes. Clinica Chimica Acta, 314(1–2), 47–53.
3. Aronoff, S. L., Berkowitz, K., Shreiner, B., and Want, L. (2004). Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. Diabetes Spectrum, 17(3), 183–190.
4. Bowen, M. E., Xuan, L., Lingvay, I., and Halm, E. A. (2015). Random Blood Glucose: A Robust Risk Factor For Type 2 Diabetes. The Journal of Clinical Endocrinology and Metabolism, 100(4), 1503–1510.
5. Clinical Methods: The History, Physical, and Laboratory Examinations. (1990). Annals of Internal Medicine, 113(7), 563.
6. da Silva, S. L., Chaar, J. da, Damico, D. C., Figueiredo, P. de, & Yano, T. (2008). Antimicrobial activity of ethanol extract from leaves of casearia sylvestris. Pharmaceutical Biology, 46(5), 347–351.
7. Egan, A. M., & Dinneen, S. F. (2019, January). What is diabetes? Medicine, 47(1), 1–4.
8. Ighodaro, O. M., Adeosun, A. M., & Akinloye, O. A. (2017). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina, 53(6), 365–374.
9. Kim, J. H. (1997). Anti-bacterial action of onion (Allium cepa L.) extracts against oral pathogenic bacteria. The Journal of Nihon University School of Dentistry, 39(3), 136–141.
10. KUSANO, S., & ABE, H. (2000). Antidiabetic Activity of White Skinned Sweet Potato (Ipomoea batatas L.) in Obese Zucker Fatty Rats. Biological and Pharmaceutical Bulletin, 23(1), 23–26.
11. Kwak, J. H., Seo, J. M., Kim, N. H., Arasu, M. V., Kim, S., Yoon, M. K., & Kim, S. J. (2017, September). Variation of quercetin glycoside derivatives in three onion (Allium cepa L.) varieties. Saudi Journal of Biological Sciences, 24(6), 1387–1391.

12. Kyung, K. H. (2012, April). Antimicrobial properties of allium species. Current Opinion in Biotechnology, 23(2), 142–147.

13. Marrelli, M., Amodeo, V., Statti, G., & Conforti, F. (2018, December 30). Biological Properties and Bioactive Components of Allium cepa L.: Focus on Potential Benefits in the Treatment of Obesity and Related Comorbidities. Molecules, 24(1), 119.

14. Nafisa PC, Chakradnar VL, Vandana SP, Suresh RN (2007). An experimental evaluation of the antidiabetic and antilipidaemic properties of a standardized Momordica charantia fruit extract. BMC Complementary Altern. Med., 7: 29-55.

15. Nathan, D. M., Turgeon, H., and Regan, S. (2007). Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia, 50(11), 2239–2244.

16. Ozougwu, Jevas. (2011). Anti-Diabetic effects of Allium cepa (ONIONS) aqueous extracts on alloxan-Induced diabetic Rattus Novergicus. Pharmacologyonline. 1. 270-281.

17. Raman, P. (2016, June 16). Environmental Factors in Causation of Diabetes Mellitus. Environmental Health Risk - Hazardous Factors to Living Species.

18. Santas, J., Almajano, M. P., & Carbó, R. (2010, February). Antimicrobial and antioxidant activity of crude onion (Allium cepa, L.) extracts. International Journal of Food Science & Technology, 45(2), 403–409.

19. Sheriff, O. L., Olayemi, O., Taofeeq, A. O., Riskat, K. E., Ojochebo, D. E., & Ibukunoluwa, A. O. (2020, January 1). A New model for Alloxan-induced diabetes mellitus in rats. Journal of Bangladesh Society of Physiologist, 14(2), 56–62.

20. Stoica, F., Aprodu, I., Enachi, E., Stănciuc, N., Condurache, N. N., Duță, D. E., Bahrim, G. E., & Râpeanu, G. (2021, October 28). Bioactive's Characterization, Biological Activities, and In Silico Studies of Red Onion (Allium cepa L.) Skin Extracts. Plants, 10(11), 2330.

21. Stolk, R., Orchard, T. J., and Grobbee, D. (1995). Why Use the Oral Glucose Tolerance Test? Diabetes Care, 18(7), 1045–1049. Stumvoll, M., Goldstein, B. J., and van Haeften, T. W. (2005). Type 2 diabetes: principles of pathogenesis and therapy. The Lancet, 365(9467), 1333–1346.

22. Tan, S. Y., Mei Wong, J. L., Sim, Y. J., Wong, S. S., Mohamed Elhassan, S. A., Tan, S. H., Ling Lim, G. P., Rong Tay, N. W., Annan, N. C., Bhattamisra, S. K., and Candasamy, M. (2019). Type 1 and 2 diabetes mellitus: A review on current

treatment approach and gene therapy as potential intervention. Diabetes and Metabolic Syndrome: Clinical Research and Reviews, 13(1), 364–372.

23. Tedesco, I., Carbone, V., Spagnuolo, C., Minasi, P., & Russo, G. L. (2015, May 22). Identification and Quantification of Flavonoids from Two Southern Italian Cultivars of Allium cepa L., Tropea (Red Onion) and Montoro (Copper Onion), and Their Capacity to Protect Human Erythrocytes from Oxidative Stress. Journal of Agricultural and Food Chemistry, 63(21), 5229–5238.

24. Teshika, J. D., Zakariyyah, A. M., Zaynab, T., Zengin, G., Rengasamy, K. R., Pandian, S. K., and Fawzi, M. M. (2018). Traditional and modern uses of onion bulb (Allium cepaL.): a systematic review. Critical Reviews in Food Science and Nutrition, 59(sup1), S39–S70.

25. Trojan-Rodrigues, M., Alves, T., Soares, G., & Ritter, M. (2012, January). Plants used as antidiabetics in popular medicine in Rio Grande do Sul, southern Brazil. Journal of Ethnopharmacology, 139(1), 155–163.

26. Wild, S., Roglic, G., Green, A., Sicree, R., King, H., 2004. Global prevalence of diabetes: estimates for 2000 and projections for 2030. Diabetes Care 27, 1047–1053.

27. Zahid, N., Claussen, B., & Hussain, A. (2008, June). Diabetes and impaired glucose tolerance in a rural area in Pakistan and associated risk factors. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 2(2), 125–130.