EFFECT OF NIPA (*NYPA FRUTICANS* WURMB.) VINEGAR ON THE INHIBITION OF INTESTINAL GLUCOSE ABSORPTION

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by

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AUC	Area under the curve
B.W.	Body weight
ELISA	Enzyme-linked immunosorbent assay
HPLC	High-performance liquid chromatography
PBS	Phosphate buffer solution
SEM	Standard error of the mean
UV	Ultraviolet

LIST OF SYMBOLS

α	Alpha
β	Beta
^{o}C	Degree celsius
G	Gram
Kg	Kilogram
mL	Millilitre
M	Molar
µL	Microlitre
mL/min	Millilitre per minute
mg/mL	Milligram per millilitre
mM	Millimolar
mL/kg	Millilitre per kilogram
mmol/L· min	Millimole per litre per minute
Mm	Millimetre
Nm	Nanometre
%	Percent

KESAN CUKA NIPAH (*NYPA FRUTICANS* WURMB.) TERHADAP PERENCATAN PENYERAPAN GLUKOSA USUS

ABSTRAK

Cuka nipa ialah cuka tradisional yang digunakan secara meluas di Asia Tenggara sebagai bahan dalam masakan dan juga diambil untuk merawat beberapa jenis penyakit. Kajian terdahulu telah melaporkan potensi aktiviti antihiperglisemik bagi ekstrak akues cuka nipah. Dalam kajian ini, kesan pengambilan cuka nipah ke atas aras glukosa selepas makan telah diselidiki. Penyediaan dan dos cuka nipah yang digunakan menyamai dengan cara penggunaannya oleh masyarakat. Kaedah *in vitro*, *ex vivo* dan *in vivo* telah digunakan. Ujian spektrofotometri dijalankan untuk melihat kesan cuka nipah terhadap perencatan dan kinetik enzim, pengambilan glukosa oleh jejunum dilakukan melalui teknik kantung terbalik dan akhirnya ujian karbohidrat oral secara *in vivo* pada tikus normoglisemik dijalankan untuk mengesahkan penemuan *in vitro* dan *ex vivo*. Kajian fizikokimia termasuk pH, kandungan keasidan dan asid organik juga dilakukan. Analisis fizikokimia mengesahkan bahawa cuka nipah mengandungi peratusan asid asetik yang lebih tinggi (2.3%) berbanding dengan asid organik yang lain (laktik, suksinik, sitrik dan malik). Cuka nipah secara sederhana menghalang aktiviti enzim karbohidrat iaitu α-glukosidase dan α-amilase secara bergantung kepada dos. Analisis kinetik menggunakan plot Lineweaver-Burk menunjukkan bahawa cuka nipah mempamer perencatan tidak kompetitif dalam ujian α-glukosidase dan perencatan kompetitif terhadap substrak dalam ujian α-amilase. Pemberian tiga dos cuka nipah (2 mL/kg B.W., 1 mL/kg B.W. dan 0.08 mL/kg B.W.) pada tikus normoglisemik menurunkan tahap glukosa darah pasca-prandial dalam ujian toleransi kanji secara oral dan mengesahkan lagi pengujian

enzimatik secara *in vitro*. Selain itu, cuka nipah menunjukkan perencatan yang ketara dalam pengambilan glukosa, seperti yang dinilai melalui teknik kantung jejunum tikus secara *ex vivo*. Berbanding dengan kawalan negatif, cuka nipah secara ketara ($p < 0.05$) merencat pengambilan glukosa dalam kantung jejunum terbalik. Tindakan ini adalah setanding dengan kawalan positif, phlorizin. Ujian toleransi glukosa oral akut mengesahkan pemerhatian *ex vivo*. Semua dos cuka nipah secara ketara ($p < 0.05$) menekan peningkatan kadar glukosa darah selepas pemberian glukosa. Dalam rawatan sub-ujian toleransi glukosa oral selama 14 hari, cuka nipah secara ketara ($p < 0.05$) merencat glukosa pasca-prandial, mengurangkan berat badan, meningkatkan insulin, dan menurunkan tahap hormon peptida-1 seperti glukagon. Cuka nipah pada dos 2 mL/kg terbukti menjadi dos yang paling berkesan. Sebagai kesimpulan, kajian *in vitro*, *ex viv*o dan *in vivo* mengesahkan potensi cuka nipah dalam merencat penyerapan glukosa usus. Hal ini menegaskan dakwaan penggunaan cuka nipah secara tradisional and potensi nutraseutikal cuka nipah dalam pengurusan diabetes.

EFFECT OF NIPA (*NYPA FRUTICANS* WURMB.) VINEGAR ON THE INHIBITION OF INTESTINAL GLUCOSE ABSORPTION

ABSTRACT

Nipa palm vinegar is a traditional vinegar widely used in Southeast Asia as a condiment and a home remedy for several ailments. Previous studies have reported the antihyperglycemic activity of aqueous extract of nipa palm vinegar. In this study, the effects of consuming nipa palm vinegar on postprandial glucose levels were scrutinized. The preparation and dose of nipa palm vinegar resembled the way the locals consume it. The investigation utilized *in vitro*, *ex vivo* and *in vivo* methods to study glucose absorption. *In vitro*, the spectrophotometric assay was conducted to study the effect of nipa palm vinegar on enzyme inhibition and kinetics and, *ex vivo,* glucose uptake by jejunum was determined via the everted sac technique. Finally, *in vivo,* oral carbohydrate tests in normoglycemic rats were performed to confirm the *in-vitro* and *ex-vivo* findings. Physicochemical studies, including pH, total acidity, and organic acid, were also performed. The physicochemical analysis confirmed that nipa palm vinegar contained a higher percentage (2.3%) of acetic acid compared with other organic acids (lactic, succinic, citric, malic). Nipa palm vinegar moderately inhibited carbohydrate hydrolysing enzymes, α-glucosidase, and α-amylase activity in a dose-dependent manner. The uncompetitive and competitive inhibition mechanism of nipa palm vinegar is due to its inhibitory effects on α-glucosidase and α-amylase, respectively. The *in-vitro* enzymatic assay was further confirmed by an *in-vivo* oral starch tolerance test. In normoglycemic rats, the administration of three doses (2 mL/kg B.W., 1 mL/kg B.W. and 0.08 mL/kg B.W.) of nipa palm vinegar significantly lowered postprandial blood glucose levels in oral

starch tolerance test. In addition to that, nipa palm vinegar showed a significant suppression in the glucose uptake, as assessed via *ex vivo* everted rat jejunum sac technique. As compared with the negative control, nipa palm vinegar significantly ($p < 0.05$) delayed glucose uptake in an everted jejunum sac. The action, which was comparable with the positive control, was phlorizin. The acute oral glucose tolerance test validated the *ex vivo* observation. All doses of nipa palm vinegar significantly ($p < 0.05$) suppressed rising blood glucose levels following glucose loading. In 14 days of sub-oral glucose tolerance test treatments, nipa palm vinegar significantly ($p<0.05$) suppressed postprandial glucose level, reduced the body weight, increased insulin, and glucagon-like protein-1 hormone levels. Nipa palm vinegar at the dose of 2 mL/kg proved to be the most effective dose. In conclusion, *in vitro*, *ex vivo* and *in vivo* studies confirmed the potential of nipa palm vinegar in suppressing intestinal glucose absorption. These assert claims are associated with its traditional and possible nutraceutical use in diabetes management.

