biologics

MDPI

Article

Abelmoschus esculentus (L.) Moench Pod Extract Revealed Antagonistic Effect against the Synergistic Antidiabetic Activity of Metformin and Acarbose upon Concomitant Administration in Glucose-Induced Hyperglycemic Mice

Md. Anamul Haque [1,†], Md. Sanower Hossain [2,3,*,†], Nur Muhammad Abu Sayed [1], Mohammad Touhidul Islam [1], Md. Robin Khan [4], Foyez Ahmmed [5], Fatama Tous Zohora [6], Duygu Ağagündüz [7], Long Chiau Ming [8] and Raffaele Capasso [9,*]

1 Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka 1207, Bangladesh; anamul.ph@diu.edu.bd (M.A.H.); nur29-863@diu.edu.bd (N.M.A.S.); touhidul.ph@diu.edu.bd (M.T.I.)
2 Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Kuantan 25200, Malaysia
3 Faculty of Science, Sristy College of Tangail, Tangail 1900, Bangladesh
4 Bangladesh Reference Institute for Chemical Measurements, Dr. Qudrat-i-Khuda Road Dhanmondi, Dhaka 1205, Bangladesh; robinkhan83845@gmail.com
5 Department of Statistics, Faculty of Science, Comilla University, Kotbari, Cumilla 3506, Bangladesh; foyez.sbi@gmail.com
6 Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Tangail 1902, Bangladesh; ftzohora.bge@gmail.com
7 Department of Nutrition and Dietetics, Faculty of Health Sciences, Gazi University, Emek, Ankara 06490, Turkey; duyguturkozu@gazi.edu.tr
8 PAP RashidahSa'adatul Bolkiah Institute of Health Sciences, Universiti Brunei Darussalam, Bandar Seri Begawan BE1410, Brunei; long.ming@ubd.edu.bn
9 Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy
* Correspondence: mshossainbge@gmail.com (M.S.H.); rafcapas@unina.it (R.C.); Tel.: +60-1169609649 (M.S.H.); +39-081-678664 (R.C.)
† These authors contributed equally to this work.

check for updates

Citation: Haque, M.A.; Hossain, M.S.; Sayed, N.M.A.; Islam, M.T.; Khan, M.R.; Ahmmed, F.; Zohora, F.T.; Ağagündüz, D.; Ming, L.C.; Capasso, R. *Abelmoschus esculentus* (L.) Moench Pod Extract Revealed Antagonistic Effect against the Synergistic Antidiabetic Activity of Metformin and Acarbose upon Concomitant Administration in Glucose-Induced Hyperglycemic Mice. *Biologics* **2022**, 2, 128–138. https://doi.org/10.3390/biologics2020010

Academic Editor: Manfredi Tesauro

Received: 11 March 2022
Accepted: 7 May 2022
Published: 10 May 2022

Abstract: *Abelmoschus esculentus* (L.) Moench, commonly known as okra, is one of the most widely used vegetable crops currently used for diabetes treatment as well. It is thought that the large amount of soluble dietary fibers present in okra is responsible for the slowing of the absorption of glucose from the gut. However, its role in concomitant administration with commonly prescribed medications, including metformin (MET) and acarbose (ACR) for diabetes, is unclear. Therefore, this study assessed the effect of *A. esculentus* pod extract (AEE) administered concomitantly with MET and ACR in the glucose-induced hyperglycemic mice model. The AEE was prepared using green okra pods. In this experiment, each male Swiss Webster mouse was administered a 2.5 gm/kg/BW dose of glucose via gastric lavage to induce hyperglycemia. The experimental animals were divided into five groups: (i) negative control, (ii) positive control, (iii) MET only, (iv) MET and ACR, and (v) MET, ACR, and AEE. The orally administered doses of the MET, ACR, and the extract were 150 mg/kg/BW, 15 mg/kg/BW, and 0.2 mL/kg/BW, respectively. We found that MET only and a combination of MET and ACR reduced glucose levels significantly ($p < 0.01$) compared to the positive control. On the other hand, when MET, ACR, and AEE were administered simultaneously, the synergistic antihyperglycemic action of the MET and ACR was diminished. After 150 min, the blood glucose level was 4.50 ± 0.189 mmol/L (iv) and 6.58 ± 0.172 mmol/L (v). This study suggests that taking AEE concurrently with MET and ACR would reduce the effectiveness of antidiabetic drugs; thereby, concomitant administration of these antidiabetic agents is not recommended. This study provides an essential basis for decision-making about the consumption of AEE with conventional medicine. Further study is required to find the molecular insight of drug interactions in combination therapy of medicinal plants for diabetes.

Keywords: animal; antihyperglycemic agent; diabetes control; in vivo; medicinal plants; okra; T2DM

1. Introduction

Diabetes is a critical challenge to global health. Approximately 537 million adults (20–79 years) lived with diabetes in 2021. According to projections, the number of individuals living with diabetes will increase to 643 million by 2030 and 783 million by 2045. The number of people with diabetes living in low- and middle-income countries is getting more severe [1]. The number of diabetic patients has quadrupled in the last 40 years. Diabetes is also the leading cause of male death, increasing by 80% since 2000. An 5% increase 5in diabetes-related premature death occurred between 2000 and 2016 [2].

Hyperglycemia, often known as elevated blood sugar, is a common complication of uncontrolled diabetes that, if left untreated, can cause catastrophic harm to many of the body's systems, including the nervous system and blood vessels. Therefore, it may cause blindness, kidney failure, heart attacks, stroke, and lower limb amputation [3]. Many drugs like sulfonylureas, meglitinides, D phenylalanine derivatives, DPP-4 inhibitors, GLP agonists, amylin analogs, thiazolidinediones, biguanides, and alpha-glucosidase inhibitors are commonly used to control hyperglycemia [4]. In general, metformin (MET) is the initial therapy for patients with type 2 diabetes mellitus (T2DM), which contributes to 90–95% of all diabetes cases [5]. MET reduces the amount of blood glucose by inhibiting glycogenolysis, gluconeogenesis, and absorption of glucose from the small intestine [6,7]. Another drug, acarbose (ACR), works by slowing the breakdown of carbohydrates into glucose, thus reducing glucose absorption in the intestine [8,9]. For many patients, controlling blood glucose is difficult and physicians suggest a combination of drugs. Commonly, MET is suggested in combination with ACR for managing hyperglycemic conditions in T2DM, because both MET and ACR work synergistically to reduce the blood glucose level significantly in T2DM. A meta-analysis reported that MET and ACR combined therapy appears to be more efficacious than MET or ACR monotherapy [10]. Many other researchers also reported similar results when MET was combined with other drugs [11–14].

