EFFECT OF QUERCETIN ON INDUSTRIAL TRANS FATTY ACIDS INDUCED GLUCOSE INTOLERANCE IN *RATTUS NORVEGICUS ALBINUS*

BY

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SCHOOL OF MEDICINE

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DECLARATION

In adherence to principles of academic honesty, I attest that the content presented in this article is not a duplication and has not been previously put forth for academic assessment in any other academic establishment.

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DEDICATION

To myself.

ABSTRACT

Glucose intolerance, a condition characterized by elevated plasma glucose concentrations above normal but below the threshold for clinical diabetes, encompasses both prediabetes and diabetes. As industrialization has led to lifestyle changes and the unsustainability of long-term physical exercise for managing prediabetes necessitates exploration of alternative preventive measures. This study aimed to determine blood glucose concentration in Wistar rats before and after feeding on industrial trans fatty acids and quercetin,determine insulin serum concentration before and after feeding industrial trans fatty acids and quercetin and examine liver and pancreas histological changes in Wistar rats with industrial trans fatty acid-induced glucose intolerance before and after treatment with quercetin. Wistar rats, aged 3-4 months and weighing 150g – 200g, were randomly divided into three groups, each consisting of 10 rats. Group one served as the control and was fed normal rat chow, Group two received a normal rat chow along with iTFA (margarine) at 50kcal of the diet for six weeks while Group three was also fed a normal diet along with iTFA (margarine) at 50kcal of the diet for six weeks, but in addition, they were administered quercetin at a dosage of 50mg/kg body weight intragastrically every twelve hours for six weeks to counteract the effects of iTFA.Random blood sugar (RBS) levels were measured at baseline and weekly in all the rats during the six weeks. At the end of the study, all animals were euthanized with chloroform inhalation, and blood samples were obtained through cardiopuncture for insulin enzyme-linked immunosorbent assay (ELISA) assays. Liver and pancreas samples were collected for histological analysis. The effects of quercetin on RBS and insulin levels were found to be statistically significant, as indicated by two-way repeated measure analysis of variance (ANOVA) results: $F(2,27) = 86.322$, $p < 0.01$, $\eta^2p = 0.865$ for RBS, and $F(2,27) = 65.613$, $p < 0.01$, $\eta^2p = 0.829$ for RBS and insulin, respectively. At the study's end, the overall mean RBS difference between the control group and experimental group 1 and experimental group 2 was 0.79 and 9.73mmol/l respectively while overall mean insulin difference among the above groups was 0.0087 and 0.2523Umol/l respectively. Histologically, quercetin prevented liver and pancreatic β cell injury induced by iTFAs. In conclusion, the study found that quercetin prevents hyperglycemia induced by iTFAs without necessitating an augmentation in insulin secretion.

TABLE OF CONTENT

LIST OF ABBREVIATIONS

ANOVA	:	Analysis of variance.
AMPK	:	AMP activated protein kinase.
CRP	:	C reactive protein.
ELISA	:	Enzyme-linked immunosorbent assay.
FOXO1	:	Forkhead box protein O1.
FBS	:	Fasting blood glucose.
GI	:	Glucose intolerance.
GLUT4	:	Glucose transporter type 4.
GRP78	:	Glucose-regulated protein 78.
HFD	:	High fructose diet.
IFG	:	Impaired fasting glucose.
IGT	:	Impaired glucose tolerance.
IL-6	:	Interleukin type six.
ITFA	:	Industrial trans fatty acids.
JNK	:	c-Jun N-terminal kinase.
MTRH	:	Moi Teaching and Referral Hospital.
TNF-α-	:	Tumour necrosis factor alpha.
STZ	:	Streptozotocin.
UPF	:	Ultra processed food.

LIST OF OPERATIONAL DEFINITIONS

Flavonoids -Secondary phenolics found in plants.

Glucose intolerance - Impaired glucose tolerance

Industrial trans fatty acids - seed oils manufactured through partial

Quercetin - the most abundant of all flavonoids and belongs to a class of flavonols

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CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Glucose intolerance (GI) comprises prediabetes and diabetes (R et al., 2018),with prediabetes indicating elevated blood glucose concentration between normal and clinical diabetes (Bansal, 2015). IFG and IGT are diagnostic parameters for prediabetes, which respectively indicate hepatic and skeletal muscle insulin resistance (Abdul-Ghani & DeFronzo, n.d.). The global burden of prediabetes is 7.3% and it is predicted to increase to 8.3% by 2045 with Africa recording a prevalence of 8.6%(Hostalek, 2019) while Kenya's prevalence was estimated at 3.1% in 2018 (Mohamed et al., 2018a).Roughly a quarter of individuals with prediabetes progresses to type 2 diabetes mellitus(T2DM) within 3-5years while up to 70% will eventualy develop T2DM in their lifespan (Tabák et al., 2012). The natural history of preDM is either the maintenance of the prediabetes state or the progression to T2DM(Gong et al., 2019). Diet intervention of increasing vegetable intake and a reduction in sugar intake for six years increases the regression of prediabetes to normal glucose regulation(NGR) by 31%(Pan et al., 1997). though the pharmacological intervention of prediabetes with Metformin and Rosiglitazone significantly reduces the progression of prediabetes to T2DM, withholding the intervention leads to a washout and regression back to prediabetes(Ramachandran et al., 2006). Lifestyle interventions in ten individuals with prediabetes can prevent one death in a 30-year follow-up period. (Sallar & Dagogo-Jack, 2020).

WHO defines diabetes as a metabolic disorder marked by prolonged hyperglycemia which is detrimental to blood vessels, heart, kidneys, nerves, and eyes in the long term .T2DM constitute majority of all diabetes cases and it is marked by insufficient insulin secretion and IR (Roden & Shulman, 2019). Modifiable factors and non-modifiable risk factors determine the epidemiology of T2DM (Galicia-Garcia et al., 2020). Diabetes affects 422 million people globally, with a million deaths attributed to it annually. Globally, the prevalence of diabetes was estimated at 9.3% in 2019 and it is forecasted to increase to 10.9% in the next three decades (Saeedi et al., 2019a).between the years 1990 and 2017, the incidence of diabetes doubled, and the increase was higher in low-middle, middle, and high-middle income countries(Lin et al., 2020).in Kenya, diabetes prevalence has increased from 2.4% in the 2015 to 3.3% in 2021

The use of ITFAs has increased rapidly in the last three decades in developing countries(Teegala et al., 2009) leading to a rise in the prevalence of glucose intolerance(Pipoyan et al., 2021). Quercetin, a naturally occurring flavonoid has demonstrated potentially beneficial effects on glucose metabolism (Peng et al., 2017)**.** However, the effects of quercetin on glucose intolerance induced by ITFAs have not been extensively studied (Bule et al., 2019a).

1.2 Statement of the Problem

For the last three decades obesity has tripled and the human genome and the total energy intake has not changed significantly hence the major contributing variables of obesity are the change in diet component and physical inactivity which has been rising parallel to industrialization(Hill & Melanson, 1999). ITFAs have been shown to disrupt glucose metabolism, leading to IR and GI (Pipoyan et al., 2021).The use of ITFAs has increased rapidly, leading to a rise in the prevalence of glucose intolerance(Teegala et al., 2009). Currently, the long-term outcome of DM in developing countries is unfavourable partly due to poverty and weak health systems('Prevention and Management of Chronic Disease', 2010).