CHAPTER 1

INTRODUCTION

1.1 Background

Diabetes mellitus is a disorder involving deficient carbohydrate metabolism and numerous aetiologies. Diabetes mellitus is characterized by persistent hyperglycaemia due to faulty carbohydrates, fats, and proteins metabolism, and it presents with absolute or relative insulin deficiency (Therasa et al., 2014). The disorder leads to complications associated with glucose toxicity, including retinopathy, neuropathy, nephropathy, and peripheral vascular insufficiency (Nistor Baldea et al., 2010). Diabetes mellitus may be managed with insulin therapy, oral antidiabetic drugs, frequent exercise, weight reduction, and diet optimization. Postprandial hyperglycemia, one of the initial identifiable anomalies of patients with type 2 diabetes, has been linked to the development of diabetes-related problems in clinical investigations (Singh, 2012). The term refers to a rapid and marked increase in blood glucose levels following a meal. According to Maffettone et al. (2018), postprandial hyperglycaemia is the main limiting factor for optimal glycaemic control in type 2 diabetes mellitus patients.

There is a critical need for creating and actualizing multisectoral methodologies to manage diabetes mellitus better. Without efficient interventions, it is projected that 578 million individuals will have diabetes in 2030 and that the number of patients will have increased by 51% (700 million) by 2045 (Saeedi et al., 2019). Achieving optimal control of postprandial hyperglycaemia is essential for the prevention of diabetes mellitus related complications. The management of postprandial hyperglycaemia involves several physiological processes: (1) the conversion of complex sugars to absorbable monosaccharides via carbohydrate-

digesting enzymes (specifically the pancreatic α-amylase and the intestinal α-glucosidase), (2) the activation of intestinal glucose carriers (e.g., sodium-glucose linked transporter-1 and glucose transporter-2 (Abid et al., 2014)), and (3) the release of incretin hormones.

Incretin hormones have been the subject of intensive research due to their role in glucose homeostasis and the pathophysiology of type 2 diabetes mellitus and, conceivably, other metabolic disorders. Gastric emptying, the incretin axis, and postprandial hyperglycaemia are widely viewed as being interdependent. The rate of gastric emptying impacts the proportions of glycaemic excursion and incretin hormone secretion (Marathe et al., 2013).

Foods that contain biologically active compounds are commonly referred to as functional foods because they may offer physiological benefits, promote one's health, and help prevent and manage chronic diseases, including diabetes mellitus. Regular consumption of functional foods has been linked to improved antioxidation, reduced inflammation, enhanced insulin sensitivity, and decreased cholesterol levels, all of which contributes to the prevention and management of type 2 diabetes mellitus (Alkhatib et al., 2017). Functional foods may be advantageous as alternative remedies because of the better tolerance, adherence, fewer side effects, and lower costs generally associated with them than traditional medications.

Vinegar is a widely used functional food worldwide and has been used for over 3,000 years. Before introducing pharmacological glucose-lowering medications, vinegar was taken with meals as a home remedy for diabetes mellitus. Human trials and animal studies have shown that vinegar intake enhances glycaemic control by altering postprandial glycemia and postprandial insulinemia (Lim et al., 2016). Nipa palm vinegar is commonly incorporated in diets and folk medicinal preparations to

achieve a range of health benefits, including antidiabetic, antilipidemic, anticancer and anti-rheumatoid arthritis effects and insomnia relief (Mohamad et al., 2018). It is made from a local alcoholic beverage known as "Nira" derived from the flower clusters (inflorescence) of nipa palm (*Nypa fruticans* Wurmb.). In our lab, we demonstrated that nipa palm vinegar and its aqueous extract significantly suppressed postprandial glucose levels (Yusoff et al., 2015a) and alleviated postprandial hyperglycaemia in normoglycemic rats (Yusoff, et al., 2015b). However, it was unclear whether nipa palm vinegar regulated intestinal glucose absorption to influence postprandial glucose levels after oral intake. Understanding the mechanisms of action is critical because lowering or normalizing postprandial glucose levels has been hypothesized to prevent or postpone the onset of diabetes mellitus and its complications (Nistor Baldea et al., 2010).

Therefore, the present study was conducted to determine whether nipa palm vinegar exerted enzymatic inhibitory effects and suppressed the activity of α-glucosidase and α-amylase. These carbohydrate-metabolizing enzymes are known to be the main enzymes responsible for the digestion of carbohydrates in the body. This work also aimed to elucidate the mode of inhibition for these enzymes when exposed to nipa palm vinegar. The study also determined the possible effect of nipa palm vinegar on glucose uptake by the everted jejunal sac. *In vivo* studies validated the effects of nipa palm vinegar on this basic mechanism involved in intestinal glucose absorption. Furthermore, after repeated doses in sub-oral glucose tolerance test, the effect of nipa palm vinegar on the release of incretin hormone was assessed.

1.2 Problem statement

Aqueous extract of nipa palm vinegar has been reported to exert a glucose-lowering effect in rats challenged with carbohydrate loading. However, studies investigating the effects of nipa palm vinegar when administered in manners similar to the traditional methods used in folk medicine have not been conducted. Further assessments may be warranted as varying doses and preparations may exert different effects.

1.3 Hypothesis

Ingestion of nipa palm vinegar will promote better blood glucose control, partially by decreasing postprandial glucose levels. It can be achieved either by inhibiting the activity of carbohydrate digestive enzymes (α-glucosidase and α-amylase), suppressing the expression of intestinal glucose transporters (sodium-glucose linked transporter-1 and glucose transporter-2) and/or modulating the secretion of incretin hormone (glucagon-like peptide-1).