Self-medication using medicinal plants or natural products has increased dramatically, particularly in developing or low-income countries, due to being safer and free from toxic effects [15,16]. Additionally, the World Health Organization (WHO) estimates that 75–80% of the world's population entirely rely on conventional medical systems for their first line of treatment, owing to concerns about the safety of synthetic medicine [17–19]. On the other hand, natural products have risen to enormous prominence as sources of polypharmacological treatments for various diseases [20,21]. Furthermore, evidence of the relevance of plants for a variety of diseases in historical scriptures caught the interest of many researchers who focused on analyzing the scientific validity of traditional claims [22–24]. Therefore, many patients have been concomitantly self-administering medicinal or supplemental food therapies with potential antihyperglycemic drugs like MET and ACR to better control glucose levels.

Among the plethora of medicinal plants, *Abelmoschus esculentus* (L.) Moench (ladies finger or okra) is a common plant that possesses high antihyperglycemic effects [25–27]. It is a very familiar perennial plant grown in all tropical and subtropical areas worldwide. Its green edible pod is known for its nutritional and health benefits such as hepatoprotective, antidiabetic, antiulcer, anticancer, anti-inflammatory, laxative, antihyperlipidemic, antifungal, and analgesic activities [28] and is used in preparing vegetable curry in everyday cuisine. Among many of its constituents, viscous soluble dietary fibers (guar gum) and flavonoids like myricetin are of great concern other than carbohydrates, protein, minerals, and vitamins [28]. Traditionally, okra has been a good choice for diabetes treatment [29–32]. Doreddula et al. [28] reported that methanolic and aqueous extracts of *A. esculentus* possess alkaloids, carbohydrates, flavonoids, phenols, proteins, terpenoids, tannins, and sterols. The primary flavonoids, isoquercitrin, and quercetin 3-*O*-gentiobioside

isolated from *A. esculentus* lowered blood glucose and serum insulin levels and increased glucose tolerance in obese mice [33]. Another study showed that the identified polyphenolic compounds oligomeric proanthocyanidins from *A. esculentus* were responsible for α-glucosidase and α-amylase inhibitory activity [34]. It is thought that the large amount of soluble dietary fibers present in okra is accountable for slowing the absorption of glucose from the gut [31]. Although MET, ACR, and okra extract exert a common action by impeding glucose absorption in the small intestine, a possible synergistic effect might be taken simultaneously. There might be a possibility of drug interaction when herbal and synthetic drugs are co-administered for better glycemic control, and the combinations might show synergistic or antagonistic effects [35,36]. It is noted that combining dietary supplements with prescribed drugs may have severe and even life-threatening consequences [37].

To our best knowledge, no study has been conducted to evaluate okra's synergistic or antagonistic effects in combination with MET and ACR. This is necessary to assess postprandial uses of MET and ACR combination simultaneously with AEE. The outcomes of this study would suggest whether okra can be taken in combination with MET and ACR or not. Therefore, the mice model combined *A. esculentus* extract with MET and ACR in this experiment to evaluate its synergism or antagonism activity.

2. Results and Discussion

Since an adequate amount of nutrition plays a significant role in preventing and controlling diabetes, taking medicinal plant extracts or food supplements along with prescription medications as a natural and safer source to improve diabetes has increased drastically over the last two decades. Some medicinal or food supplements can enhance the medication's efficacy, while others can reduce it. Therefore, combining dietary supplements with prescribed drugs may have severe consequences [37]. *Abelmoschus esculentus* (L.) Moench (okra) is traditionally used for diabetes prevention and improvement, even with pharmaceutical drugs [29–32]. The effectiveness of an aqueous extract of okra against hyperglycemia in different in vitro and in vivo studies has been scientifically proven [38–42]. Okra can slow glucose absorption from the gut due to having a high amount of soluble dietary fibers [31]. Previous studies suggested that AEE should not be taken concurrently with MET or ACR in controlling diabetes mellitus [43,44]. Therefore, here we evaluated the effectiveness of AEE in combination with MET and ACR only, other than AEE with MET and AEE with ACR in vivo settings. To assess the preventive measure of glucose level in blood, we performed glucose p.o. in Swiss Webster mice after they were administered the plant extract/drugs. We mainly observed whether AEE plays a role as a synergistic partner or an antagonistic partner. Synergistic partners increase drug effectiveness, whereas antagonistic partners decrease the effectiveness of pharmaceuticals due to drug–drug interactions that block or reduce the effectiveness of one or more drugs [45].

It has previously been shown that AEE produces hypoglycemic effects by interfering with many cellular signaling pathways, such as blocking the activity of a glucose metabolizing enzyme (DPP-4). Furthermore, AE regulates the AMPK/mTOR, PI3K, and mitochondrial pathways, which may reduce palmitate-induced apoptosis of β cells [46]. AE is rich in several bioactive compounds that have been implicated for their traditional use in DM. These bioactive compounds were categorized as polyphenols, glycosides, alkaloids, polysaccharides, and volatile oils [47–49]. Shen and colleagues showed that the fruits' flavonoid fractions had a concentration-dependent α-glucosidase and α-amylase inhibitory effect [50]. In another study, researchers identified polyphenolic compounds oligomeric proanthocyanidins from *Abelmoschus esculentus* as active compounds responsible for α-glucosidase and α-amylase inhibitory activity [34]. Similarly, myricetin showed a dose-dependent decrease in the plasma glucose concentration and improved insulin sensitivity in obese Zucker rats [51]. The observed antidiabetic activity of pure polyphenolic compounds could imply that these compounds are partly responsible for the antidiabetic activity of the crude extracts of *A. esculentus* [52]. Besides that, polysaccharides derived from AEE can improve insulin sensitivity by correcting insulin signaling defects, most of

which are related to the PI3K/AKT signaling pathway [53]. Moreover, the antioxidant-rich substances in okra fruit (e.g., carotenoids, riboflavin, ascorbic acid, thiamine, and nicotinic acid) may be linked to the antiglycation capabilities of the fruit, which is connected to the hypoglycemic effects of okra [54]. Fan and colleagues [33] found that the primary flavonoids in okra extract, isoquercitrin and quercetin 3-*O*-gentiobioside, lowered blood glucose and serum insulin levels, and increased glucose tolerance in obese mice, indicating that okra might be used as a dietary treatment for hyperglycemia.