1.3 Justification

ITFAs cause a slower development of PreDM through dysregulation of lipid oxidation, ER stress, inflammation and oxidative stress (Zhu et al., 2019a). Other studies have demonstrated a protective effect of quercetin against glucose intolerance induced by streptozotocin in animal models(Bule et al., 2019b). With lifestyle changes accompanying industrialization and unsustainable long-term physical exercise in managing PreDM (Thorsen et al., 2022), DM incidence is projected to increase rapidly(Saeedi et al., 2019b). Thus necessitating a need to investigate other potential long-term preventive measures.

1.4 Significance of the Study

The study's findings underscore the critical importance of targeted interventions to curb the deleterious effects of ITFAs, paving the way for more informed preventive measures and public health initiatives

1.5 Objectives

1.5.1 Main objective

To assess the effect of quercetin on industrial Trans fatty acids induced glucose intolerance in Wistar rats.

1.5.2 Specific Objectives

1. To determine blood glucose concentration in Wistar rats before and after feeding on industrial trans fatty acids and quercetin.
2. To determine insulin serum concentration in Wistar rats before and after feeding industrial trans fatty acids and quercetin.
3. To examine liver and pancreas histological changes in Wistar rats with industrial trans fatty acid-induced glucose intolerance before and after treatment with quercetin.

1.6 Hypothesis

1.6.1 Null Hypothesis

There is no difference in the mean of blood glucose, insulin levels and tissue histologic changes among group one two and three.

H_O: $\mu 1=\mu 2=\mu 3$

CHAPTER TWO

LITERATURE REVIEW

2.1 Pathophysiology of Type Two Diabetes Mellitus

Long-term epidemiological studies indicates that elevated levels of FBS and PPBS are predictors of future diagnosis of T2DM, indicating underlying insulin resistance (Abdul-Ghani & DeFronzo, 2009). In the onset of pathogenesis, the β cells counteract hyperglycemia by increasing insulin secretion. Hyperinsulinemia, in turn, stimulates lipolysis in white adipose tissue (WAT), leading to an increase in NEFA that enter the liver and stimulate gluconeogenesis (Roden & Shulman, 2019). Insulin resistance (IR) is a state of decreased action of insulin response by insulin-sensitive tissues resulting in hyperinsulinemia which has been associated with obesity, which activates forkhead box protein O1 (FOXO1) and disrupts glucose transporter type 4 (GLUT4),an insulin-dependent transporter present in adipocytes and myocetes that facilitates glucose diffusion into the cells. Downregulation of GLUT4 results in postprandial hyperglycemia (Czech, 2017).

An alternate hypothesis has been also proposed that hyperinsulinemia is the aetiology of IR and not the other way round as described above. The hypothesis is based on the observation made of non-diabetic obese people who present with hyperinsulinemia without hyperglycaemia that is physiologically expected to occur. Hyperinsulinemia activates inflammatory processes in the adipocytes and increases lipolysis and a subsequent increase in the substrates of gluconeogenesis(Czech, 2017). A unifying concept of the development of T2D takes multiple organs approach involving the liver, skeletal muscle, pancreas, and gut with modulation from the brain via. leptin (Galicia-Garcia et al., 2020)

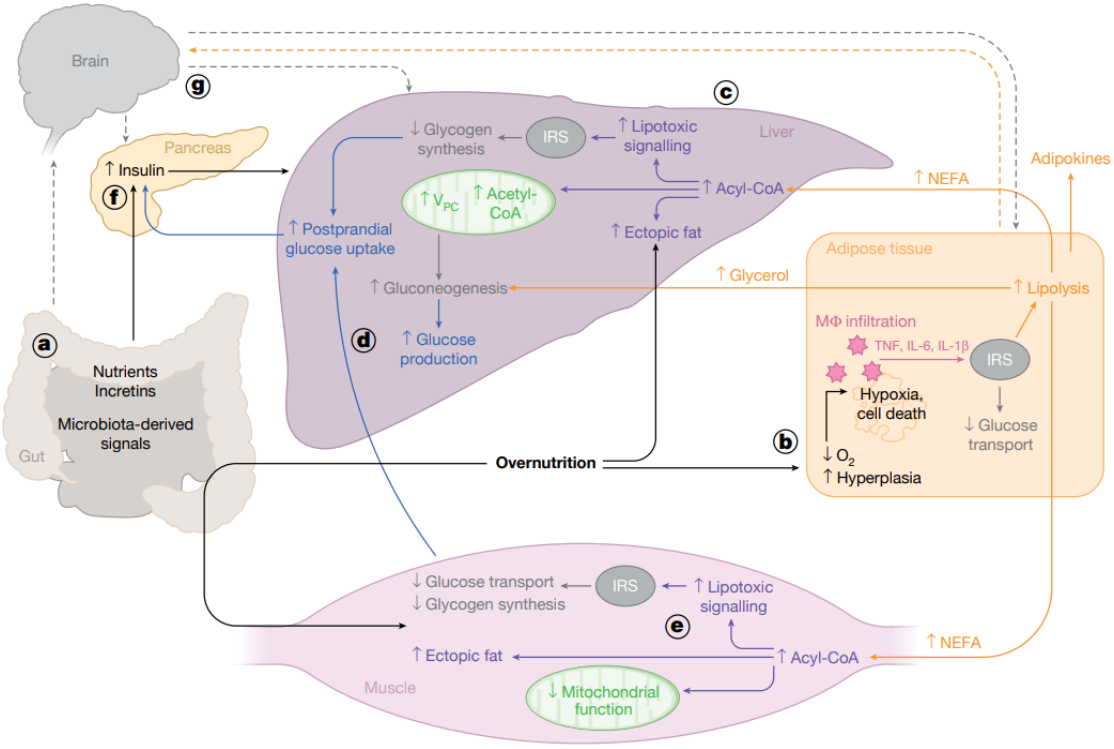

Figure 2.1: insulin resistance process in humans

Adapted from (Galicia-Garcia et al., 2020).

2.1.1 Factors associated with type 2 diabetes

A 2016 umbrella review of 86 epidemiological studies on the association between modifiable factors and T2DM found a strong and significant association between lifestyle and dietary factors, comorbidity, proxies of obesity, and serum biomarkers with the development of T2DM. Consumption of processed food, sugar-sweetened drinks, and dietary heme iron are positively associated with T2DM. These dietary factors are also linked to other factors such as physical inactivity, increased BMI and smoking. Whole-grain product consumption is negatively associated with T2DM independent of BMI (Bellou et al., 2018).