1.4 Significance of study

Ingesting nipa palm vinegar can improve blood glucose regulation by lowering postprandial glucose levels. This study will report on the physiological mechanisms involved in nipa palm vinegar antihyperglycemic activity. Ultimately, it generates evidence-based knowledge as to whether nipa palm vinegar inhibits the action of the carbohydrate digestive enzymes, α-glucosidase, and α-amylase, modulates expression of glucose transporter and/or regulates incretin hormonal secretion, glucagon like peptide-1. As ‘nira’, the raw material used to prepare nipa palm vinegar is native to Malaysia, the potential of developing a native superfood backed by evidence-based data can have positive effects on the community in more

than one way. This work seeks to offer an alternative evidence-based therapy for controlling postprandial glucose levels.

1.5 Objectives of the study

The primary goal of this research is to investigate the effects of nipa palm vinegar on intestinal glucose absorption and incretin secretion utilizing a variety of laboratory and *in vivo* methodologies. The specific objectives of the study are as follows:

1. To determine the effect of nipa palm vinegar on the enzymatic inhibitory and kinetic against the digestive enzymes α-amylase and α-glucosidase.
2. To investigate the effects of nipa palm vinegar on glucose uptake using the everted jejunal sac technique.
3. To demonstrate the antihyperglycemic effects of nipa palm vinegar in terms of the attenuation of postprandial glycaemia in normoglycemic rats using oral starch and glucose tolerance tests.
4. To determine the effect of nipa palm vinegar on the release of the incretin hormone, glucagon like peptide-1, upon repeated dosing for 14 days in sub-oral glucose tolerance test.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes cases globally

Diabetes is a long-term, natural condition that has a substantial impact on people's lives, families, and social orders all over the world. It is one of the world's most critical public health threats, posing a huge global public health and socioeconomic development burden. Despite the fact that the number of diabetics has begun to fall in some countries, diabetes has become more prevalent in most other industrialised and rising economies in recent decades (Lin et al., 2020). In 2017, 451 million adults worldwide have diabetes, according to the International Diabetes Federation. If no effective preventative strategies are implemented, that figure is predicted to climb to 693 million by 2045 (Cho et al., 2018). Diabetes is one of the top 10 causes of death worldwide. Diabetes types 1 and 2 are becoming more frequent in children and adolescents. More than one million children and adolescents under the age of 20 have type 1 diabetes (Dabelea et al., 2014). Diabetes patients have a 2–3 fold higher risk of death overall (Yang et al., 2019). Infection, cardiovascular disease, stroke, chronic renal disease, chronic liver disease, and cancer fatalities are all associated to diabetes. One out of every two people with diabetes is said to be completely uninformed of their condition (Bragg et al., 2017). Overall, diabetes is a global problem.

2.2 Diabetes cases in the community

Diabetes is a major public health concern in Malaysia, linked to an increase in macro and microvascular complications and premature and avoidable mortality. As Malaysia proceeds with its formative advancement as a country both socially and

financially, infection patterns and weights change to reflect changes in its population's way of life and dietary examples. It is very much perceived that diabetes in Malaysia has gotten progressively risky alongside other cardiovascular conditions, such as hypertension, coronary illness, and stroke. The prevalence of diabetes in Malaysians aged 20 to 79 are expected to account for 17.5 percent of the population, putting the country third in the Asia-Pacific region. In 2030, Malaysia is anticipated to have 2.48 million diabetics, up from 0.94 million in 2000, a 164 percent increase (Aljunid et al., 2019). According to the National Health and Morbidity Surveys 2019, Diabetes affects one out of every five persons in Malaysia, or roughly 3.9 million people aged 18 and up (Institute for Public Health NHMS, 2019). Malaysia has one of Asia's highest prevalence rates of type 2 diabetes, and this trend is projected to continue (Abdullah et al., 2017).

2.3 Blood glucose regulation

Blood glucose modulation is the mechanism wherein the body retains blood sugar levels, mostly glucose, within a small range (4–6 mM). Insulin and glucagon are the hormones that control this process. Pancreatic endocrine hormones include insulin and glucagon, which are both released by the pancreas (Röder et al., 2016). Figure 2.1 demonstrates the intimate relationship and mechanism that exists between insulin and glucagon. As seen in figure 2.1, insulin affects a variety of cells, including muscle, red blood cells, and fat cells. Rising blood glucose levels after a meal are the primary stimulus for insulin synthesis in β-cells. The facilitative glucose transporter glucose transporter 2 absorbs circulating blood glucose and is present on the surface of β-cells (Komatsu et al., 2013).

In reaction to insulin, cells absorb glucose from the bloodstream, driving high blood glucose levels back into a good range. Glucagon is secreted by α-cells of the pancreatic similarly to insulin, but with opposite action. Glucagon is generated in high levels when blood glucose falls below a specific level, for instance, between meals or during exercise. Similar to insulin, glucagon influences numerous cells in the body, but most notably the liver (Rajak et al., 2021).

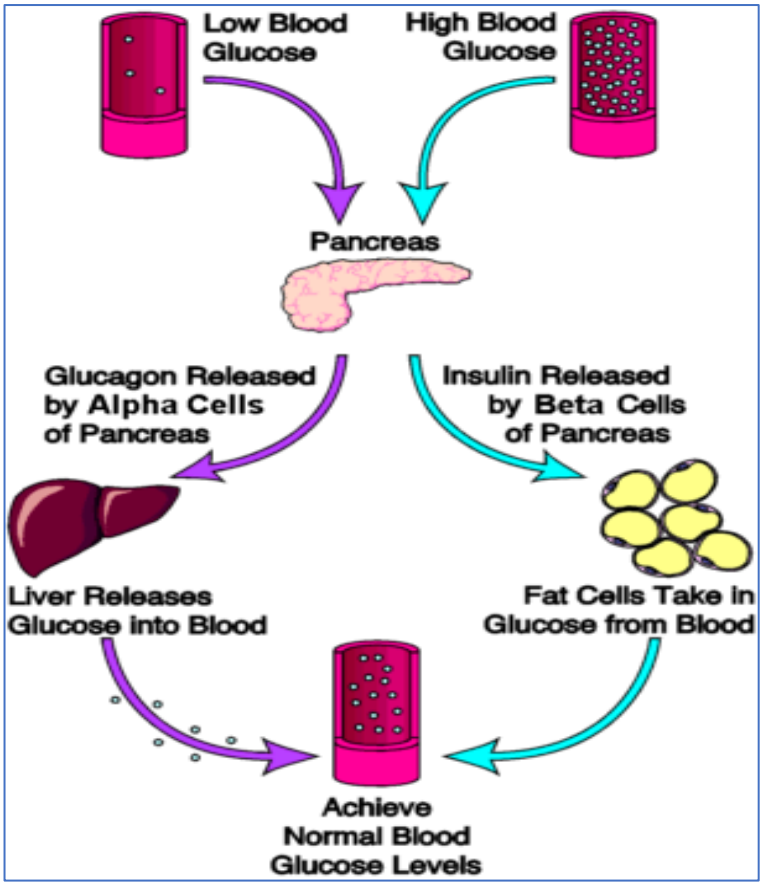

Figure 2.1 Regulation of blood glucose (Adopted by Endocrine Web, Retrieved on 2016, from https://www.endocrineweb.com/images/sugar.gif)

