IN VIVO HYPOGLYCEMIC ACTIVITY OF N-HEXANE, CHLOROFORM, ETHYLACETATE, ACETONE AND AQUEOUS FRACTIONS OF *HUNTERIA UMBELLATA* LEAF IN STREPTOZOTOCIN INDUCED DIABETIC RATS.

**** Abubakar, Asmau Niwoye***

Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria

ORCID: 0000-0003-1298-9891

Saidu, Ndaman Abubakar

Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria

ORCID: 0000-0001-9254-6534

Akanya, Helmina Oluwafunmilayo

Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria

ORCID: 0000-0002-1993-5457

Evans, Chidi Egwim

Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria

ORCID: 0000-0002-2532-5106

ABSTRACT

Globally, diabetes is a major health problem causing serious issues among the public. In spite the various drugs available, the challenge of controlling diabetes still remains. This study was designed to evaluate the hypoglycemic potentials of various fractions of *Hunteria umbellata* leaf in streptozotocin induced diabetic rats. Partitioning of the crude methanol extract was performed using solvent-solvent extraction method. Diabetes was induced in experimental rats by a single dose of intra-peritoneal injection (45mg/kg body weight) of streptozotocin dissolved in 0.1 mL fresh cold citrate buffer at pH 4.5. The various fractions were administered in doses 200 and 400 mg/kg body weight. The result of the hypoglycemic effect showed that rats administered Glibenclamide had 65.10 % ± 2.36 activity while rats administered 400mg/kg body weight of chloroform fraction had the highest percentage glucose reduction of 61.58 % ±4.56 followed by ethylacetate fraction (58.83±2.45), aqueous (53.95 % ±2.56), acetone fraction 52.73 % ±1.56 and n- hexane (19.32 % ±3.56) had the least activity. All fractions of *Hunteria umbellata* caused a significantly ($p<0.05$) hypoglycemic effect in dose dependent manner when compared with the diabetic untreated rats (-21.83±2.36). In animals treated with 200mg/kg body weight, the hypoglycemic effect of the Chloroform fraction was significantly ($p<0.05$) higher than the remaining fractions. *Hunteria Umbellata* leaf has some hypoglycemic potentials and could be further processed towards the management of diabetes mellitus.

Keyword: *Hunteria umbellata*, Streptozotocin, Intraperitoneal, Glibenclamide, Diabetes

INTRODUCTION

Diabetes is a complex metabolic disorder characterized by dysfunction or destruction of pancreatic beta cells responsible for the production of insulin which causes insulin deficiency, insensitivity or total lack of insulin. The main symptom of type 2 diabetes mellitus is insulin resistance coupled with relative or absolute lack of secretion, which eventually leads to disorders of carbohydrate, fat, and protein metabolism.(Saidu et al., 2014; Abubakar et al., 2019) The clinical manifestations of diabetes type II include persistent hyperglycemia, increased hunger, high urinary frequency, dehydration and weight loss, with various complications like retinopathy, neuropathy, and nephropathy, cardiovascular and cerebrovascular diseases resulting in high mortality (Festa et al

2017; Pavlon et al., 2018). Several medicinal plants have been reported to be useful in treating diabetes globally and are used as anti-hyperglycemic remedy. The hypoglycemic effect of the plants could be due to their ability to reinstate the function of pancreatic tissues by causing an increase in insulin production or inhibition of the intestinal glucose absorption or activation of insulin receptors. Synthetic drugs such as meglitinides, biguanides, sulfonylureas, and thiazolidinediones used for treating diabetes showed some side effects like weight gain, toxicity, hypoglycemia, and drug resistance (Sagbo et al., 2015; Satar et al., 2019). This necessitate researchers to focus on natural products like plants with minimal or no side effects that is cost effective and could be involved in future drug development strategies (Chukuma etal., 2019).