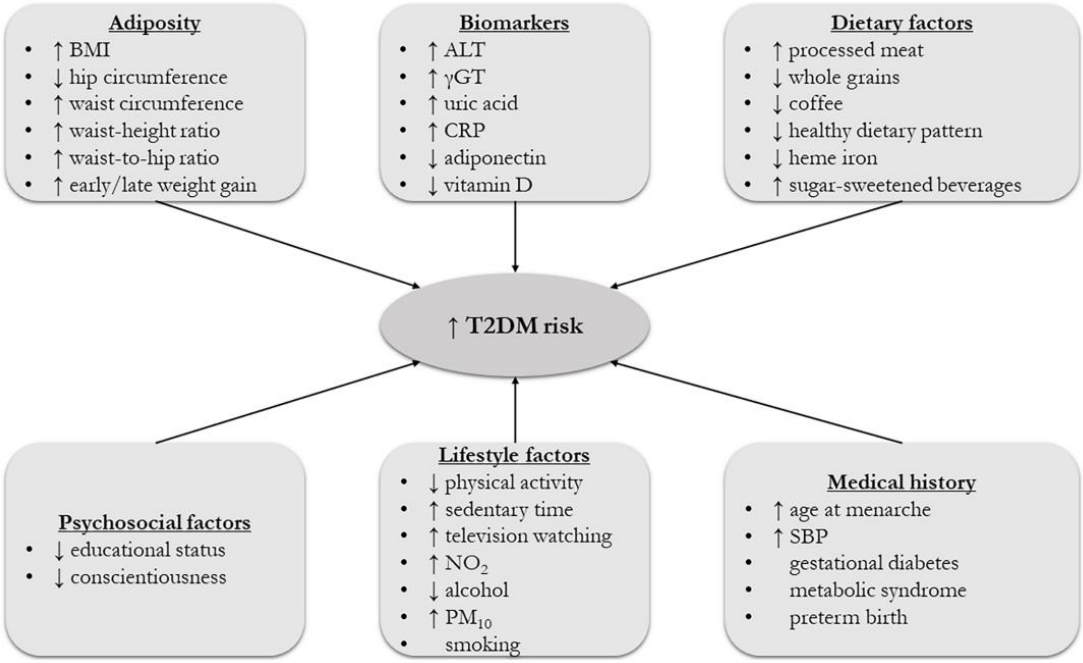

Figure 2.2: A unified concept of insulin resistance in humans

Adapted from (Bellou et al., 2018).

Meta-analysis of studies conducted in Ghana indicated that family history of diabetes, obesity, lack of physical activity, and age above 40 years are significant risk factors for diabetes (Asamoah-Boaheng et al., 2019). The above findings correlates with study conducted in Kanungu District, Uganda where family history, BMI, alcoholism, and smoking as significant risk factors for diabetes(Asiimwe et al., 2020). 2015 Kenya STEPs survey reported that lack of formal education and high total cholesterol are predictors of pre-diabetes while old age, high blood pressure, and obesity are associated with diabetes(Mohamed et al., 2018b).

In May 2022 WHO declared the obesity epidemic in Europe with a prevalence of 59% in adults and 28% in children. This increase has been attributed to a highly digitalized society that is associated with the marketing of unhealthy foods and video games among children. Increasing prevalence of T2DM and the incidence of obesity appears to follow a parallel trajectory (Xu et al., 2013), this relationship between industrialization, obesity and T2DM is evident in the disproportionate higher of T2DM in towns compared to countryside inhabitants (Uloko et al., 2018). Obesity is now recognised as the single major independent factor of T2DM (Zheng et al., 2018). The above relationship was reinforced by repeated observational studies done after the mid-1990s Cuban crisis where during the crisis, the average population's body weight had decreased by 5kgs and a rapid decline in diabetes was

recorded in the same period and after the crisis, the population's average weight exceeded the pre-crisis level accompanied by a surge in the cases diabetes (Franco et al., 2013).

Consumption of ultra-processed food(UPF), a dietary pattern associated with a western diet that is common in industrialized countries and urban populations is an established risk factor for T2DM among these populations (Srour et al., 2020).UPF undergoes several physical, biological and chemical processes such as hydrogenation in an attempt to make the final product more palatable. This modification process can lead to the production of new compounds which together with the additives added can alter the body's metabolic process(Monteiro et al., 2018). The Mediterranean diet pattern is a traditional diet common among the natives of countries around the Mediterranean Sea and it has been associated with reduced weight gain and a low incidence of both obesity and T2DM(Medina-Remón et al., 2018).

meta-analysis of long-term prospective observational studies shows an inverse association between the consumption of plant products and the incidence of T2DM(Neuenschwander et al., 2019). Diet has a dual effect in modulating the development of T2DM through specific components(Cheung et al., 2018). The diversity of dietary fats and the accompanying nutrients make some dietary fats a risk factor for T2DM (J. H. Y. Wu et al., 2019), while others appear to not affect the development of T2DM(Brown et al., 2019). Polyphenols which are mostly obtained from a plant-based diet have a protective effect against the effect of other dietary T2DM dietary risk factors (Kang et al., 2019).

2.2 Industrial Trans Fatty Acids

Industrial trans fatty acids(iTFA) are manufactured by hydrogenating and refining seeds oil (Martin et al., 2007), the final product has a longer shelf life, palatable taste and is relatively cheaper, and it is marketed as 'vegetable oils'(Bhardwaj et al., 2011).frying of food at temperatures above 180℃ also leads to the formation of iTFA through isomerization of *cis* unsaturated fatty acids (Gotoh et al., 2018).WHO recommends below one percent total energy intake of iTFA equivalent to 2.2g/day in a 2000 caloric diet based on the evidence linking iTFA and cardiovascular diseases(Organization, 2018). An association exist between plasma iTFA and cases of diabetes among the adult population (Liu et al., 2018). CRP, an inflammatory marker has also been positively associated with the consumption of iTFA (Mazidi et al., 2017). A large prospective observation from Study showed there is a positive

association between iTFA intake and proinflamatory markers such as tumour necrosis factor alpha, interleukin subtype six and C-reactive protein (Mozaffarian et al., 2004).

Caloric-controlled feeding of monkeys with iTFA (*trans'* 18:1, 8% energy) compared with *cis* mono-saturated fatty acid (MUFA) for six years increases intra-abdominal fat deposition, insulin resistance(IR) and hyperinsulinemia(Kavanagh et al., 2007). A study using mice to compare a highly fatty diet and iTFA demonstrated the latter induces severe obesity and IR the through down-regulation of IRS-1 in hepatocytes(Zhao et al., 2016). Feedin iTFA to lactating Wistar rats causes IR in adult progeny suggesting of a delayed effect later in life caused by early exposure(Osso et al., 2008). *In vitro* studies reinforce the above *in vivo* studies where exposure of adipocytes to elaidic acid (C18 iTFA) impairs cells glucose intake and suppresses attachement of GLUT4 storage vesicles with the cell membrane(Ishibashi et al., 2020). The proposed mechanisms by which iTFA causes metabolic syndrome include dysregulation of lipid oxidation, ER stress, inflammation and oxidative stress (Oteng & Kersten, 2020).

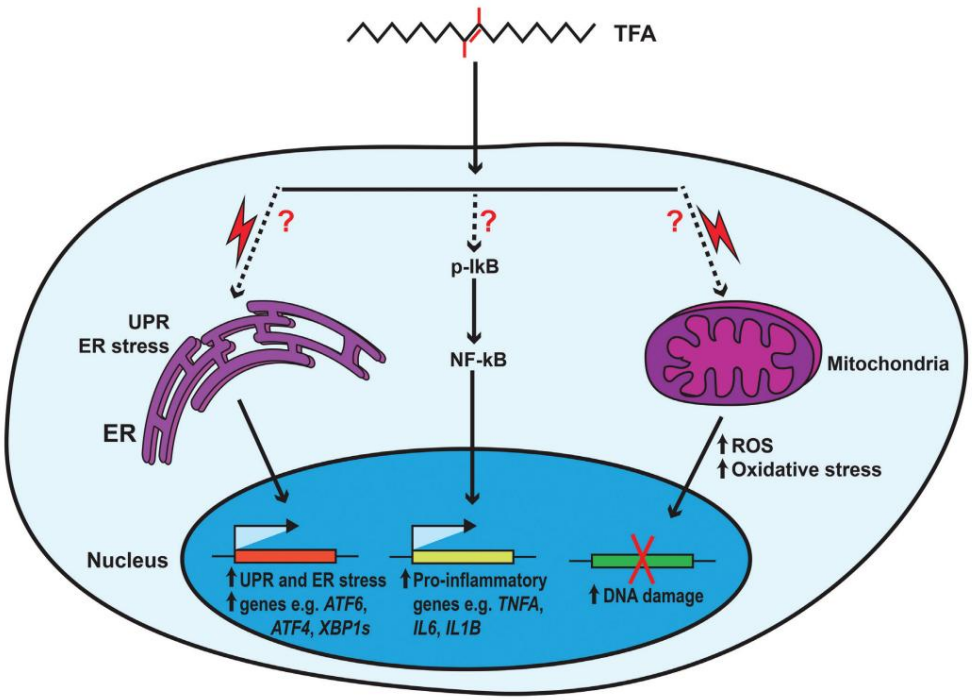

Figure 2.3: Mechanisms of Trans fatty acids

Adapted from (Oteng & Kersten, 2020).