2.4 Pathophysiology of diabetes mellitus

Diabetes mellitus is caused by a disruption in glucose metabolism and its consequences for other metabolic pathways. Diabetes pathophysiology is a complicated process involving numerous hormones (insulin, glucagon, and growth hormone) and the body's ability to utilise these hormones. When blood glucose levels rise, the pancreatic β-cells normally release insulin. In type 1 diabetes, there is almost no insulin at all due to destruction of β-cells, whereas in type 2 diabetes, the peripheral tissues resist insulin's effects (Moini, 2019). Hyperinsulinemia is a condition strongly associated with diabetes mellitus in that it resulted from aberrant insulin sensitivity, leading to the loss of β-cells and decreased glucose tolerance. On the other hand, long-term hyperglycemia frequently results in various microvascular and macrovascular diabetic complications, leading to diabetes-related morbidity and mortality (Banday et al., 2020). In figure 2.2 microvascular and macrovascular complications of diabetes are described. Major microvascular complications include conditions such as retinopathy, nephropathy, neuropathy, and macrovascular complications are conditions such as stroke, coronary heart disease, peripheral vascular disease.

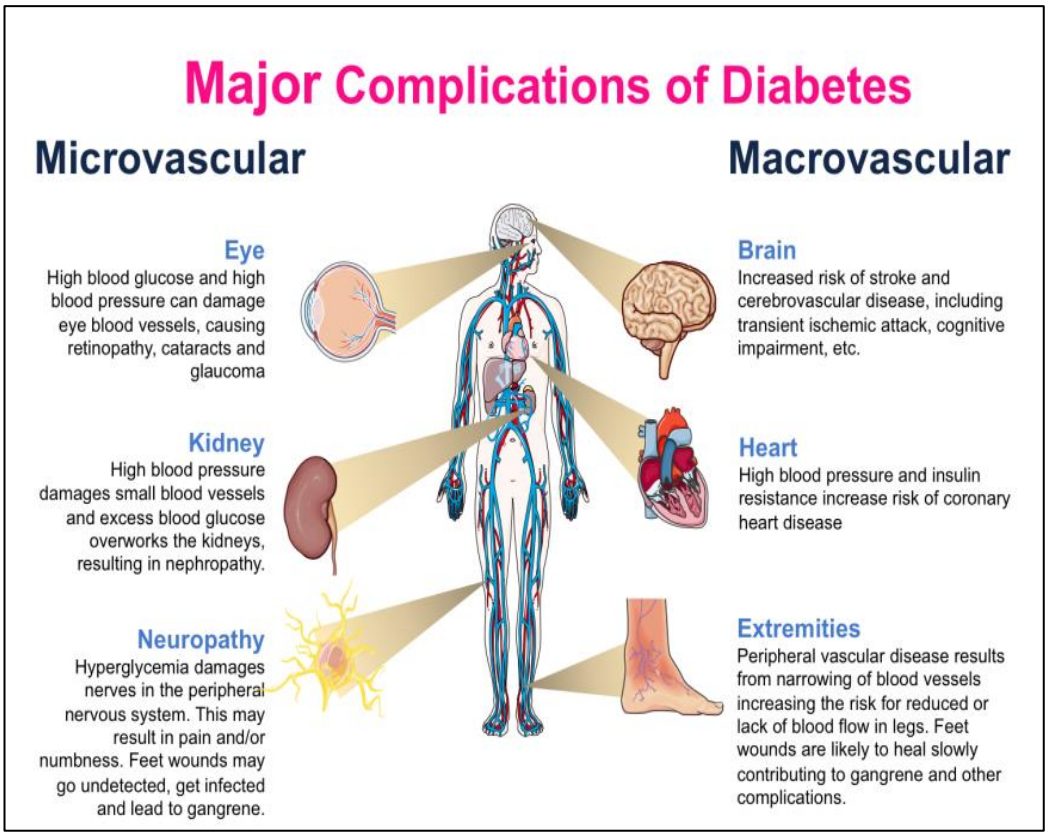

Figure 2.2 Major microvascular and macrovascular complications associated with diabetes mellitus (Adapted by Educational portal of PDB-101, from https://cdn.rcsb.org/pdb101/global-health/diabetes-mellitus/files/diabetes mellitus - Micro-Macro-Complicns.png)

2.5 Intestinal glucose absorption

Glucose reaches the bloodstream through absorptive cells in the small intestine. The steps involved in the transportation of glucose from the enteric lumen into the porous cell are as follows:

Sodium ions are transferred from the cells to the interstitial fluid in the first step. This results in low sodium concentrations within the cells (Zheng et al., 2012).

Secondly, due to the low sodium concentrations, sodium ions are transferred from the intestinal lumen to the cells by facilitated diffusion using transport proteins. The ions are used to have the glucose shipped.

Glucose is actively transported into the epithelium by the sodium-glucose co-transporter in the brush border membrane.

Glucose within the epithelium escapes into the blood through the facilitated glucose transporter-2 and diffusion across the plasma membrane. Figure 2.3 depicts the glucose absorption across intestinal membrane (Wright et al., 2018).