Hunteria umbellata (K. Schum) belongs to the family Apocynaceae. The plant is locally known as 'Abeere' among the Yoruba (South-West Nigeria) and known as Nkpokiri among the Igbo's. In African folk medicine, various parts like the leaves, roots, barks and seeds are highly used for the treatment of various human diseases and veterinary. Decoction made from the plant stem and root is apparent for its antihelminthic activities and in the treatment of swellings (Gill, 1992). The plant leaves and pulp are equally used by West African traditional midwives to treat pregnancy related ailments and to induce labour at term (Falodun *et al.,* 2006)

3.1 Reagents and chemicals

Streptozotocin (STZ) was purchased from Sigma Aldrich in Germany through Bristol scientific company (BSC) from Sigma Chemical Co St. Louis M.O., USA. Organic solvents such as Ethylacetate, Chloroform, Methanol, Acetone and N-hexane used for extraction of the plant materials were of analytical grade purchased from reputable companies like JHD, BDH and Qualikems.

3.2 Experimental animals

Adult Wistar rats of both sexes weighing between 120 -200g were used for the *in vivo* anti diabetic studies. The animals used were obtained from the Animal House of National Institute for Trypanosomiasis Research (NITR) Kaduna and Ibrahim Badamasi Babangida University, Lapai (IBBUL). They were housed in plastic cages under standard environmental condition of 12 hour light-dark cycle, temperature 27± 2 °C, and relative humidity of 50 % ± 10% .They were fed with standard rat pellets and water *ad libitum*. The animals were also allowed to acclimatize for two weeks before commencement of the experiment. A good laboratory practice in strict compliance for laboratory animal use and care as contained in the Federal University of Technology Minna animal care guidelines and protocol review of laboratory animals was followed.

3.3 Collection of plant materials

Fresh leaves of *Huntaria umbellata* was obtained from Orlu Local Government, Imo State Nigeria. The plant was collected on the basis of ethno botanical uses and information provided by some herbal practitioners on the plants used locally to treat diabetes. The Plant was authenticated by Mr. Abdullateef Abdulhakeem (Botanist) of the Herbarium Department of National Institute of Pharmaceutical Research and Development (NIPRD), Abuja and were assigned voucher number *Hunteria umbellata* (NIPRD/H/6871/).

3.4 Plant preparation and extraction

Fresh leaves of *Hunteria umbellata* were washed thoroughly under running tap and air dried in shade at room temperature (26°C± 2°C) for two weeks before pulverized into powdery form with an electronic blender. Powdered leaves (100 g) of all the selected plants were extracted separately in 400mls of 70 % methanol for three hours at 60ºC. The extracts were filtered by Muslin cloth and Whatman's no 1 filter paper, concentrated under reduced pressure by a Rotary Evaporator and subsequently in water bath (Nyakmo *et al.,* 2013). The dried extract were weighed, kept air tight in sterile bottles and refrigerated until required. The percentage yield of the extracts was calculated using the formula below:

$$\% \text{ Yield} = \frac{\text{Weight of the Crude Extract (g)}}{\text{Weight of Dried Sample (g)}} \times 100$$

Tsado et al 2015; Abubakar et al., 2019

3.5 Induction of diabetes in experimental animals

The animals were fasted for 12–14 hours with free access to water prior to the induction of diabetes. Induction of diabetes was done by single intraperitoneal injection of streptozotocin dissolved in 0.1 mL fresh cold citrate buffer at pH 4.5 at a dose of 45 mg/kg body weight (Burcelin *et al.,* 1995). On the third day of STZ injection, fasting blood glucose was determined by taking blood from tail artery of the rats on to the glucose strips placed in a glucometer (Rheney and Kirk, 2000). Rats with blood glucose greater than 200 mg/dl group were considered for the study (Tanko *et al.,* 2013). The bodyweight (g) of rats were obtained using a sensitive weighing balance prior to diabetic induction and during treatment periods (Aniague*t al.,* 2005). Each of the fraction was administered orally into the rats throughout the treatment period comparing two different doses.

3.6 Experimental design

Animals were divided into five groups consisting of six rats each namely;

Normal (Non Diabetic) and were administered normal saline

Diabetic + Standard Drug (Treated with 5mg/kg body weight of Glibenclamide)

Diabetic + 200 mg/kg bodyweight of AOC, HU, PG, PB and VP

Diabetic + 400 mg/kg bodyweight of AOC, HU, PG, PB and VP

Negative Control (Diabetic Untreated)

3.7 Preparation of stock solution

The partitioned fractions were reconstituted in 40 % DMSO at a concentration of 100 mg/mL, when needed.