This study used 2.5 mg/kg/BW glucose p.o. to induce hyperglycemia in mice. In all groups, the fasting blood glucose was determined at 4.90–5.39 mmol/L (Figure 1a). Over 2.5 h, the blood glucose level was 4.92–5.18 mmol/L in group 1, even after taking a standard pellet diet and water ad libitum. This indicates that the mice were healthy enough to control blood glucose levels. Because of the effects of insulin, blood sugar levels in people who do not have diabetes rebound to a near-normal range within 1–2 h of eating [55]. After administration of glucose in the positive control group, the diabetic status of mice was highest at 11.44 ± 0.387 mmol/L at 30 min. The blood glucose level was gradually reduced over time, and it was lowest at 7.26 ± 0.311 mmol/L at 150 min (Figure 1b). These values were over the minimum clinical value for diabetes according to the American Diabetes Association's defined value. The defined normal fasting plasma glucose is <5.6 mmol/L, and a normal 2 h plasma glucose is <7.8 mmol/L [56]. The gradually decreasing glucose level in the blood over time indicates the mice's self-regulation of glycolysis in improving the diabetic condition. The mice reached normal glucose levels at 150 min even though they were allowed a standard pellet diet and water ad libitum (Group 2).

The effectiveness of drugs/AEE on the hyperglycemic mice is shown in Table 1. All treatment groups efficiently reduced the blood glucose level compared to positive control. At 0 min, there was no significant difference among the study groups, and fasting blood glucose level was below 5.6 mmol/L, a normal level of glucose [56]. However, after 30 min of glucose administration, the glucose level was increased beyond the normal glucose level range of all groups. The results suggested that all mice experienced induced hyperglycemia upon glucose administration. Unfortunately, treatment groups also could not control the diabetes level at 30 min, but they reduced glucose significantly compared to the diabetes control group (Table 1). However, they were statistically insignificant among the treatment groups at different time points, except for group 3 and group 4 at 90 and 150 min (Tukey HSD; $p < 0.05$).

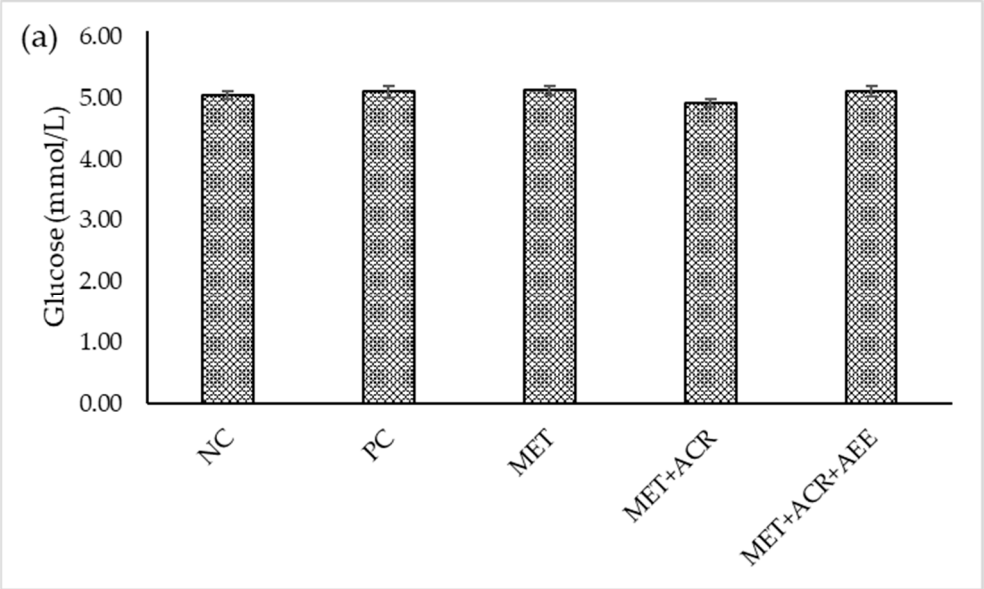

Figure 1. *Cont.*

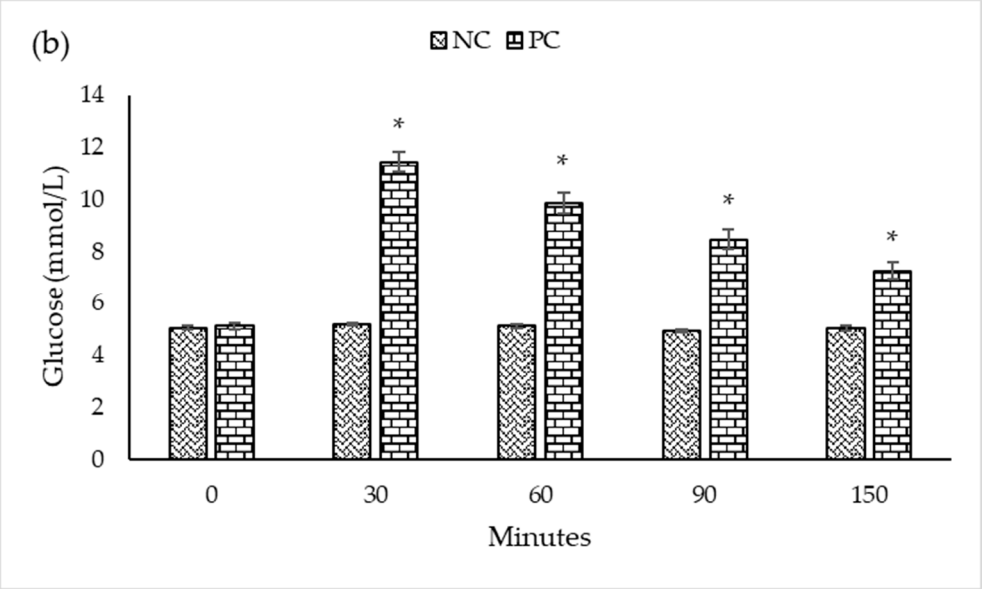

Figure 1. Fasting blood glucose level in mice groups (**a**) and a relative glucose level of negative and positive control groups until 150 min (**b**). The error bar shows the standard error of the mean. The value of the positive (diabetes) group showed a statistically significant difference (Tukey HSD; $p < 0.05$) compared to the negative control group in all-time points, except for 0 min. NC: negative control; PC: positive control; MET: metformin; ACR: acarbose; AEE: *Abelmoschus esculentus* pod extract. * denoted statistically significant at $p < 0.05$ between NC and PC.

Table 1. Effects of therapeutic doses of different drugs either alone or in combination in mice model.

Experimental Groups	*n*	Blood Glucose Levels (mmol/L) at a Different Time (Minutes) Intervals				
		0	30	60	90	150
		Mean ± SEM				
PC	5	5.10 ± 0.105 [a]	11.44 ± 0.387 [a]	9.86 ± 0.408 [a]	8.46 ± 0.387 [a]	7.26 ± 0.311 [a]
MET	5	5.12 ± 0.066 [a]	9.64 ± 0.227 [b]	8.54 ± 0.172 [b]	7.32 ± 0.139 [b]	5.32 ± 0.102 [b]
MET + ACR	5	4.92 ± 0.058 [a]	9.04 ± 0.178 [b]	7.82 ± 0.188 [b]	6.36 ± 0.154 [c]	4.50 ± 0.095 [c]
MET + ACR + AEE	5	5.10 ± 0.084 [a]	9.28 ± 0.128 [b]	8.60 ± 0.089 [b]	7.76 ± 0.121 [ab]	6.58 ± 0.086 [a]

SEM: Standard error of mean; NC: negative control; PC: positive control; MET: metformin; ACR: acarbose; AEE: *Abelmoschus esculentus* extract. According to Tukey HSD, different letters in a column indicate a significant ($p < 0.05$) difference between groups at a 95% confidential interval.