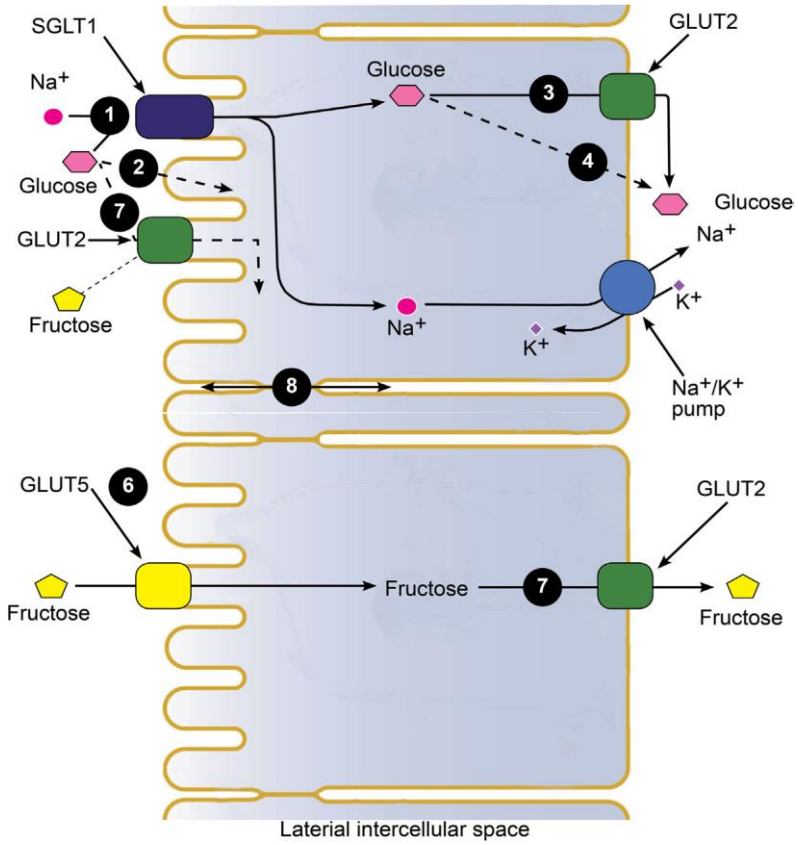

Figure 2.3 Model of glucose absorption across intestinal epithelium (Wright et al., 2018).

2.6 Effect of glucose homeostasis in healthy and diabetic people

Glucose homeostasis is essential for a healthy life (Yang, 2014). In healthy people, it sustains an internal environment where cellular processes occur in a manner that sustains life (Wang et al., 2019). In other words, the cells function to regulate blood glucose levels (Figure 2.4). When insulin binds with its receptors, a cascade of events results in a reaction on the cells surfaces to open glucose channels and enable the entry of glucose. Conversely, glucose homeostasis is impaired in abnormal conditions, causing blood glucose levels to rise and fall unchecked. If blood glucose levels are then not managed, they may become dangerously high or morbidly low. Diabetes mellitus can cause major complications such as diabetic

retinopathy, retinal degeneration in older adults, neurotoxicity, blindness, renal failure, myocardial disease, and mortality due to persistent hyperglycemia and transitory hypoglycaemia (Huang et al., 2014).

Insulin in the bloodstream does not bind to its receptors properly in type 2 diabetes. Hence, glucose channels do not open, leaving high glucose levels in the blood while cells starve (Figure 2.4). Thus, the internal environment is maintained in a suboptimal condition; and enzymes cannot work efficiently (Bano, 2013).

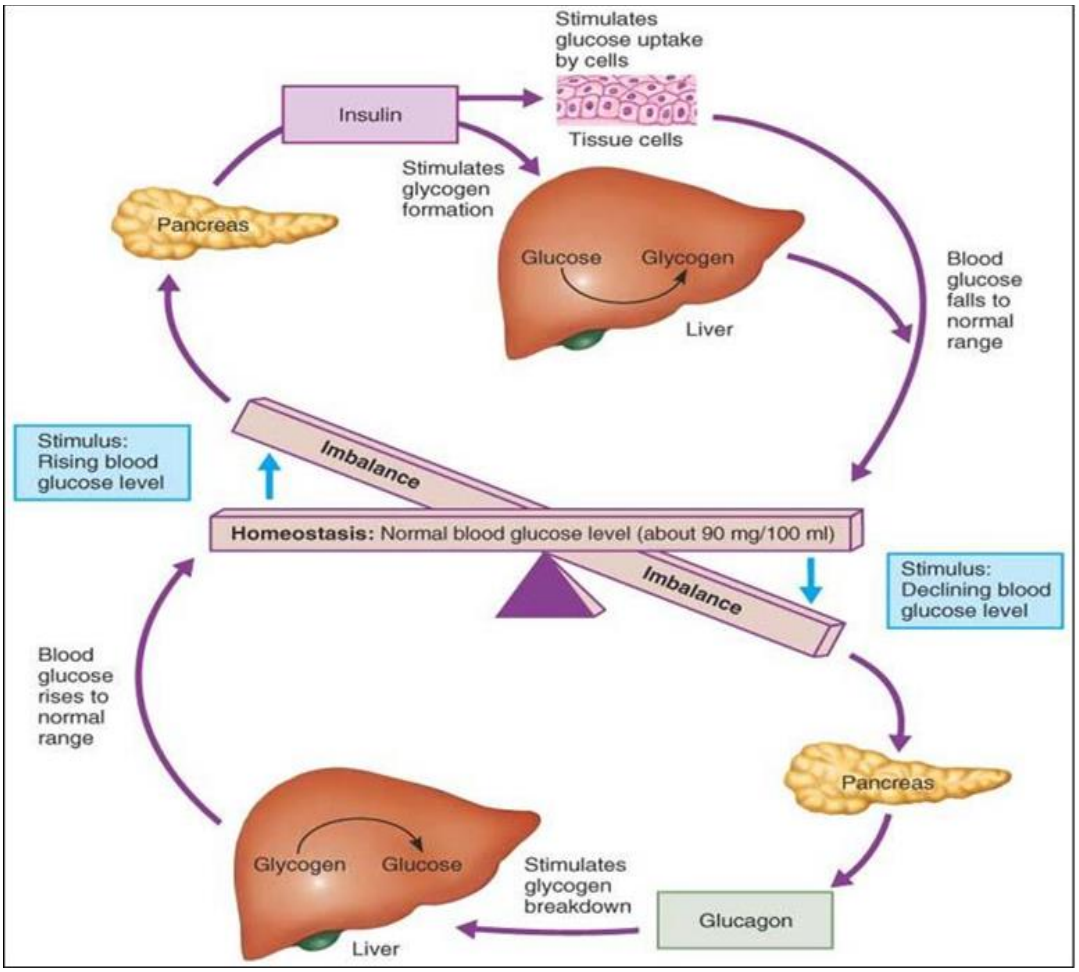

Figure 2.4 Glucose Homeostasis (Adopted from Human Lab Biology. Retrieved on April 12, 2021, from http://humanbiologylab.pbworks.com).