Administration of ITFA to Wistar rats results in hepatic lipotoxicity by increasing oxidative stress while reducing levels of plasma antioxidants (Dhibi et al., 2011). In addition,

incubating human cells with elaidic acid leads to apoptosis with the concomitant production of ROS (Zapolska-Downar et al., 2005). Moreover, the upregulation of GRP78 and the phosphorylation of JNK indicate that WAT is the primary site of endoplasmic reticulum stress (Zhu et al., 2019b).

2.2.1 Industrial trans fatty acids effects on the liver and pancreas

iTFA induces histological changes in the liver and pancreas, including increased liver fat accumulation, pancreatic inflammation, and fibrosis (Li et al., 2015)These changes may contribute to the genesis of glucose intolerance and IR. Rats fed a high-fat diet containing iTFA showed histological changes in the liver, including hepatocyte ballooning, steatosis, and inflammation (Kucera & Cervinkova, 2014)These changes were associated with increased insulin resistance and impaired glucose metabolism. iTFA consumption induced pancreatic inflammation and fibrosis in rats, associated with beta-cell dysfunction and impaired glucose tolerance (Estadella et al., 2013)

2.3 Flavonoids

Flavonoids are secondary metabolites that can be found in all vascular plants and tend to accumulate in the fruits, leaves, and stems (Karak, 2019). They are composed of two benzene rings connected by a heterocyclic pyran ring C, forming a 15-carbon skeleton (Heim et al., 2002).

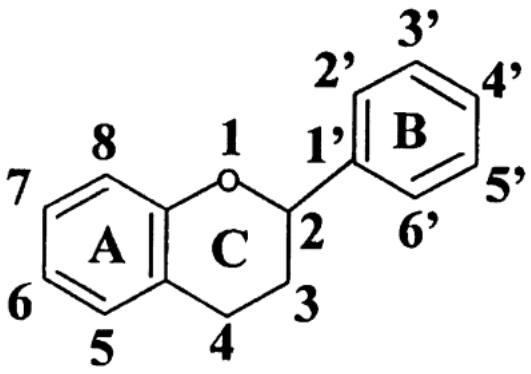

Figure 2.4: Structure of flavonoids

Adapted from (Heim et al., 2002)

Flavonoids are classified based on the position of attachment of the B ring on the C ring and the level of saturation of the C ring(Panche et al., 2016) as shown in *figure 5.*

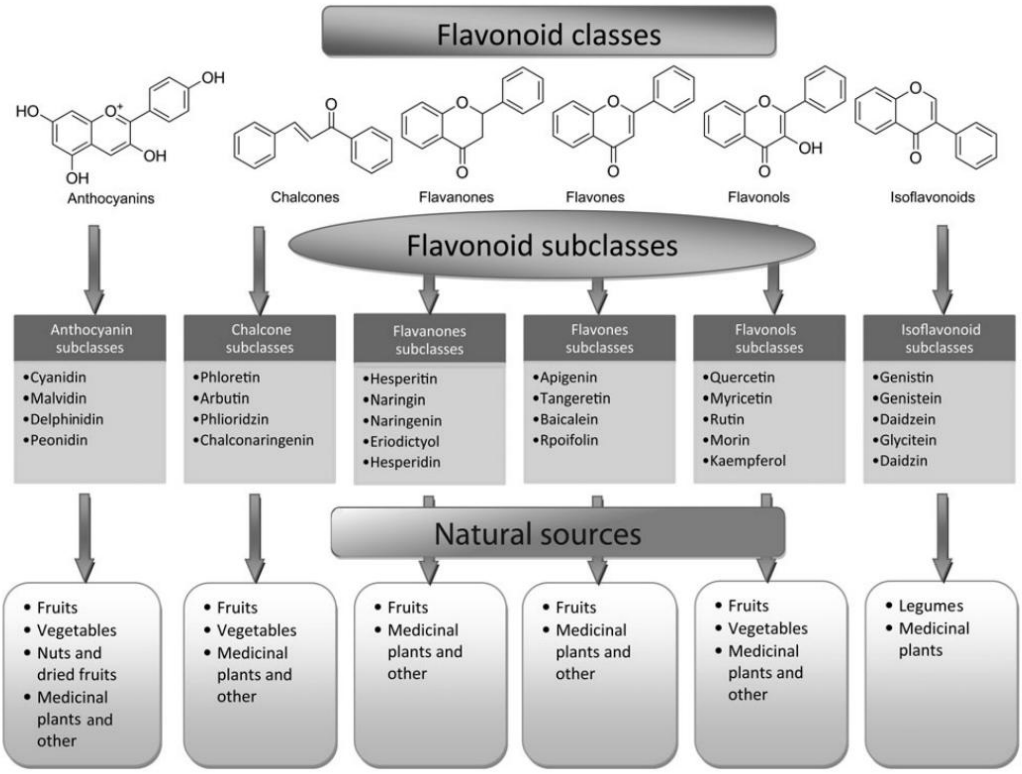

Figure 2.5: Classification of flavonoids

Adapted from (Panche et al., 2016)

Quercetin is the most abundant of all flavonoids and belongs to a class of flavonols which have five hydroxyl groups(Lakhanpal & Rai, 2007) .Fruits and vegetables such as apples, barriers, onions and kales are The major dietary source of quercetin (P. Portillo, 2011). oral bioavailability varies from 25% in humans to 17% in mice(Lakhanpal & Rai, 2007) .Water solubility increases with an increase in sugar groups which in turn increases oral bioavailability(Shi et al., 2019).

A survey among the adult Chinese population reported an average quercetin intake of 20.9±2.32mg/day mainly from apples, oranges and tea, after adjustment for potential confounders, an inverse proportion exist between the intake and the prevalence of T2DM. Meta-analysis of trials involving patients with metabolic syndrome, the combined results did not show a significant reduction in glycemic parameters. However, the subgroup analysis revealed that supplementation with quercetin for at least eight weeks and a dosage of 500mg per day or higher led to a reduction in FBS (Ostadmohammadi et al., 2019). In vivo studies conducted on diabetic Wistar rats induced with streptozotocin found that administering

quercetin for 21 days improved the hyperglycemia caused by STZ (Ahmad et al., 2017). A similar model study on the impact of quercetin on pro-inflammatory markers in STZ-induced diabetic Wistar rats demonstrated a reduction of TNF-α and IL-6 (Dokumacioglu et al., 2018). Analysis of rat studies reported that quercetin effectively lowered serum glucose levels induced by STZ at dose of 50mg/kg bw. (Bule et al., 2019b).

Hypoglycaemic mechanisms of quercetin act via the liver, pancreas, skeletal muscles and small intestine(P. Portillo, 2011) as demonstrated in figure 6.