Group 2 demonstrated that the glucose level was reduced significantly until 1 h post-administration of glucose p.o. However, later it was not significant, and the difference between 0 min and 150 min was statistically significant ($p = 0.002$) (Supplementary Table S1). Treatment groups 3 to 5 effectively reduced the blood glucose level. The difference among these three groups was not statistically significant until 1 h. However, at 90 min, glucose level reduction between groups 3 and 4 was significant, but groups 3 and 5 were not significant. Additionally, the difference between groups 4 and 5 was significant. At the 150 min, the glucose level was within the normal range in group 3 (5.32 ± 0.102) and group 4 (4.50 ± 0.095), but it was higher in group 5 (Table 1).

This experiment observed that administration of MET and ACR simultaneously showed more incredible antihyperglycemic action than that of the MET alone, and effectiveness was comparatively better after 1 h of treatment. This might be due to the synergistic effects of MET and ACR. A meta-analysis (75 studies were included) reported that, through direct comparison, the glucose-lowering effects of MET monotherapy and ACR monotherapy are the same; however, MET is marginally superior by indirect comparison [57]. However, because of their distinct and complementary modes of action,

the combination of MET and ACR is a sensible medication that provides good glucose management with extra cardiovascular advantages while minimizing adverse effects [12]. A recent study also demonstrated that a combination of MET and ACR treatment showed a 95% survival rate in COVID-19 infected T2DM patients. However, this study suggested that inpatient use of MET and ACR together or alone during hospitalization should be studied in randomized trials [58].

In many earlier studies [31,32], the antihyperglycemic property of okra was found in alloxan/streptozotocin-induced diabetic rats/mice. Some studies were also performed to assess the impact of MET and ACR combined therapy vs monotherapy [13,14]. Another study was also carried out to observe the pharmacodynamics of MET when co-administered with okra extracts [44]. It is well established that MET is highly potent as an antidiabetic agent, and in a combination with ACR, it augments the antihyperglycemic effect. However, the synergism of MET and ACR was reversed when concomitantly administered with AEE (Group 5). Table 1 demonstrated that even though the glucose level was significantly decreased in group 5, the glucose level (6.58 ± 0.086) was not within the normal range at 150 min. The value was statistically insignificant in the diabetes control group (Group 2). Similarly, this value was significantly higher than the monotherapy of MET (Group 3) and combination therapy of MET and ACR (Group 4). This result suggested that AEE showed antagonistic activity against the antihyperglycemic activity of MET and ACR. It was suggested that myricetin in viscous AEE shows its action by inhibiting glucose transporter 2 (GLUT2) at the intestinal brush border [59] and increasing glucose utilization [48]. MET also reduces glucose uptake from the gut and increases GLUT 4 mediated glucose transport to the muscle cell.

In contrast, ACR is an alpha (α) glucosidase inhibitor that delays intestinal absorption of glucose. MET and ACR are often prescribed in combination for better glycemic control in T2DM. Some researchers surmised that gum-like soluble fibers might interfere with the activity of metformin [60]. MET, ACR, and AEE all have proven antidiabetic properties. Thus, concomitant administration may increase the glucose-lowering effect. Our finding also reported that AEE resists the synergism of MET and ACR combination therapy. We assumed that the presence of gums and mucilage in AEE may interfere with the function of MET and ACR. Group 5 showed antagonistic effects against hyperglycemic mice that support the hypothesis of other researchers [44,60].

3. Materials and Methods

3.1. Collection of Materials

The green okra pods were collected from a rural area in the Kishoreganj district of Bangladesh and authenticated by botanist Muhammad Mahfuz Hasan, Department of Botany, University of Dhaka. MET and ACR (Sunman Birdem Pharma and Pacific pharmaceuticals, Bangladesh Ltd., Dhaka, Bangladesh) and a digital glucometer (Safe touch, HMD Biomedical Inc., Hsinchu, Taiwan) were purchased.

3.2. Preparation of Extract

The green okra pods were used to prepare the *Abelmoschus esculentus* pod extract (AEE) following the protocol described by Yaradua et al. [61]. Briefly, from the collected samples, approximately 200 gm of fresh *A. esculentus* (okra) was appropriately washed with fresh water and dried to a constant weight at room temperature. After that, these were cut horizontally into slices, and the seed was discarded. Then those pieces were soaked into a jar with 250 mL of distilled water overnight. In the morning, the slightly viscous liquid portion was collected and filtrated. After this, the extract was stored at 4 °C for further experimentation.

3.3. Animal Experiment

The animal experiment was conducted in the animal room at Daffodil International University, Bangladesh. About 2–3 month old Swiss Webster strain male mice weighing

25–30 gm was obtained from the Jahangirnagar University, Bangladesh. The mice were acclimatized for three days in standard laboratory conditions. The experimental condition of the laboratory was controlled, maintaining a temperature of 24 °C to 26 °C, a humidity of 65–70%, and a 12 h light and dark cycle. All animals could access a standard pellet diet and water ad libitum. The experiment was conducted as per the approval of the ethical committee (Ref. FAHS-REC/DIU/2020/1003), Faculty of Allied Health Sciences, Daffodil International University, Dhaka, Bangladesh.

3.3.1. Sample Size Calculation

The alternative of the power analysis approach for determining the sample size, the resource equation approach, is highly recommended for exploratory animal study [62]. According to this approach, the permissible range of degrees of freedom (DF) for the error term in an analysis of variance (ANOVA) is between 10 and 20. This study also considered the 3R formula for animal study [63]. Therefore, the sample size was calculated following the resource equation approach described by Arifin and Zahiruddin [64]. Following this approach, this study determined a minimum (10/3 + 1 = 4.3 rounded up) of 5 animals/group and a maximum (20/5 + 1 = 7.7 rounded down) of 7 animals/group to compare the effectiveness of drugs/extracts among three treatment groups (excluding negative control and positive control for sample size calculation). It is noted that the numbers of animals per group are rounded up or down to keep DF within limits (e.g., DF = 12 for 5, and DF= 18 for 7) [64]. Therefore, minimum animal numbers were chosen for this exploratory animal experimental study regarding the 3R recommendation.