3.8 Solvent partitioning of the leaf extracts of *Hunteria umbellata*

Hunteria umbellata methanol extracts Partitioning into fraction was carried out by solvent-solvent extraction as described by Ogbadoyi *et al*. (2011). Fifty grams of the extract was dissolved in 50 mL of distilled water in a beaker each. The mixture of the extract was poured into 500 mL separating funnel and was successfully partitioned by fractionation using *n*-haxane, chloroform, ethylacetate, acetone and aqueous. This gave five fractions, this procedure was repeated six times so as to obtain enough yield for the animal studies

3.9 STATISTICAL ANALYSIS

Data were analyzed using SPSS 16.0. One way analysis of variance (ANOVA) coupled with Duncan (post-hoc test). The result was considered significant at 95% confidence level and $p<0.05$.

4.0 RESULTS

Table 4.1 Fractional Yields of *Hunteria umbellata* Leaves in Different Solvents

Solvent	Yield (%) of Fractions
***N*- hexane**	1.90
Chlorofoam	13.82
Ethylacetate	8.54
Acetone	21.76
Aqueous/residue	52.38

4.0 Hypoglycemic activity of the fractions of *Hunteria umbellata* leaf

The effect of *Hunteria umbellata* fractions on plasma glucose concentration was in a dose dependent manner (Figures 4.1 and 4.2). Chloroform fraction was more potent when compared to other fractions at doses 200 and 400 mg/kg body weight (260±1.32 -120±1.19 mg/dL) and (288.33±1.20 to 110.33±2.60 mg/dl) respectively. The blood glucose concentration of the negative control diabetic rats rises from (260.00±18.55 to 324.67±40.71 mg/dl). However, rats treated with glibenclamide had decrease in glucose levels from (298.67±2.60 to 104.00±4.16 mg/dl).

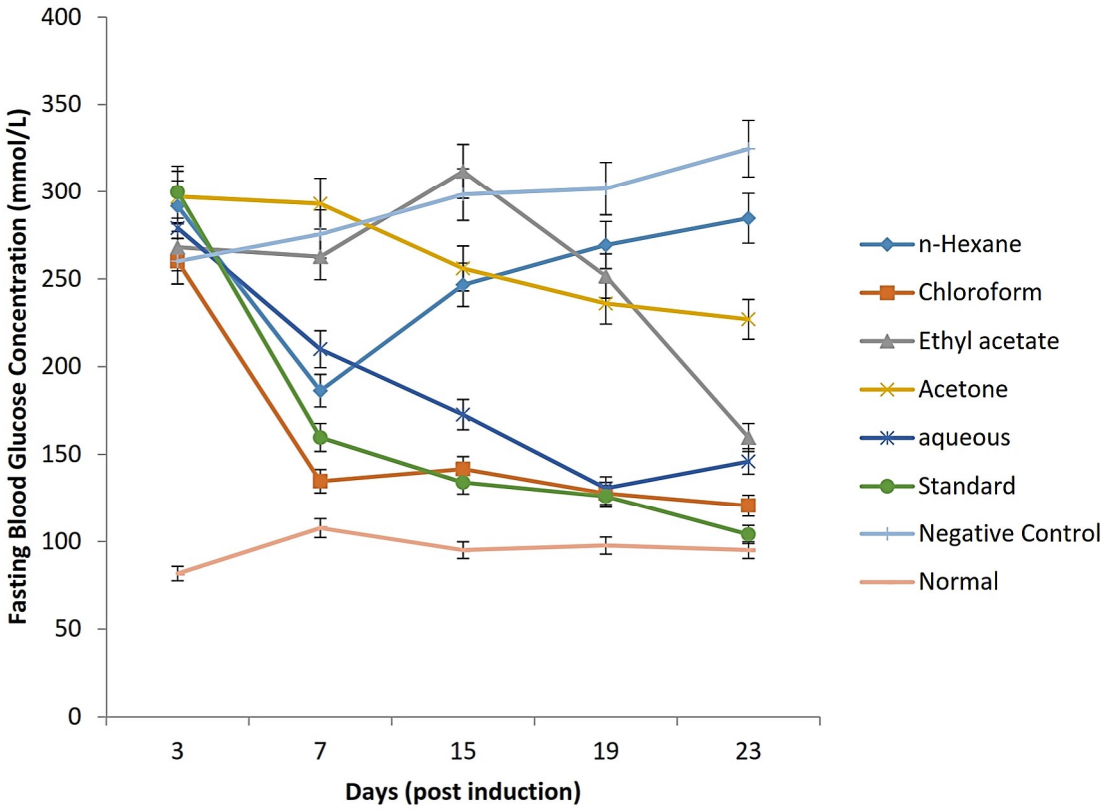

Figure 4.1: Effect of *Huntaria umbellata* leaf Fractions Administered at 200 mg/kg Body weight on Fasting Blood Glucose Concentration in Streptozotocin induced Diabetic Rats