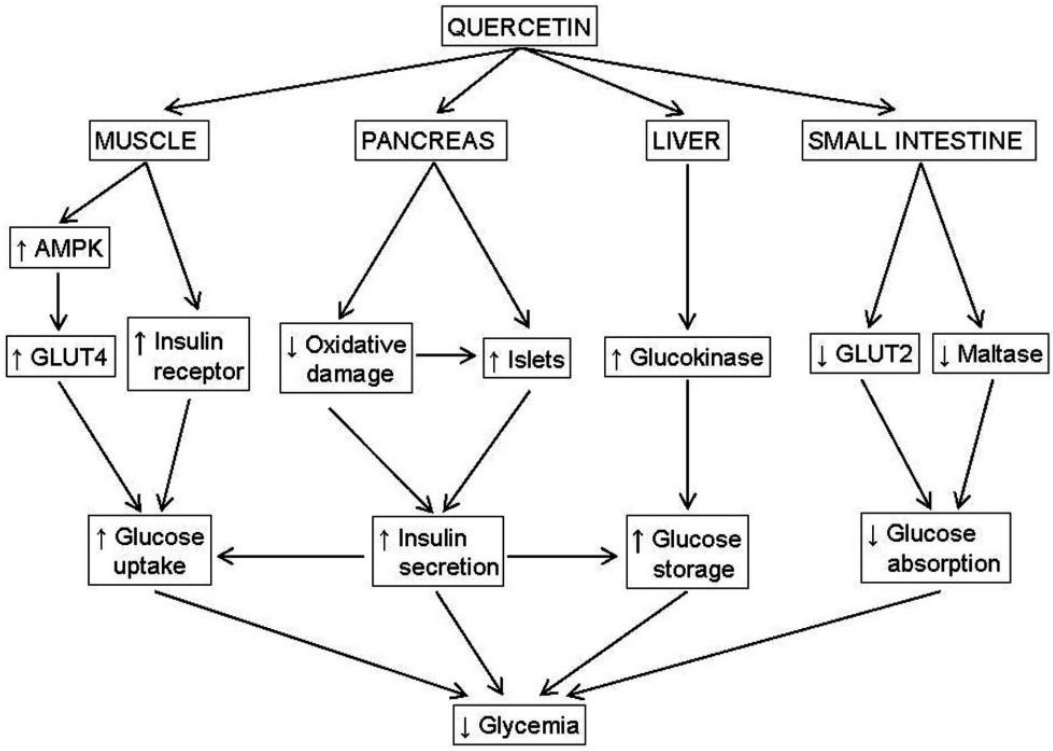

Figure 2.6: Anti-diabetic effects of Quercetin

Adapted from (P. Portillo, 2011)

In vitro studies on L6 myotubes have demonstrated that quercetin affects glucose uptake by activating the AMPK pathway. This pathway is activated by an increase in intracellular AMP: ATP, leading to the translocation of GLUT4 from the cytosol to the cell membrane. Interestingly, this pathway is not dependent on the insulin signaling pathway (Dhanya et al., 2017). In STZ-induced diabetic rats, orally administered quercetin at dose of 50 mg/kg body weight for four weeks improved glucose storage by alleviating the disrupted activity of hepatic glucose-6-phosphate and hexokinase. The same study also reported an increase in the activity of pancreatic glutathione, catalase, and superoxide dismutase (Oyedemi et al., 2020). Quercetin was found to reduce ER stress induced by lipid peroxidation in rats with T2DM.

Administration of quercetin orally for a duration of six weeks effectively reduced ER stress induced by STZ, and the effect persist even after the discontinuation of intervention (Suganya et al., 2018) The above protective effect of quercetin extends to the progeny when it is administered to lactating wistar rats (Z. Wu et al., 2014)

CHAPTER THREE
METHODOLOGY

3.1 Study Area

University of Eldoret, Kenya

3.1.1 Study Design

Randomized controlled experimental study.

3.1.2 Sample Size Determination

Glenn D. Israel's statistical formula (Israel, 1992): $n = (Z^2 1\text{-}\alpha/2 * S2) / d2$, where n is the number of animals per group. Z1-α/2 is the Z-value at the α-level of significance, which is 1.96 when α=0.05 (as per statistical tables). S is the SD of blood glucose levels for FBS and PPBS in rats, which are 3.95+/-1.31 and 5.65+/-1.63 mmol/L, respectively, as estimated by (Wang et al., 2010). with a precision of 1.6mmol/L. d is the margin of error of the mean we are willing to tolerate, set at 1mmol/L. Thus giving a sample size of 10 animals per group.

3.1.3 Hyperglycaemia in Rats

Hyperglycaemia in rats is diagnosed when there is a sustained random plasma glucose concentration of 16mmol/L or more (Lukačínová et al., 2008).

3.2 Materials

Male Wistar rats (*Rattus norvegicus Albinus*), industrial Tran's fatty acid (margarine with 50% ITFA), quercetin with purity of 95 % was purchased from Otto Chemie pvt.limited

3.3 Sampling Procedure

30 male albino Wistar rats, age 3-4 months weighing 150g – 200g were randomly selected from University of Nairobi Chiromo Campus animal house and transported to the University of Eldoret Biological Sciences Department animal house (the lab is certified for care and use of laboratory animals) and allowed to acclimatize for two weeks. The animals were then randomly assigned to the three treatment groups with ten (n=10) per group.

3.4Treatment Protocol

Each animal was weighed, given a serial number and labelled. Baseline RBS was measured with a glucometer (On Call - ACON LABS INC) and recorded. The experiment was conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines for experimental animal use and care. Three rats were housed per cage where drinking water was provided *ad libitum* with 12:12 hour day-night cycle.

3.4.1 Group 1(control group)

10 ats – were fed on a normal rat chow for six weeks and acts as the control group

3.4.2 Group 2(experimental group 1)

10 rats – were fed on a normal rat chow plus iTFA (margarine) 50kcal of the diet for six weeks to induce hyperglycemia.

3.4.3 Group 3(experimental group2)

10 rats – Will be placed on a normal diet plus iTFA(margarine) 50kcal of the diet for six weeks to induce hyperglycemia and quercetin 50mg/kg b.w is administered intragastrically twelve hourly for six weeks .

3.5 Inclusion and Exclusion Criteria

3.5.1 Inclusion Criteria

Male albino Wistar rats weighing 180g±20g and 4 months old.

3.5.2 Exclusion Criteria

Animals with RBS≥16mmol/L.

3.6 Data Collection

3.6.1Blood Glucose concentration

The baseline blood glucose concentration was measured at the outset of the study, using a glucometer (On Call - ACON LABS INC 2016 series).Subsequently, blood glucose concentration will be measured weekly throughout the study. The choice of RBS is based on the need to assess postprandial glucose concentration and minimize stress confounding associated with measuring FBS.

3.7 Blood Insulin concentration

On the commencement of the study,one milliliter of blood was drawn from the rat's tail, centrifuged at 2000 RMP for four minutes and serum stored at -20℃ freezer. All the animals were euthanized with chloroform inhalation and blood sample obtained through cardiopuncture at the end of the study.one milliliter of blood sample was centrifuged 2000 RMP for four minutes and serum rum stored at -20℃ freezer. Insulin serum concentration 4was analyzed at MTRH lab withZ insulin ELISA (Cobas 600 E601-Roche Hitachi).

3.7.1 Histological studies

The euthanized animals were dissected to obtain tissue samples of liver and pancreas.The tissues were rinsed and fixed with 10% neutral-buffered formalin for 24 hours to maintain their structure. The fixed tissues were dehydrated, embedded in paraffin wax, and cut into 3 µm sections using a microtome. These sections were mounted onto glass slides and dyed with hematoxylin and eosin for an overview of the tissue morphology. The stained sections were examined under a light microscope to identify any histological alterations. (tissue processing was done at Pathlabs,Kisumu)

3.8 Data Analysis

The assembled data was analysed using SPSS version 26.0. A repeated measures ANOVA and partial eta-squared (η^2p) from ANOVA were performed followed by Tukey's HSD post-hoc test. A confidence interval of 95% was used in all analyses.