3.3.2. Experimental Design

The simplified experimental design is shown in Figure 2. Swiss Webster strain male mice weighing 25–30 gm were used. Overnight fasted animals were divided into five groups (n = 5). Group 1 served as a negative control: the animals in this group were not induced hyperglycemia but were free to access a standard pellet diet and water ad libitum. Group 2 served as a positive control: the animals in this group received glucose at 2.5 gm/kg/BW*Per os* (p.o.) for hyperglycemia induction but were not allowed any medications. Group 3 served as MET only: the animals in this group received MET (150 mg/kg/BW p.o.). Group 4 served as MET and ACR: The animals of this group received MET (150 mg/kg/BW p.o.) and ACR (15 mg/kg/BW p.o.) simultaneously. Group 5: the animals in this group received MET (150 mg/kg/BW p.o.), ACR (15 mg/kg/BW p.o.), and AEE (0.2 mL/kg BW p.o.). In this study, the AEE dose was selected based on Aligita et al. [32], who reported that okra water extract (50–200 mg/kg BW) effectively reduced blood glucose levels significantly in a diabetic mice model. Glucose-induced hyperglycemia was made on the normoglycemic experimental mice (n = 25) through glucose administration following 2.5 gm/kg/BW p.o.

In this study, the glucose solution (2.5 gm/kg/BW p.o.) was administered 30 min after plant extract/drug administration. A blood sample (around 0.5 µL) was taken through the puncture of a vein in the tail, and glucose level was measured using a digital glucometer (Safe touch, HMD Biomedical Inc., Hsinchu 30548, Taiwan). Blood samples were collected at 0, 30, 60, 90, & 150 min after glucose administration.

3.4. Data Analysis

An analysis of variance (ANOVA) followed by the Tukey HSD test was used to determine the differences among the treatment groups. Before conducting the ANOVA, a test of normality and a test of Homogeneity of Variances were run to observe the normal distribution of data sets. There was no significant difference in the data sets, even between groups at a 99% confidential interval. The results were presented as mean ± SEM (standard error of the mean) with different letters to indicate the significance level at a 95% confidential interval. The analysis was done using SPSS (version IBM 25; Armonk, NY, USA: IBM Corp.).

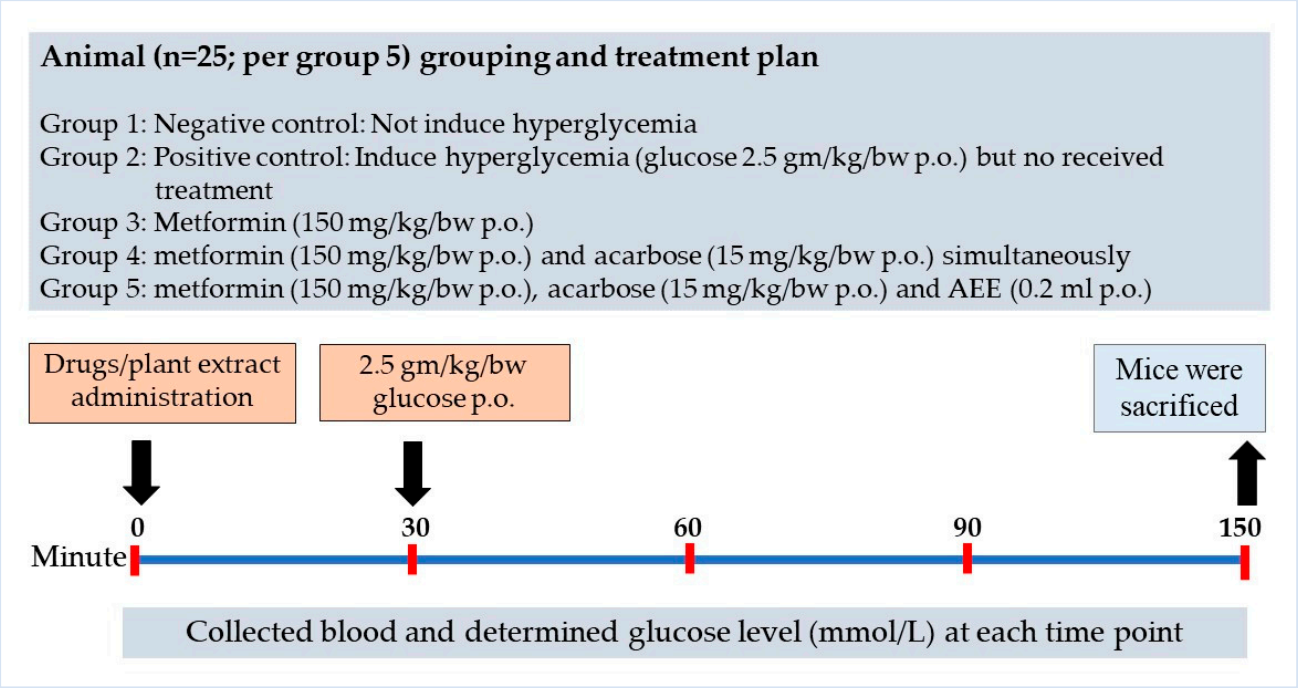

Figure 2. Animal experimental design and treatment regime. p.o.: *Per os*.

4. Conclusions

A. esculentus (L.) Moench (okra) is known for its nutritional values and antidiabetic properties. Thus, it improves the postprandial glycemic index, even concomitantly with mono- or combination pharmaceutical therapy. Our study found that *A. esculentus* pod extract may hamstring the action of MET and ACR when taken simultaneously and attenuate the beneficial effects of the combination of these drugs (Group 4). Therefore, based on this finding, it can be suggested that those who take a combination of MET and ACR should not take *A. esculentus* concomitantly. Nonetheless, further study should be carried out to elucidate the molecular mechanisms involved in the antagonistic role of *A. esculentus* pod extract against the synergism of MET and ACR while administered concomitantly.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biologics2020010/s1, Table S1: Multiple comparisons among the groups at different time intervals in the hyperglycemic mice model.