2.7 Incretin hormones

Incretins make up a group of gut-peptide hormones that belong to the glucagon superfamily. They function to invigorate insulin release in response to nutrients intake (primarily in response to glucose and fat). Incretins are a significant part of the gastrointestinal physiology in the human body because they trigger pancreatic insulin secretion in a glucose-dependent manner. They are, therefore, essential components for the regulation of blood glucose levels (Saini & Badole, 2015a).

2.7.1 Incretin hormones classification

Glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 are the two forms of incretin hormones found in humans. They are also referred to as the upper (glucose-dependent insulinotropic polypeptide, K cells) and lower (glucagon-like peptide-1, L cells) intestinal incretin hormones. Endocrine cells in the digestive system discharge these two hormones into the bloodstream to bind to their receptors (Nauck & Meier, 2018). The glucose-dependent insulinotropic polypeptide receptors are mainly found in pancreatic β-cells, adipose tissues, and the central nervous system. On the other hand, the glucagon-like peptide-1 receptors are expressed in the islets of α-cells and β cells (where they are particularly abundant), and in the gastrointestinal tract, the central nervous system, the heart, the lungs, and the kidneys (Ruozi et al., 2017).

2.7.2 Mechanism of action of incretin hormone

Incretin production is initiated when glucose is found in the small intestines. Incretins are released and transported via the bloodstream to the pancreatic cells

(Figure 2.5). When incretins stimulate β-cells, they secrete more insulin to respond to the quantities of glucose reaching the circulation (Saini & Badole, 2015a).

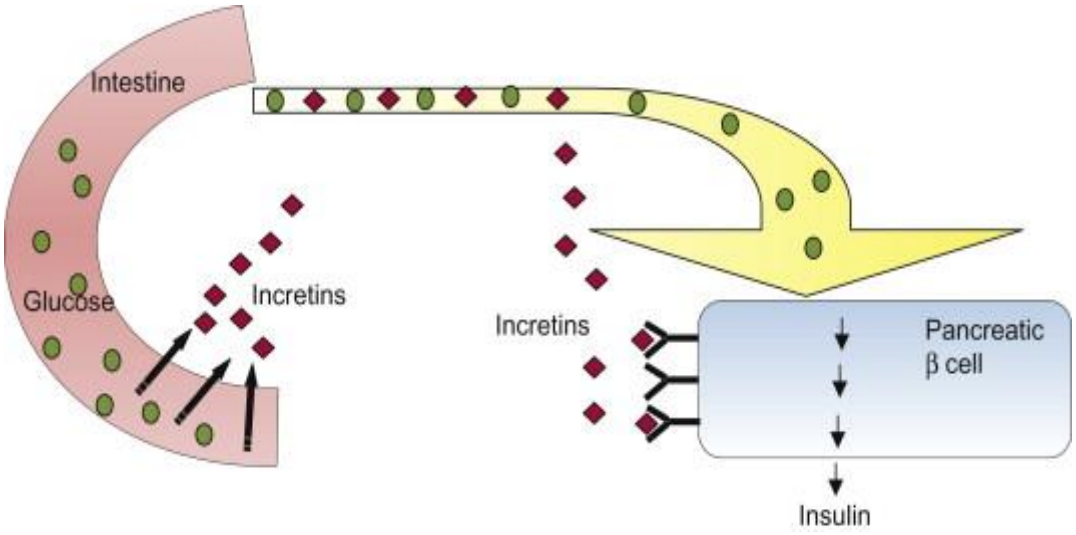

Figure 2.5 Mechanism of actions of incretin hormones (Saini & Badole, 2015a).

2.7.3 Diabetes medicine that acts by controlling incretin hormones

When β cells are unable to produce adequate amounts of insulin in a diabetic patient due to malfunctioning glucose regulation, several physiological functions fail. Increased glucagon secretion results in glucose reabsorption in the kidneys, while glucagon-like peptide-1 and gastrointestinal inhibitory polypeptides are reduced (Kalin et al., 2017).

Patients with type 1 and type 2 diabetes mellitus can use a variety of therapies to achieve optimal glycaemic control. In patients having low insulin levels, the Diabetes Control and Complications Trial Research Group hold that insulin therapy can effectively prevent the complications associated with diabetes mellitus, including diabetic retinopathy, nephropathy, and neuropathy (Liu et al., 2019). However, long-

term insulin administration has been linked to adverse events, such as extreme hypoglycaemia and weight gain. Incretin-based medications are a viable way to improve glucose regulation while avoiding the negative effects of existing treatments (Campbell & Drucker, 2013). Incretin-related treatments are classified into two categories based on their mode of action: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. Glucagon-like peptide-1 receptor agonists have a longer half-life than naturally occurring glucagon-like peptide-1 molecules and target the glucagon-like peptide-1 receptors. Inhibitors of dipeptidyl peptidase-4, across the other hand, increase endogenous glucagon-like peptide-1 activity by preventing its breakdown by the enzyme (ADA, 2015). Incretin-based hypoglycaemic agents of the glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitor classes have been approved for type 2 diabetes mellitus treatment. They seem to have similar effectiveness and pose a low risk of hypoglycaemia compared with other non-insulin-based antidiabetic drugs. These medications may also be advantageous because they do not result in significant body weight gain (Roberto et al., 2019). Some exert their therapeutic activity by increasing glucagon-like peptide-1 activity, which plays an essential role in glucose homeostasis (White, 2009). Combination therapy involving a drug such as glucagon-like peptide-1 receptor agonists or a dipeptidyl peptidase-4 inhibitor, along with basal insulin, has been shown to improve glucose control in type-1 diabetes mellitus patients (Lili Liu et al., 2019).