3.9 Ethical Considerations

The study was authorized by institutional Scientific Ethic Review Committee (ISEREC), University of Eastern Africa Baraton (UEAB) and school of graduate studies, Maseno University.

3.10 Wistar rats

The selection of Wistar rats as the primary animal model in this research is underpinned by several justifications that are pivotal to the successful execution of the study. Wistar rats have a calm and docile temperament, making them easy to handle and work with in laboratory settings, and their cooperative behaviour significantly reduces stress during experiments, thereby facilitating precise data collection(Corredor et al., 2022). Furthermore, inbred strains of Wistar rats exhibit genetic stability, offering consistent genetic backgrounds crucial for controlling genetic variables within experiments thus enhancing validity and reproducibility of research outcomes(Nakanishi et al., 2015). Additionally, the adaptability of Wistar rats to diverse environmental conditions, dietary regimens, and experimental setups is essential to the study(Sudakov et al., 2021). Moreover, opting for male Wistar rats contributes to enhanced hormonal stability within the research framework by reducing hormonal variability and potential inconsistencies in the experimental results.

CHAPTER FOUR

RESULTS

4.1 Quercetin effects on random blood sugar concentration in Wistar rats

A two way repeated measure ANOVA was performed to evaluate the effect of quercetin on RBS concentration in Wistar rats. A summary ANOVA table is shown in Table 4.1 bellow. Mauchly's test indicated a violation of sphericity χ^2 =135.39,p=0.01, hence the degree of freedom corrected using Greenhouse-Geisser estimates of sphericity (ε=0.278).the effect of quercetin on RBS was significant,F(2,27)=86.322,p=<0.01, η^2p =0.865.post-hoc pairwise comparison with Tukey's HSD indicated a significant mean RBS concentration between experimental group 2, experimental group 1 and the control group (p=0.01). However, the control group and experimental group did not have any significant mean RBS concentration difference (p=0.918) as illustrated graphically in figure 4.1 and table 4.2 showing mean RBS changes among the groups from week 0 throughout to week 6 of the study.

Table 4.1: Two-way repeated measure ANOVA of RBS

Source	**df**	**F**	**sig.**
η^2p			
Between-subject effects			
Groups	2	86.322	<0.01
0.865			
Within-subject effects			
Time	1.668	61.282	<0.01
0.694			
Time*groups	3.337	52.299	<0.01
0.772			

Table4.2: Mean RBS changes from week 0 to week 6.

	Week o	Week 1	Week 2	Week 3	Week4	Week5	Week 6
Control group	4.98	4.45	4.73	4.55	4.36	4.27	4.77
Exp.group1	4.53	4.12	4.44	4.44	4.88	4.99	5.56
Exp.group2	4.87	4.35	3.87	6.08	8.04	10.68	15.29

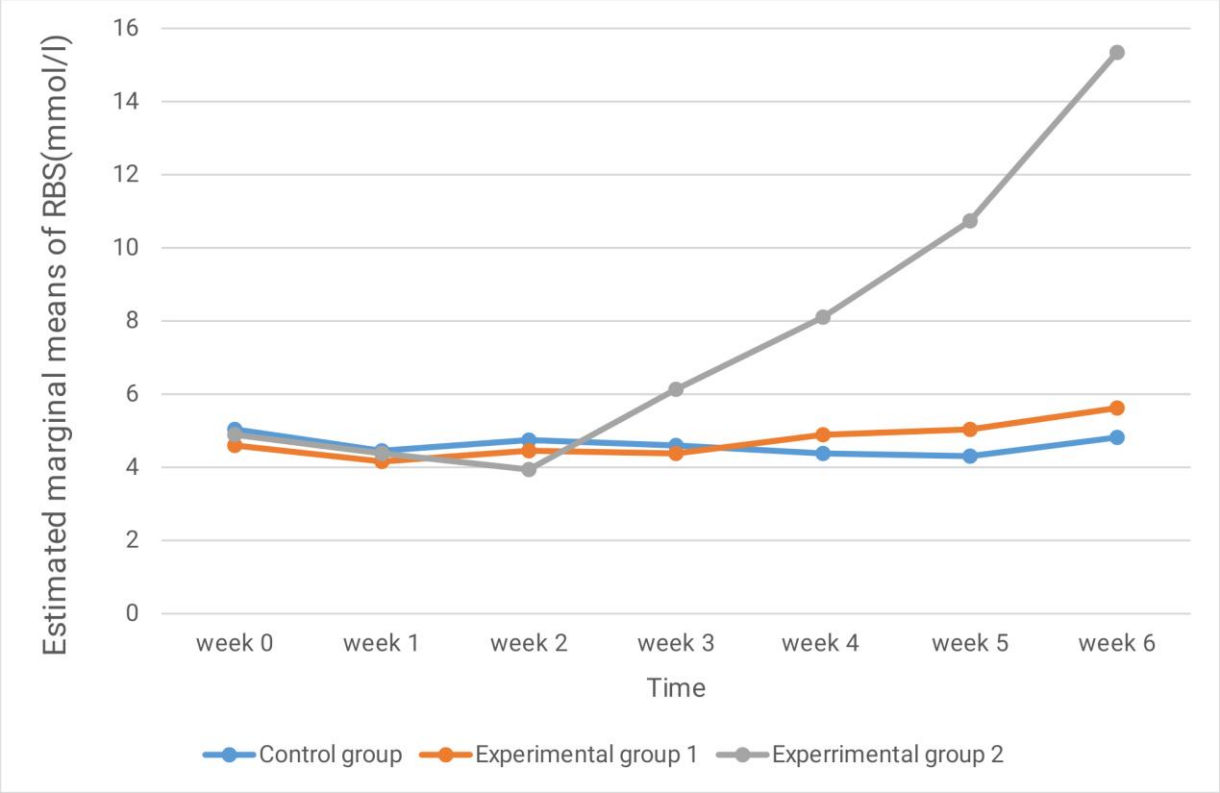

Figure 4.1: Estimated marginal means of RBS

4.2Quercetin effects on random blood insulin concentration in Wistar rats

Furthermore, similar results were observed in the evaluation of insulin concentration in response to quercetin treatment.The effect of quercetin on insulin was significant, $F(2,27)=65.613, p=0.01$, $\eta^2p = 0.829$ as shown in table 4.1 below. Table 4.2 illustrate a trend changes in mean insulin concentration at baseline and at the studys end.post-hoc pairwise comparison with Tukey's HSD indicated a significant mean insulin concentration between experimental group 2, experimental group 1 and the control group (p=0.01). However, the control group and experimental group did not have any significant mean RBS concentration difference (p=0.837) as illustrated in Figure 4.1.

Table 4.3:Two-way repeated measure ANOVA of insulin

Source	df	F	sig. η^2p
Between-subject effects			
Groups	2	65.613	<0.01 0.829
Within-subject effects			
Time	1	196.853	<0.01 0.879
Time*groups	2	152.457	<0.01 0.919

Table 4.4 Mean insulin changes between week 0 and week 6.