Author Contributions: Conceptualization, M.A.H. and M.S.H.; methodology, M.A.H., N.M.A.S., M.T.I. and M.R.K.; validation, M.S.H.; formal analysis, M.S.H. and F.A.; investigation, M.A.H., M.S.H., N.M.A.S., M.T.I. and M.R.K.; resources, M.A.H. and M.S.H.; data curation, M.A.H. and M.S.H.; writing—original draft preparation, M.A.H. and M.S.H.; writing—review and editing, M.A.H., M.S.H., M.T.I., M.R.K., F.A., F.T.Z., D.A., L.C.M. and R.C.; visualization, M.S.H. and M.T.I.; supervision, M.A.H. and M.S.H.; project administration, M.A.H.; funding acquisition, M.S.H., L.C.M. and R.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board of the Faculty of Allied health sciences, Daffodil International University, Daffodil Smart City, Savar, Dhaka, with the reference number FAHS-REC/DIU/2020/1003 in January 2020.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The Authors would like to thank the Department of Pharmacy, Daffodil International University, for providing all the technical facilities during the study.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviation

T2DM	Type 2 Diabetes mellitus
NIDMI	Non-Insulin dependent diabetes mellitus
IDDM	Insulin-dependent diabetes mellitus
NC	Negative (Fasting) Control
PC	Positive (Hyperglycemic) control
MET	Metformin only group
AE	*Abelmoschus esculentus* pod extract
ACR	Acarbose
MET + ACR	Metfomin + Acarbose group
MET + ACR + AEE	Metformin + Acarbose + *Abelmoschus esculentus* pod Extract group
p.o.	*Per os*
BW	Bodyweight

References

1. IDF. Diabetes around the world in 2021. Available online: https://diabetesatlas.org/ (accessed on 10 April 2022).
2. WHO. *Improving Diabetes Outcomes for All, a Hundred Years on from the Discovery of Insulin: Report of the Global Diabetes Summit*; World Health Organization and the Government of Canada: Geneva, Switzerland, 2021; p. 14.
3. WHO. Diabetes. Available online: https://www.who.int/news-room/fact-sheets/detail/diabetes (accessed on 22 January 2022).
4. Riddle, M.C. Oral pharmacologic management of type 2 diabetes. *Am. Fam. Physician* **1999**, *60*, 2613–2620. [PubMed]
5. Rahman, K.M.H.; Hossain, M.S.; Haque, N.; Razak, T.B.A.; Ahmad, H. Apolipoprotein E gene polymorphism influenced glycemic status among Malaysians. *Res. Ther.* **2019**, *6*, 3307–3314. [CrossRef]
6. Hunter, R.W.; Hughey, C.C.; Lantier, L.; Sundelin, E.I.; Peggie, M.; Zeqiraj, E.; Sicheri, F.; Jessen, N.; Wasserman, D.H.; Sakamoto, K. Metformin reduces liver glucose production by inhibition of fructose-1-6-bisphosphatase. *Nat. Med.* **2018**, *24*, 1395–1406. [CrossRef] [PubMed]
7. Hundal, R.S.; Krssak, M.; Dufour, S.; Laurent, D.; Lebon, V.; Chandramouli, V.; Inzucchi, S.E.; Schumann, W.C.; Petersen, K.F.; Landau, B.R.; et al. Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* **2000**, *49*, 2063–2069. [CrossRef] [PubMed]
8. Hanefeld, M. Cardiovascular benefits and safety profile of acarbose therapy in prediabetes and established type 2 diabetes. *Cardiovasc. Diabetol.* **2007**, *6*, 20. [CrossRef] [PubMed]
9. McIver, L.A.; Tripp, J. Acarbose. StatPearls [Internet]. Available online: https://www.ncbi.nlm.nih.gov/books/NBK493214/ (accessed on 22 January 2022).
10. Liu, Z.; Zhao, X.; Sun, W.; Wang, Y.; Liu, S.; Kang, L. Metformin combined with acarbose vs. single medicine in the treatment of type 2 diabetes: A meta-analysis. *Exp. Ther. Med.* **2017**, *13*, 3137–3145. [CrossRef] [PubMed]
11. Wu, H.; Liu, J.; Lou, Q.; Liu, J.; Shen, L.; Zhang, M.; Lv, X.; Gu, M.; Guo, X. Comparative assessment of the efficacy and safety of acarbose and metformin combined with premixed insulin in patients with type 2 diabetes mellitus. *Medicine* **2017**, *96*, e7533. [CrossRef]
12. Joshi, S.R.; Ramachandran, A.; Chadha, M.; Chatterjee, S.; Rathod, R.; Kalra, S. Acarbose plus metformin fixed-dose combination in the management of type 2 diabetes. *Expert Opin. Pharmacother.* **2014**, *15*, 1611–1620. [CrossRef]
13. Wang, J.S.; Huang, C.N.; Hung, Y.J.; Kwok, C.F.; Sun, J.H.; Pei, D.; Yang, C.Y.; Chen, C.C.; Lin, C.L.; Sheu, W.H.; et al. Acarbose plus metformin fixed-dose combination outperforms acarbose monotherapy for type 2 diabetes. *Diabetes Res. Clin. Pract.* **2013**, *102*, 16–24. [CrossRef]
14. Zhilun, Z. Application of acarbose combined with metformin in treatment of newly diagnosed type 2 diabetes patients. *Heilongjiang Med. J.* **2011**, *24*, 223–225.
15. Ekor, M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Front. Pharmacol.* **2014**, *4*, 177. [CrossRef]
16. Ismail, N.A.; Hossain, M.S.; Mohd Mustafa, N.H.; Phang, I.C. Morpho-Physiological Characteristics, Selected Macronutrient Uptake, and Oxidative Stress Level of *Andrographis paniculata* Under Saline Condition. *J. Teknol.* **2015**, *77*, 135–140. [CrossRef]
17. Hossain, M.S.; Urbi, Z. Effect of Naphthalene Acetic Acid on the Adventitious Rooting in Shoot Cuttings ofAndrographis paniculata (Burm.f.) Wall. ex Nees: An Important Therapeutical Herb. *Int. J. Agron.* **2016**, *2016*, 1–6. [CrossRef]
18. Hossain, M.S.; Urbi, Z.; Sule, A.; Rahman, K.M.H. Andrographis paniculata (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. *Sci. World J.* **2014**, *2014*, 274905. [CrossRef]
19. Urbi, Z.; Zainuddin, Z. Standardization of Surface Sterilization Protocol of Field Grown *Stevia rebaudiana* Prior to *In Vitro* Clonal Propagation. *J. Teknol.* **2015**, *77*. [CrossRef]