1. Glucagon-like peptide-1 receptor agonists

In the human body, the enzyme dipeptidyl peptidase-4 rapidly destroys native glucagon-like peptide-1, with a half-life of roughly 2 minutes. The activity of dipeptidyl peptidase-4 enzymes can be inhibited by glucagon-like peptide-1 receptor

agonists which has similar structure and characteristic as glucagon like peptide 1 (Lund et al., 2014). Glucagon-like peptide-1 receptor agonist has a similar structure and characteristics as glucagon-like peptide-1, which is responsible for stimulating glucose-dependent insulin release from pancreatic β-cell islets by binding to the glucagon-like peptide-1 receptors. Synthetic glucagon peptide-1 receptor agonists have a longer half-life than glucagon peptide-1 because they are resistant to degradation. Mostly as a result, glucagon peptide-1 receptor agonists can help with glucose control in type 2 diabetes (Jonas et al., 2011). To avoid degradation by the dipeptidyl peptidase-4 enzymes and make a glucagon-like peptide-1 receptor agonist effective, there are two different ways involved in its synthesis. There are two strategies involved in the synthesis of a glucagon-like peptide-1 receptor agonist to prevent its degradation by the dipeptidyl peptidase-4 enzymes and make it effective (Lund et al., 2014):

a) The first approach uses the structure of a native glucagon-like peptide-1 molecule and adds a few amino acid substitutions to shield the synthetic molecules from degradation.
b) The second strategy uses naturally occurring proteins, including exendin-4 (isolated from the saliva of the lizard *Heloderma suspectum*), to provide glucagon-like peptide-1 receptor agonist with the same potency as a native glucagon-like peptide -1.

2. According to their structures and pharmacokinetic profiles, glucagon-like peptide-1 receptor agonists can be divided into groups:

a) Short-Acting glucagon-like peptide-1 receptor agonist (plasma half-life 2-4h)
b) Long-Acting glucagon-like peptide-1 receptor agonists (prolonged half-life)

Long-acting drugs have a significant advantage in terms of glycemic management because they require less frequent administration and are often well tolerated (Buse et al., 2013). On the other hand, short-acting compounds are ideal for ameliorating postprandial glucose excursions due to periodic activation of glucagon- like peptide-1 receptors, which maintains a decelerating effect on gastric emptying. This effect appears to vanish when glucagon peptide-1 receptor agonists constantly excite glucagon-like peptide-1 receptors, as with long-acting medications. However, blood pressure and body weight are significantly reduced with both short-acting and long-acting compounds (Lund et al., 2014). Agonist for the Gglucagon-like peptide-1 receptor increases insulin levels and reduce glucagon secretion when plasma glucose levels are raised in diabetic patients. These methods are known to prolong the gastric emptying time and increase insulin sensitivity, which positively counters the polyphagia observed in patients and reduces the overall food intake. Ultimately, this contributes to better blood glucose management with moderate weight loss (Kshirsagar et al., 2020).

3. Dipeptidyl peptidase-4 inhibitors

The dipeptidyl peptidase-4 blockers, which inhibit the main enzyme responsible for the breakdown of endogenous glucagon-like peptide-1, are the other family of incretin-based drugs (Vella, 2012). Hindering the activity of dipeptidyl peptidase-4 molecules improves β-cell responsiveness as well reduces glucagon quantities in the bloodstream, which leads to an increase in the glucagon-like peptide-1 and gastric inhibitory polypeptide levels. Blood glucose levels are reduced as a result of these effects. In patients with type 2 diabetes and autoimmune diabetes, studies have demonstrated that a dipeptidyl peptidase-4 inhibitor has a significant protective impact in protecting β-cell function (Zhao et al., 2014).

2.8 Carbohydrate digesting enzyme: α-amylase and α-glucosidase

One therapeutic approach for treating early-stage diabetes is to decrease postprandial hyperglycemia. To retard glucose absorption, the carbohydrate-hydrolyzing enzymes α-amylase and α-glucosidase can be inhibited in the digestive tract. Inhibitors of these enzymes (α-glucosidase and α-amylase) cause a reduction in the rate of glucose absorption and, consequently, cause a blunting of postprandial plasma glucose level rises. Inhibition of carbohydrate hydrolyzing enzymes, which are involved in carbohydrate digestion, can considerably lower blood glucose levels after meals, making it an important method for regulating blood glucose concentrations in patients with type 2 diabetes and indeterminate (Gong et al., 2020).

2.8.1 Diabetes treatment by inhibition of α-glucosidase

Alpha glucosidase inhibitors are a class of drugs used to treat type 2 diabetic patients with low glucose tolerance and hypoglycemia risk. They reduce postprandial glucose concentrations by around 3 mmol/L by slowing the absorption of carbohydrates in the gastrointestinal tract (Akmal & Wadhwa, 2021). An α-glucosidase inhibitors competitively inhibits α-glucosidase, making these inhibitors particularly effective for lowering postprandial hyperglycaemia and insulin levels. They have a minor effect on glycosylated haemoglobin levels (Zhang et al., 2019). The United States Food and Drug Administration has approved some α-glucosidase inhibitors to treat type 2 diabetes mellitus. Despite showing some benefits when administered in type 1 diabetic patients and women with gestational diabetes, the Food and Drug Administration has not approved any α-glucosidase inhibitors for use in such patients (Gao et al., 2018). The most popular drug in the class is acarbose. Voglibose and miglitol are also members of the α-glucosidase inhibitors class.

Acarbose and voglibose, which is not approved by Food and Drug Administration, are molecules that are poorly absorbed in the intestines, have low bioavailability, and are excreted in the stools (Akmal & Wadhwa, 2021).

In contrast, miglitol, which secured Food and Drug Administration approval in 1999, is fully absorbed in the gut and is transferred to the urine through the renal route (Kumar et al., 2011). Alpha glucosidase inhibitors reduce carbohydrate absorption in the proximal gut, resulting in the delivery of large amounts of undigested carbohydrates to the distal gut. This makes α-glucosidase inhibitors reduce glucose-dependent insulinotropic polypeptide secretion from the K-cells while increasing glucagon-like peptide-1 secretion from the L-cells (Min et al., 2018).

2.8.2 Diabetes treatment by inhibition of α-amylase

Alpha-amylase is a vital enzyme involved in converting starch to simple sugars. The inhibitors of a-amylase enzyme molecules slow the digestion of carbohydrates and lower the rate of glucose absorption, which results in reduced postprandial blood glucose levels. Hence, the use of α-amylase inhibitors may help diabetic patients enhance their glucose tolerance. The inhibitors may be useful in treating type 2 diabetes due to their robust therapeutic design (Bashary et al., 2019). First endorsed by the Food and Drug Administration in 1995 under the brand name Precose, Acarbose exhibits activity against both pancreatic α-amylase and α-glucosidase. However, α-amylase inhibitors are not widely utilized because they have little impact on haemoglobinA1c levels. Moreover, they must be administered in a threefold-dosing system every day, and they pose a risk of extreme gastrointestinal adverse events.