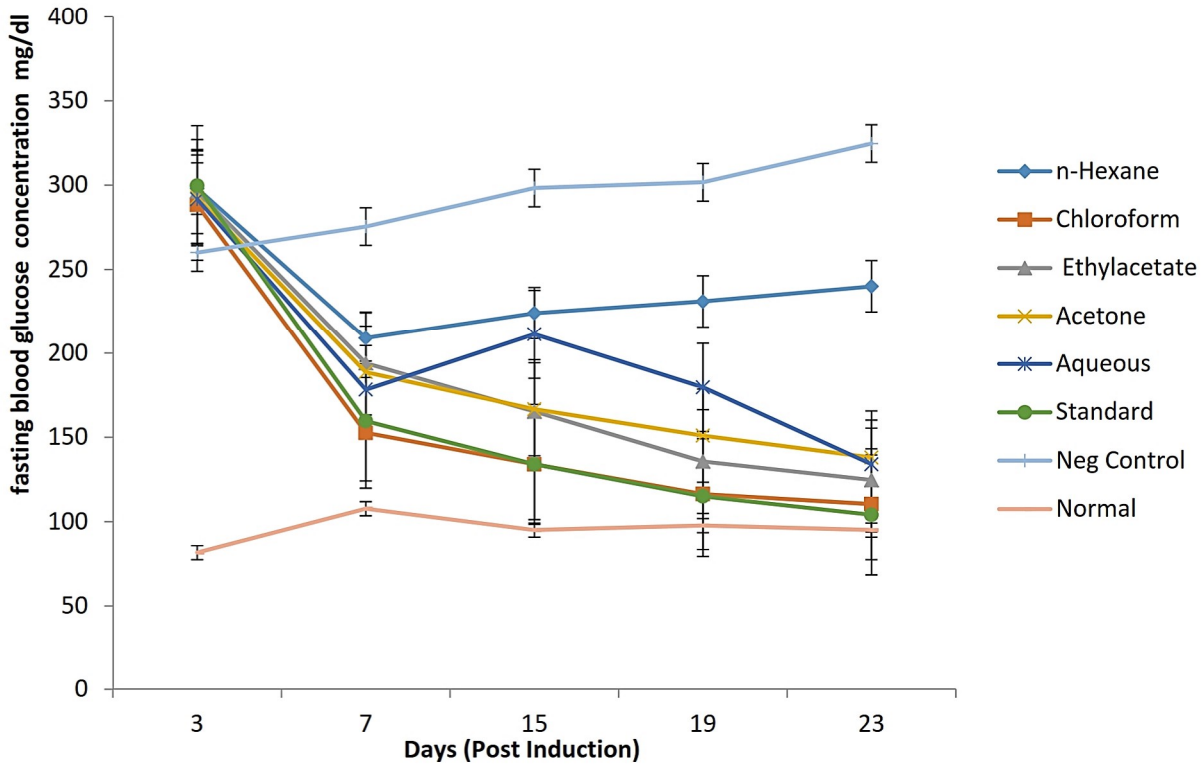

Figure 4.2: Effect of *Huntaria umbellata* Leaf Fractions Administered at 400 mg/kg Body weight on Fasting Blood Glucose Concentration in Streptozotocin Induced Diabetic Rats

4.3 Percentage glucose reduction of the fractions of *Hunteria umbellata* leaf

The percentage hypoglycemic effects of the various fractions of *Hunteria umbellata* is shown in Table 4.1. Chloroform fractions of *Hunteria umbellata* extracts (61.58±4.56 %) had the highest percentage blood glucose reduction in rats treated with 400 mg/kg body weight, followed by ethylacetate fraction (58.83±2.45 %), aqueous fraction (53.95±2.56 %) while acetone fraction had (52.73±1.56 %) and *n*-hexane fraction of *Hunteria umbellata* (2.40±4.56 %) possesses the lowest hypoglycemic activity among the rats treated with fractions. Rats administered glibenclamide had better percentage glucose reduction of (65.10±2.36 %) when compared with the extract group.