20. Farooq, U.; Khan, T.; Shah, S.A.; Hossain, M.S.; Ali, Y.; Ullah, R.; Raziq, N.; Shahid, M.; Capasso, R. Isolation, Characterization and Neuroprotective Activity of Folecitin: An In Vivo Study. *Life* **2021**, *11*, 825. [CrossRef]
21. Hossain, M.S.; Urbi, Z.; Karuniawati, H.; Mohiuddin, R.B.; Moh Qrimida, A.; Allzrag, A.M.M.; Ming, L.C.; Pagano, E.; Capasso, R. *Andrographis paniculata* (Burm. f.) Wall. ex Nees: An Updated Review of Phytochemistry, Antimicrobial Pharmacology, and Clinical Safety and Efficacy. *Life* **2021**, *11*, 348. [CrossRef]
22. Hossain, M.S. The Effect of Salinity Stress on the Morpho-physiology and Protein Profile of *Andrographis Paniculata*. Master's Thesis, International Islamic University Malaysia, Kuantan, Pahang, Malaysia, 2016.
23. Urbi, Z.; Hossain, M.S.; Rahman, K.M.H.; Zayed, T.M. Grape: A medicinal fruit species in the holy Qur'an and its ethnomedicinal importance. *World Appl. Sci. J.* **2014**, *30*, 253–265.
24. Hossain, M.S.; Sharfaraz, A.; Dutta, A.; Ahsan, A.; Masud, M.A.; Ahmed, I.A.; Goh, B.H.; Urbi, Z.; Sarker, M.M.R.; Ming, L.C. A review of ethnobotany, phytochemistry, antimicrobial pharmacology and toxicology of Nigella sativa L. *Biomed. Pharm.* **2021**, *143*, 112182. [CrossRef]
25. Durazzo, A.; Lucarini, M.; Novellino, E.; Souto, E.B.; Daliu, P.; Santini, A. *Abelmoschus esculentus* (L.): Bioactive Components' Beneficial Properties-Focused on Antidiabetic Role-For Sustainable Health Applications. *Molecules* **2018**, *24*, 38. [CrossRef]
26. Dantas, T.L.; Alonso Buriti, F.C.; Florentino, E.R. Okra (*Abelmoschus esculentus* L.) as a Potential Functional Food Source of Mucilage and Bioactive Compounds with Technological Applications and Health Benefits. *Plants* **2021**, *10*. [CrossRef]
27. Nikpayam, O.; Safaei, E.; Bahreini, N.; Saghafi-Asl, M. The effects of okra (*Abelmoschus esculentus* L.) products on glycemic control and lipid profile: A comprehensive systematic review. *J. Funct. Foods* **2021**, *87*, 104795. [CrossRef]
28. Doreddula, S.K.; Bonam, S.R.; Gaddam, D.P.; Desu, B.S.; Ramarao, N.; Pandy, V. Phytochemical analysis, antioxidant, antistress, and nootropic activities of aqueous and methanolic seed extracts of ladies finger (*Abelmoschus esculentus* L.) in mice. *Sci. World J.* **2014**, *2014*, 519848. [CrossRef]
29. Sabitha, V.; Ramachandran, S.; Naveen, K.R.; Panneerselvam, K. Antidiabetic and antihyperlipidemic potential of *Abelmoschus esculentus* (L.) Moench. in streptozotocin-induced diabetic rats. *J. Pharm. Bioallied Sci.* **2011**, *3*, 397–402. [CrossRef]
30. Prabhune, A.; Sharma, M.; Ojha, B. *Abelmoschus esculentus* (Okra) potential natural compound for prevention and management of diabetes and diabetic induced hyperglycemia. Tamil Nadu. *Int. J. Herb. Med.* **2017**, *5*, 66–68.
31. Onuoha, N.O.; Iroegbu, L.U.; Uwaezuoke, N.J. Antidiabetic effects of okra [*Abelmoschus esculentus* (L.) Moench] fruits in alloxan-induced diabetic rats. *Biokemistri* **2017**, *29*, 94–101.
32. Aligita, W.; Muhsinin, S.; Susilawati, E.; Pratiwi, D.S.; Aprilliani, D.; Artarini, A.; Adnyana, I.K. Antidiabetic activity of okra (*Abelmoschus esculentus* L.) fruit extract. *Rasāyan J. Chem.* **2019**, *12*, 157–167. [CrossRef]
33. Fan, S.; Zhang, Y.; Sun, Q.; Yu, L.; Li, M.; Zheng, B.; Wu, X.; Yang, B.; Li, Y.; Huang, C. Extract of okra lowers blood glucose and serum lipids in high-fat diet-induced obese C57BL/6 mice. *J. Nutr. Biochem.* **2014**, *25*, 702–709. [CrossRef]
34. Lu, Y.; Demleitner, M.F.; Song, L.; Rychlik, M.; Huang, D. Oligomeric proanthocyanidins are the active compounds in *Abelmoschus esculentus* Moench for its α-amylase and α-glucosidase inhibition activity. *J. Funct. Foods* **2016**, *20*, 463–471. [CrossRef]
35. Kaur, R.; Afzal, M.; Kazmi, I.; Ahamd, I.; Ahmed, Z.; Ali, B.; Ahmad, S.; Anwar, F. Polypharmacy (herbal and synthetic drug combination): A novel approach in the treatment of type-2 diabetes and its complications in rats. *J. Nat. Med.* **2013**, *67*, 662–671. [CrossRef]
36. Gupta, R.C.; Chang, D.; Nammi, S.; Bensoussan, A.; Bilinski, K.; Roufogalis, B.D. Interactions between antidiabetic drugs and herbs: An overview of mechanisms of action and clinical implications. *Diabetol. Metab. Syndr.* **2017**, *9*, 59. [CrossRef] [PubMed]
37. FDA. Mixing Medications and Dietary Supplements Can Endanger Your Health. Available online: https://www.fda.gov/consumers/consumer-updates/mixing-medications-and-dietary-supplements-can-endanger-your-health (accessed on 30 December 2021).
38. Peter, E.L.; Nagendrappa, P.B.; Ajayi, C.O.; Sesaazi, C.D. Total polyphenols and antihyperglycemic activity of aqueous fruits extract of Abelmoschus esculentus: Modeling and optimization of extraction conditions. *PLoS ONE* **2021**, *16*, e0250405. [CrossRef] [PubMed]
39. Roy, A.; Shrivastava, S.L.; Mandal, S.M. Functional properties of Okra *Abelmoschus esculentus* L. (Moench): Traditional claims and scientific evidences. *Plant Sci. Today* **2014**, *1*, 121–130. [CrossRef]
40. Elkhalifa, A.E.O.; Al-Shammari, E.; Adnan, M.; Alcantara, J.C.; Mehmood, K.; Eltoum, N.E.; Awadelkareem, A.M.; Khan, M.A.; Ashraf, S.A. Development and Characterization of Novel Biopolymer Derived from *Abelmoschus esculentus* L. Extract and Its Antidiabetic Potential. *Molecules* **2021**, *26*. [CrossRef]
41. Sabitha, V.; Panneerselvam, K.; Ramachandran, S. In vitro α–glucosidase and α–amylase enzyme inhibitory effects in aqueous extracts of *Abelmoscus esculentus* (L.) Moench. *Asian Pac. J. Trop. Biomed.* **2012**, *2*, S162–S164. [CrossRef]
42. Daliu, P.; Annunziata, G.; Tenore, G.C.; Santini, A. Abscisic acid identification in Okra, *Abelmoschus esculentus* L. (Moench): Perspective nutraceutical use for the treatment of diabetes. *Nat. Prod. Res.* **2020**, *34*, 3–9. [CrossRef]
43. Husen, S.A.; Winarni, D.; Wahyuningsih, S.P.A.; Ansori, A.N.M.; Hayaza, S.; Susilo, R.J.K.; Doong, R.A.; Darmanto, W. Antioxidant potency of various fractions of okra pods extract to ameliorate liver structure and function in diabetic mice. *Ann. Biol.* **2020**, *36*, 154–158.
44. Khatun, H.; Rahman, A.; Biswas, M.; Islam, A.U. Water-soluble Fraction of *Abelmoschus esculentus* L. Interacts with Glucose and Metformin Hydrochloride and Alters Their Absorption Kinetics after Coadministration in Rats. *Int. Sch. Res. Not.* **2011**, *2011*, 260537. [CrossRef]