Some clinically utilized medications like Naphazoline, Fluconazole, Astemizole, Fluoxetine, Clarithromycin, and ampicillin have been demonstrated to have specific α-amylase inhibitory endeavours (Rodda et al., 2014). According to Yasmin et al. (2021), α-amylase was substantially inhibited by vinegar, which can be introduced into the diet to lower the glycemic index of meals and help both diabetes and those at risk of diabetes.

2.8.3 Medications of type 1 and type 2 diabetes

Food and Drug Administration approved medications for managing type 1 diabetes mellitus and type 2 diabetes mellitus are summarized below in Table 2.1.

Table 2.1 Approved diabetic medications by Food and Drug Administration (FDA)

Class of drug	**Names of drugs**	**Route of Administration**	**Year**
α-Glucosidase inhibitor	Miglitol	Oral	1999
Dipeptidyl peptidase-4 inhibitors	Alogliptin benzoate	Oral	2013
	Sitagliptin and simvastatin	Oral	2011
	Linagliptin	Oral	2011
Dipeptidyl peptidase-4 inhibitor and biguanide combination	Sitagliptin and metformin HCl extended release	Oral	2012
	Linagliptin plus metformin hydrochloride	Oral	2012
	Linagliptin and metformin hydrochloride	Oral	2012
	Alogliptin and metformin hydrochloride	Oral	2013
Glucagon-like	Dulaglutide	Subcutaneous injection	2014

peptide-1 receptor agonists	Albiglutide	Subcutaneous injection	2014
	Liraglutide	Subcutaneous injection	2010
	Lixisenatide	subcutaneous injection	2016
	Liraglutide	Subcutaneous injection	2010
Insulin types	Insulin degludec	Subcutaneous	2015
	Glargine U300	Subcutaneous	2015
	Insulin Aspart	Subcutaneous injection	2017
	Lyumjev (insulin lispro-aabc)	Subcutaneous injection	2020
	Semglee (insulin glargine)	Subcutaneous injection	2020
	Empagliflozin/linagliptin	Oral	2015
Sodium-glucose co-transporter-2 inhibitor and biguanide combination	Canagliflozin and metformin hydrochloride	Oral	2014
	Empagliflozin and metformin hydrochloride	Oral	2015
	Dapagliflozin and metformin hydrochloride, extended release	Oral	2014
Sodium-glucose co-transporter-2 inhibitors	Empagliflozin	Oral	2014
	Dapagliflozin	Oral	2014
	Canagliflozin	Oral	2013

(Gourgari et al., 2017)

2.9 Vinegar

Vinegar is a liquid product of fermentable carbohydrate sources, including wine, rice, dates, and maple syrup. The term vinegar gets from "vin and aigre" or acrid wine, from the French word. The accompanying response includes the change of ethanol (CH_3CH_2OH) and oxygen (O_2) to acidic corrosive (CH_3COOH) (Bule et al., 2020):

$$CH_3CH_2OH + O_2 \rightarrow CH_3COOH + H_2O$$

Vinegar is the result of a two-stage maturation; ethanolic fermentation and acetic acid maturation. In the initial step, yeast anaerobically changes sugars (glucose, fructose) into ethanol. In the subsequent level, ethanol is oxidized into acetic acid (vinegar) vigorously by Acetobacter and Gluconobacter microorganisms. Many types of vinegar are differed according to their source of carbohydrates. Types of vinegar are given below (Figure 2.6) (Sankpal, 2019).

2.9.1 Classification of Vinegar

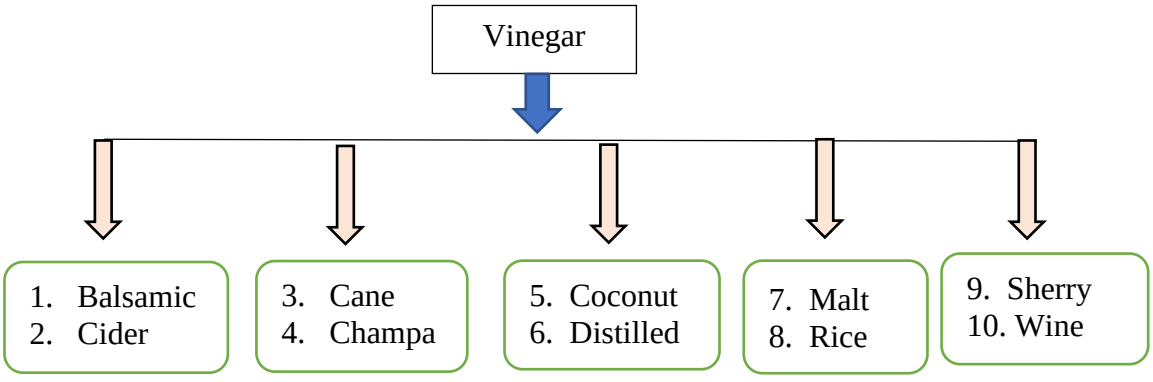

Figure 2.6 Classification of vinegar

2.9.2 Folkloric uses of vinegar

Vinegar old stories are as beautiful as it is pragmatic. People in ancient times did not understand the vinegar fermentation process, but they were aware of the end-product of vinegar and alcohol implications. As wine or other alcoholic beverages went sour, they fermented into vinegar, which was then used as a drink, preservative, and medicine. The wine was the first and oldest vinegar in the history of vinegar. Vinegar was used by most of the population in ancient times because it was believed to have various medicinal effects that improved health. Vinegar was one of the most commonly used drugs to treat diabetes in the 17th and 18th centuries (Bray, 2014).

For over 2000 years, vinegar has been used as condiments in foods, a home remedy against several ailments, and a cleaning and disinfecting agent. In Hippocrates's time, vinegar was used to treat injuries, implying that vinegar is one of the antiquated nourishments used as people medication (Ali et al., 2017). Hippocrates and his colleagues and physicians today suggested Oxymel, a well-known old remedy made from nectar and vinegar, for difficulties in breathing, constipation, fever, pneumonia, and pleuritic infections. Jews community used vinegar to treat toothaches, wounds, and dandruff, among other illnesses. Vinegar was used as a food preservative in China during the Zhou, Han, and Qin eras. The Heinz corporation first introduced vinegar bottles to the marketplace in 1880.

2.9.3 Antidiabetic activity of various kinds of vinegar

Many recent scientific investigations have documented that vinegar ingestion reduces the glucose response to a carbohydrate load in healthy adults and individuals with diabetes. The acetic acid in vinegar elicits these beneficial effects by altering metabolic processes in the gastrointestinal tract and the liver (Johnston et al., 2010).