	Week 0	Week 6
Control group	0.2467	0.2565
Experimental group 1	0.2519	0.2652
Experimental group 2	0.2432	0.5088

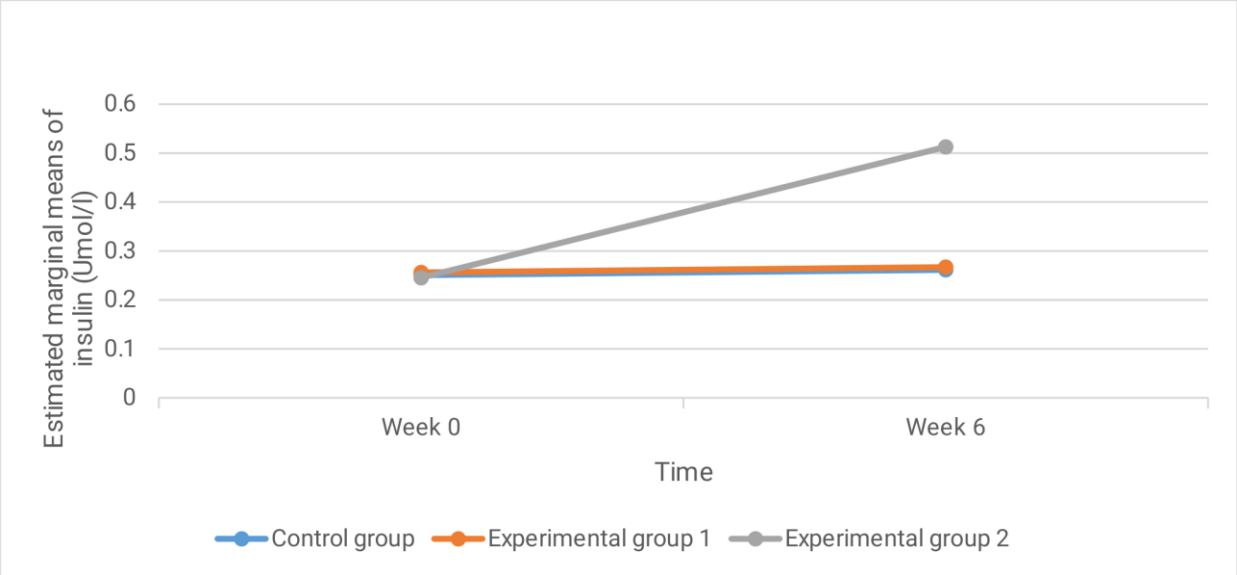

Figure 4.2: Estimated marginal means of insulin

4.3 Quercetin effects on liver and pancreas histological changes in Wistar rats

Histological analysis indicated fat deposition in the liver with foamy hepatocytes and moderate degeneration of islets of Langerhans in the pancreas of experimental group two compared to the control group and experimental group one as shown in figure 4.2 and 4.3.

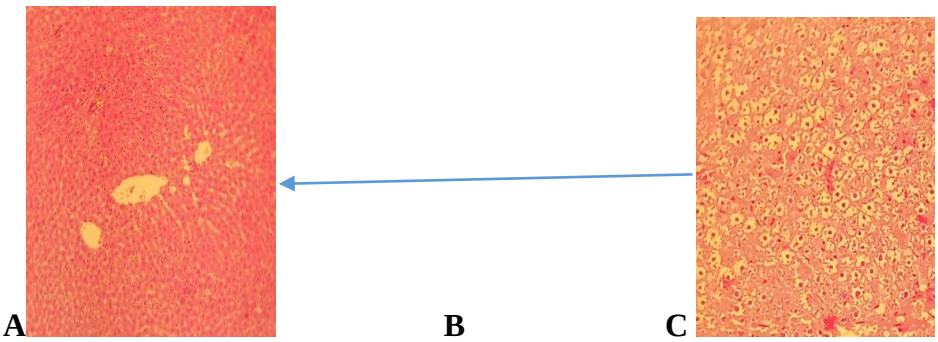

Figure 4.3: photomicrographs of the liver.

Photomicrograhs A (control group) and B (experimental group1) shows normal histology of hepatocytes and central vein while C (experimental group 2) depicts fat deposition and foamy cytoplasm of the hepatocytes

A B C

Figure 4.4: photomicrographs of the pancreas.

Photomicrographs A (control group), B (experimental group 1) shows a well distribution of equal cells in the islets of Langerhans while and C (experimental group 2). Shows scanty cytoplasm and degeneration of islets of Langerhans.

CHAPTER FIVE

DISCUSSION

Industrial trans fatty acids (ITFAs) have been identified as culprits in the slow development of pre-diabetes, attributed to dysregulated lipid oxidation, endoplasmic reticulum (ER) stress, inflammation, and oxidative stress. The trends in Random Blood Glucose (RBS) levels from baseline to week six reveal noteworthy distinctions among the three experimental groups. Initially, at the study's outset, all three groups exhibited consistent and comparable RBS levels. This uniformity at baseline served as a foundation for the subsequent observations. However, a pivotal shift in RBS trajectories becomes evident starting at week three. Notably, experimental group two experienced a substantial alteration in RBS levels, marked by the emergence of hyperglycemia. This hyperglycemia displayed a gradual escalation throughout the study's duration, signifying a progressive increase in blood glucose concentrations within this particular group.

In stark contrast, both experimental group one and the control group maintained RBS levels that closely mirrored their baseline measurements over the entire study period. This stable RBS trajectory indicated a sustained metabolic state in these groups, signifying the absence of hyperglycemia or significant fluctuations in blood glucose levels. These distinct RBS trends underscore the differential impact of the experimental conditions, particularly the consumption of industrial trans fatty acids (ITFAs), on glucose metabolism. While experimental group two displayed a consistent rise in RBS indicative of ITFA-induced hyperglycemia, experimental group one and the control group remained relatively unaffected, maintaining steady blood glucose levels akin to their initial baseline values

Normal glucose homeostasis is a precisely regulated physiological process that maintains blood glucose levels within a narrow range to ensure the consistent supply of energy to cells while avoiding hyperglycemia and hypoglycemia. In a state of equilibrium, the pancreas plays a central role, with beta cells releasing insulin in response to elevated blood glucose levels, promoting the uptake and storage of glucose in cells, primarily in the liver and muscle tissues. Concurrently, alpha cells in the pancreas release glucagon when blood glucose levels drop, stimulating the release of stored glucose into the bloodstream, allowing for glucose availability.

Initially, at the study's outset, all three experimental groups, including the control group, exhibited consistent and stable Random Blood Glucose (RBS) levels, indicative of a state of

glucose homeostasis. This initial uniformity served as a reference point for assessing subsequent changes. However, the critical juncture in the study occurred at week three when ITFA consumption began to exert its disruptive effects. Particularly, in experimental group two, exposure to ITFAs led to a pivotal disruption in glucose regulation. ITFAs, known for their detrimental impact on glucose metabolism, have been implicated in impairing insulin sensitivity, promoting inflammation, and contributing to lipid deposition in tissues.

This disruptive trend in glucose regulation continued to evolve, signifying a sustained and progressive disruption of glucose homeostasis due to ITFA consumption. These artificial trans fats appeared to perpetuate insulin resistance, impeding the ability of insulin to facilitate glucose uptake into cells and maintaining elevated blood glucose levels. In stark contrast, experimental group one which was concurrently fed qurcetin and the control group, which abstained from ITFA consumption, maintained stable RBS levels that closely mirrored their baseline measurements throughout the study period. This contrast underlines the pivotal role of ITFAs in inducing hyperglycemia and glucose dysregulation. These findings collectively emphasize that ITFA consumption is a crucial factor in the induction of hyperglycemia, disrupting the normal physiological processes that maintain blood glucose within a narrow range. The intricate and multifaceted mechanisms underlying ITFA-induced hyperglycemia include insulin resistance, inflammation, lipid accumulation, and potential mitochondrial dysfunction, all of which collectively contribute to glucose dysregulation in the presence of ITFAs.