Table 4.1: Percentage Glucose Reduction of Diabetic Rats treated with Fractions of *Hunteria umbellata.*

Treatment groups	(%)Glucose Reduction
n-hexane 200	2.40±4.56
n-hexane 400	19.32±3.56
Chloroform 200	53.84±2.46
Chloroform 400	61.58±4.56
Ethylacetate 200	40.67±3.45
Ethylacetate 400	58.83±2.45
Acetone 200	23.56±2.34
Acetone 400	52.73±1.56
Aqueous 200	47.67±4.32
Aqueous 400	53.95±2.56
Standard	65.10±2.36
Negative Control	-21.83±2.36

Data are Mean ± SEM of triplicate determination

DISCUSSION

The variation in the separating solvent polarity (which is responsible for the solubility of the active components in the different fractions) might have caused the localization of bioactive ingredients in the chloroform fraction as well as vary the activity of each extract. Although the hypoglycemic effect demonstrated by the crude extract was significantly ($p<0.05$) higher than that of the chloroform fraction. (Abubakar et al., 2019; Xue-Qin et al., 2022). This may likely be as a result of the synergistic effect of the bioactive ingredients which coexist together in the crude extract or some components present in the crude extract which may have a positive modulating effect on the bioactivity of the active ingredient but lost upon purification. A similar reduction in activity have been observed by a number of researchers such as Ugwu *et al.* (2011) and Kabiru *et al.* (2013). However because the chloroform extract displays more than 60 % hypoglycemic activity, it suggests that there is prospect for better hypoglycemic activity upon further purification and isolation of the bioactive ingredients. Chloroform fraction of *Hunteria umbellata* also showed a better protective effect on weight loss of diabetic rats compared to the other fractions, this might be as a result of stimulation of certain enzymes or hormones associated with weight or better ability to ultilize glucose energy.

CONCLUSION

In this research work the various fractions *of Hunteria Umbellata* evaluated leaf evaluated possess significant hypoglycemic potentials, the mechanism of action of the most active subfractions of the chloroform fraction will be further studied. The bioactive compound could be elucidated and may be processed to a novel drug towards the management of diabetes mellitus.

RECOMMENDATION

Further research on the antimicrobial activity of the leaf extract is suggested and extraction of individual phytoconstituents for their biological activity.

ACKNOWLEDGEMENT

The authors wish to thank the Centre for Genetic engineering and Biotechnology and Department of Biochemistry Federal University of Technology Minna for providing the enabling environment.

REFERENCES

Abubakar A.N. *, Saidu AN., Akanya H.O. and Egwim E.C. Chidi (2019). Antioxidants and Hypoglycemic Effect of Some Medicinal Plants *GSC Biological and Pharmaceutical Sciences* 08(02), 070–080

Aniagu, S. O., Nwinyi, F. C., Akumka, D. D., Ajoku, G. A., Dzarma, S., Izebe, K. S., & Gamaniel, K. (2005). Toxicity Studies in Rats fed Nature Cure Bitters. *African Journal of Biotechnology*, *4*(1), 72-78.

Abubakar, Asmau Niwoye *, Zainab Bolajoko Lawal, Elisha Japeth, Ibrahim Olatunji Yunus and Rahinat Garba (2021). *In vitro* Antidiabetic Potentials of Crude Saponins Extract from *Leptodenia hastata* and *Adansonia digitata* leaves. *GSC Advanced and Reviews* 06(03), 061–066

Burcelin, R., Eddouks, M., Maury, J., Kande, J., Assan, R., & Girard, J. (1995). Excessive Glucose Production, rather than Insulin Resistance, accounts for Hyperglycaemia in Recent-onset Streptozotoci*n*-Diabetic Rats. *Diabetologia*, 38(3), 283-290.