45. Tekin, E.; Beppler, C.; White, C.; Mao, Z.; Savage, V.M.; Yeh, P.J. Enhanced identification of synergistic and antagonistic emergent interactions among three or more drugs. *J. R. Soc. Interface* **2016**, *13*, 20160332. [CrossRef]
46. Huang, C.-N.; Wang, C.-J.; Lee, Y.-J.; Peng, C.-H. Active subfractions of *Abelmoschus esculentus* substantially prevent free fatty acid-induced β cell apoptosis via inhibiting dipeptidyl peptidase-4. *PLoS ONE* **2017**, *12*, e0180285. [CrossRef]
47. Arapitsas, P. Identification and quantification of polyphenolic compounds from okra seeds and skins. *Food Chem.* **2008**, *110*, 1041–1045. [CrossRef]
48. Saha, D.; Jain, B.; Jain, V.K. Phytochemical evaluation and characterization of hypoglycemic activity of various extracts of *Abelmoschus esculentus* Linn. fruit. *Int. J. Pharm. Pharm. Sci.* **2011**, *3*, 183–185.
49. Agnes Jenitha, X.; Kanimozhi, R. Evaluation of phytochemicals and antidiabetic activity of abelmoschus esculentus. *Int. J. Res. Pharmacol. Pharmacother.* **2021**, *5*, 142–152.
50. Shen, D.-D.; Li, X.; Qin, Y.-L.; Li, M.-T.; Han, Q.-H.; Zhou, J.; Lin, S.; Zhao, L.; Zhang, Q.; Qin, W. Physicochemical properties, phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes of okra (*Abelmoschus esculentus*) fruit at different maturation stages. *J. Food Sci. Technol.* **2019**, *56*, 1275–1286. [CrossRef]
51. Liu, I.M.; Tzeng, T.-F.; Liou, S.-S.; Lan, T.-W. Improvement of insulin sensitivity in obese Zucker rats by myricetin extracted from Abelmoschus moschatus. *Planta Med.* **2007**, *73*, 1054–1060. [CrossRef]
52. Guasch-Ferré, M.; Merino, J.; Sun, Q.; Fitó, M.; Salas-Salvadó, J. Dietary polyphenols, Mediterranean diet, prediabetes, and type 2 diabetes: A narrative review of the evidence. *Oxidative Med. Cell. Longev.* **2017**, 6723931. [CrossRef]
53. Liao, Z.; Zhang, J.; Liu, B.; Yan, T.; Xu, F.; Xiao, F.; Wu, B.; Bi, K.; Jia, Y. Polysaccharide from Okra (*Abelmoschus esculentus* (L.) Moench) Improves Antioxidant Capacity via PI3K/AKT Pathways and Nrf2 Translocation in a Type 2 Diabetes Model. *Molecules* **2019**, *24*. [CrossRef]
54. Krinsky, N.I. Carotenoids as antioxidants. *Nutrition* **2001**, *17*, 815–817. [CrossRef]
55. Petznick, A. Insulin management of type 2 diabetes mellitus. *Am. Fam. Physician* **2011**, *84*, 183–190.
56. Genuth, S.; Alberti, K.G.; Bennett, P.; Buse, J.; Defronzo, R.; Kahn, R.; Kitzmiller, J.; Knowler, W.C.; Lebovitz, H.; Lernmark, A.; et al. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* **2003**, *26*, 3160–3167. [CrossRef]
57. Gu, S.; Shi, J.; Tang, Z.; Sawhney, M.; Hu, H.; Shi, L.; Fonseca, V.; Dong, H. Comparison of glucose lowering effect of metformin and acarbose in type 2 diabetes mellitus: A meta-analysis. *PLoS ONE* **2015**, *10*, e0126704. [CrossRef]
58. Li, J.; Wei, Q.; McCowen, K.C.; Xiong, W.; Liu, J.; Jiang, W.; Thomas, R.L.; Hepokoski, M.; He, M.; Shyy, J.Y.J.; et al. Inpatient use of metformin and acarbose is associated with reduced mortality of COVID-19 patients with type 2 diabetes mellitus. *Endocrinol. Diabetes Metab.* **2022**, *5*, e00301. [CrossRef]
59. Liu, I.M.; Liou, S.-S.; Lan, T.-W.; Hsu, F.-L.; Cheng, J.-T. Myricetin as the Active Principle of Abelmoschus moschatus to Lower Plasma Glucose in Streptozotocin-Induced Diabetic Rats. *Planta Med.* **2005**, *71*, 617–621. [CrossRef] [PubMed]
60. Kwon, O.; Eck, P.; Chen, S.; Corpe, C.P.; Lee, J.H.; Kruhlak, M.; Levine, M. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. *FASEB J.* **2007**, *21*, 366–377. [CrossRef] [PubMed]
61. Yaradua, I.; Ibrahim, M.; Matazu, K.I.; Nasir, A.; Matazu, N.U.; Zainab, A.S.; Abdul Rahman, M.B.; Bilbis, L.; Abbas, A.Y. Antidiabetic activity of *Abelmoschus esculentus* (Ex-Maradi Okra) fruit in alloxan-induced diabetic rats. *Niger. J. Biochem. Mol. Biol.* **2017**, *32*, 44–52.
62. Charan, J.; Kantharia, N.D. How to calculate sample size in animal studies? *J. Pharmacol. Pharmacother.* **2013**, *4*, 303–306. [CrossRef] [PubMed]
63. Hubrecht, R.C.; Carter, E. The 3Rs and Humane Experimental Technique: Implementing Change. *Animals* **2019**, *9*, 754. [CrossRef]
64. Arifin, W.N.; Zahiruddin, W.M. Sample Size Calculation in Animal Studies Using Resource Equation Approach. *Malays. J. Med. Sci.* **2017**, *24*, 101–105. [CrossRef]