At the inception of the study, all three study groups exhibited equivalent and consistent insulin concentrations, establishing a stable baseline against which subsequent variations would be assessed. However, as the study advanced, a significant alteration in insulin dynamics came to the forefront, leading to distinctive discrepancies among the groups by the study's culmination.

By the study's end, a significant disparity materialized. Experimental group three, which had been subjected to ITFA exposure, presented a notably heightened insulin concentration in comparison to both the control group and experimental group one, which were not exposed to ITFAs. This disparity in insulin levels suggests a substantial increase in insulin secretion within experimental group three, serving as a compensatory response to the hyperglycemia triggered by ITFAs. The elevated insulin levels within this group are indicative of

hyperinsulinemia, a mechanism often observed as a compensatory measure to counteract the elevated blood glucose levels linked with insulin resistance.

In contrast, experimental group one, treated with quercetin, maintained insulin levels akin to those observed at the study's outset. This observation underscores the effectiveness of quercetin in averting the development of hyperglycemia without necessitating an escalation in insulin secretion. This outcome is of significant importance, as it suggests that quercetin's mechanisms of action do not rely on stimulating the pancreas to produce additional insulin. The prevention of hyperglycemia without an accompanying elevation in insulin levels positions quercetin as a distinctive and effective agent in maintaining insulin equilibrium in the context of ITFA-induced metabolic challenges. The heightened insulin concentration in response to ITFAs within experimental group three signifies the presence of hyperinsulinemia as a compensatory mechanism. Conversely, quercetin treatment in experimental group one adeptly averted the development of hyperglycemia without necessitating increased insulin secretion, highlighting its unique role in preserving insulin balance. These findings further underscore the distinct mechanisms of action of quercetin within the realm of glucose regulation and insulin dynamics, presenting it as a potential shield against the complications of ITFA-induced hyperglycemia without invoking compensatory hyperinsulinemia.

The histological analysis revealed distinct structural alterations, notably in experimental group two. These findings carry essential implications in understanding the contrasting effects of ITFAs and the protective actions of quercetin on tissue integrity and overall metabolic health.In the liver, the histological examination unveiled the presence of fat deposition and the occurrence of foamy hepatocytes in experimental group two. This histological evidence aligns with the established understanding that ITFAs can contribute to lipid accumulation within hepatic tissues, a characteristic hallmark of NAFLD. Such lipid deposition in the liver can potentially disrupt its normal functionality, particularly concerning the regulation of glucose and lipids, which are pivotal to overall metabolic health.

Within the pancreas of experimental group two, the histological analysis revealed moderate degeneration of the islets of Langerhans. These islets are home to the pancreatic beta cells, responsible for the synthesis of insulin. The degeneration of these islets is a critical observation, as it indicates structural impairments that could compromise the pancreas's insulin-producing capacity. Given the central role of insulin in glucose regulation, these

structural changes in the pancreas align with the observed hyperglycemia and insulin imbalance in this study.

When viewed in the broader study context, these histological findings underscore the adverse impact of ITFAs on tissue integrity, particularly in the liver and pancreas. The fat deposition and foamy hepatocytes within the liver signify the potential of ITFAs to disrupt hepatic function, thereby impacting both lipid and glucose metabolism. Additionally, the degeneration of the islets of Langerhans in the pancreas reinforces the link between ITFA consumption and insulin imbalance.

Conversely, the protective effects of quercetin come into sharp relief. In experimental group one, where quercetin was administered, these histological alterations in the liver and pancreas were conspicuously absent. Quercetin's mechanisms, including its role in mitigating inflammation, reducing endoplasmic reticulum stress, and acting as an antioxidant, likely contribute to the preservation of tissue integrity in these organs. By shielding the liver and pancreas from the structural impairments associated with ITFA consumption, quercetin emerges as a pivotal guardian in maintaining metabolic health.

In summary, the histological findings within the liver and pancreas of experimental group two accentuate the adverse effects of ITFAs on tissue integrity and their role in promoting conditions like NAFLD and pancreatic islet degeneration. These structural alterations closely correspond with the observed hyperglycemia and insulin imbalance, further highlighting the interconnectedness of tissue integrity, glucose regulation, and insulin dynamics. The protective role of quercetin, as demonstrated in experimental group one, offers a compelling counterpoint by preserving the structural integrity of these vital organs, underscoring its potential as a safeguard against the metabolic challenges induced by ITFAs.

The property of quercetin in prevention of hyperglycemia development induced by ITFAs is consistent with other studies that demonstrated its protective effects against STZ induced hyperglycemia (Dokumacioglu et al., 2018). Additionally,the findings on hyperinsulinemia prevention is invariable with other animal model experiments induced by alloxan.The possible underlying mechanism could be through activation of the AMPK pathway, facilitating the translocation of GLUT4 from the cytosol to the cell membrane independently of the insulin signaling pathway(Dhanya et al., 2017). Moreover, quercetin protects the histological integrity of the liver and pancreas against ITFAs toxicity perhaps via the activity of key pancreatic antioxidants such as glutathione, catalase, and superoxide dismutase

(Oyedemi et al., 2020) . In the case of Quercetin, it also plays a pivotal role in reducing ER stress induced by lipid peroxidation in rats with Type 2 Diabetes Mellitus (Suganya et al., 2018).

Collectively, these mechanisms enhance glucose disposal while safeguarding the integrity of pancreatic β cells. This dual action of Quercetin not only prevents pre-diabetes but does so without necessitating compensatory hyperinsulinemia.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, quercetin demonstrates a preventive effect on hyperglycemia triggered by ITFAs, highlighting its potential as a therapeutic agent in managing glucose homeostasis in the presence of these harmful fatty acids. Secondly, the compound exhibits a protective influence against hyperinsulinemia, a condition often linked to the elevated blood sugar levels induced by ITFAs. This suggests that quercetin not only addresses the immediate glycemic impact but also contributes to maintaining insulin sensitivity in the context of ITFA-induced metabolic disturbances. Furthermore, the study reveals that quercetin plays a crucial role in preserving the histological integrity of vital organs such as the liver and pancreas in the face of ITFA toxicity.

6.2 Recommendations

1. Human trials on prediabetes high risk population
2. Human trials on a population with obesity but without metabolic syndrome
3. Human trials on a high risk population for Non-alcoholic fatty liver disease(NAFLD).

6.3 Study limitations

1. Blood sample collection from rat tail is time consuming due to small size of the animals
2. Regular relebaling of animals due to oily fur

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APPENDICES

APPENDIX 1 BUDGET ESTIMATES

ITEM	QUANTITY/NUMBER	PRICE/UNIT COST	TOTAL COST[ksh]
Wistar rats	30 Animals	@1000	30000
Glucometer	1	@2000	2000
Glucometer strips	4*25's	@2000	8000
Rat food pellets	3*5kg	@3000	9000
Microfine syringes with needles	1*100's	@2000	2000
ITFA(Margarine)	1*500g	@400	400
Quercetin powder	1*500g	@3000	3000
Clean drinking water	5*5l	@500	2500
Invitro-anticoagulant[EDTA] tubes	10ml*100	15	1500
Blood tubes without anticoagulant	10ml*100	15	1500
Sample analysis cost			64600
Weighing balance[Digital]	1	4000	4000
Labour costs	2*assistants	@10000	20000
Subsistence costs			30000
Total cost			150000