Chukwuma, C. I. Matsabisa, M. G. Ibrahim, M. A. Erukainure, O. L. Chabalala, M. H. and Islam, M. S. (2019). "Medicinal plants with concomitant anti-diabetic and anti-hypertensive effects as potential sources of dual acting therapies against diabetes and hypertension: a review," Journal of Ethnopharmacology, vol. 235, pp. 329–360,

Falodun, A., Nworgu, Z. A., & Ikponmwonsa, M. O. (2006). Phytochemical Components of Hunteriaumbellata (*K. schum*) and its Effect on Isolated No*n*-pregnant Rat Uterus in Oestrus. *Pakistan Journal of Pharmaceutical Sciences*, 19(3), 256-258.

Festa, A., Heller, S. R., Seaquist, E., Duan, R., Hadjiyianni, I. and Fu, H. (2017). Association between mild and severe hypoglycemia in people with type 2 diabetes initiating insulin," Journal of DiabetesanditsComplications,vol. 31, no.6,pp.1047–1052.

Gill, L. S. (1992). *Ethnomedical uses of plants in Nigeria*. Benin: Uniben Press, 377-387.

Kabiru, A., & Por, L. Y. (2013). Elephantopus species, traditional uses, pharmacological actions and chemical composition. *Advance Life Science Technology*, 15, 6-13

Nyakmo, E. A., Iwara, I. A., Bob, M. I., Godwin, O., Godwin, E. E., & Patrick, E. (2013). *Achyranthe saspera* (Agadha), Herbs that improves pancreatic function in alloxan induced rats. *International Journal of Phytomedicine,* 5(2), 159-177

Ogbadoyi, E. O., Garba, M. H., Kabiru, A. Y., Mann, A., & Okogun, J. I. (2011). Therapeutic Evaluation *Acacia nilotica* (Linn) stem bark extract in experimental African trypanosomiasis. *International Journal of Applied Research in Natural Products,* 4 (2), 11-18

Pavlou, D.I. Paschou, S.A. Anagnostisetal. P."Hypertension in patients with type 2 diabetes mellitus: targets and management," (2018). Maturitas, vol. 112, pp. 71–77.

Rheney, C. C., & Kirk, J. K. (2000). Performance of three blood glucose meters. *Annals of Pharmacotherapy*, *34*(3), 317-321.

Sagbo, I. J. (2015). Phytochemical analysis and antibacterial properties of aqueous and ethanolic extracts of Brachylaena elliptica and Brachylaena ilicifolia. MSc Dissertation [Dissertation, thesis], University of FortHare.

Saidu, A.N., Abubakar A.N., Daniel, M.U. and Kabiru, A.Y. (2014). Phytochemical screening and effects of Methanolic Extract of *Azadiractha indica* leaf in Alloxan induced diabetic rats. Journal of Pharmacy and Biological Sciences, 9 (1) 16-20. www.iostjournals.org

Sattar N. Advances in the clinical management of type 2 diabetes: a brief history of the past 15 years and challenges for the future. BMC Med (2019). 17(1):2-5.

Tanko, Y., Muhammad, A., Emokpae, L.V., Mohammed K. A., Jimoh, A., Sada, N. M.,Abdulrazak A., Yerima M., & Mohammed, A. (2013). A. Effects of Ethyl acetate and *n*-Butanol Fractions of *Acacia nilotica*methanol Leaves Extract on Lipid profile and Liver Enzyme of Alloxan - Induced Diabetic Wistar rats. *Journal of Applied Pharmaceutical Sciences,* 3(12), 103-108.

Tsado, A. N., Lawal, B., Mohammed, S. S., Famous, I. O., Yahaya, A. M., & Shuaibu, M. (2015).Phytochemical composition and antimalarial activity of methanol leaf extract of *Crateva adansonii* in *Plasmodium berghei* Infected Mice. *British Biotechnology Journal*, 6, 165-173.

Ugwu, C. E., Olajide, J. E., Alumana, E. O., & Ezeanyika, L. U. S. (2011). Comparative effects of the leaves of Vernonia amygdalina and Telfairia occidentalis incorporated diets on the lipid profile of rats. *African Journal of Biochemistry Research*, 5(1), 28-32.

Xue-Qin Li, Shan-Shan Jia, Ke Yuan , and Song-Heng Jin (2022). Hypoglycemic Effect of the N-Butanol Fraction of Torreya grandis Leaves on Type 2 Diabetes Mellitus in Rats through the Amelioration of Oxidative Stress and Enhancement of βCell Function Hindawi *BioMed Research International* Volume 2022, Article ID 5648896, https://doi.org/10.1155/2022/5648896