Evaluation of Antidiabetic Potential of Whole Camel Milk and Camel Whey Protein in Streptozotocin Induced Diabetic Rats

By: Zainab Sohail
Registration No: NUST00000328609
MS Industrial Biotechnology

Supervised by:
Dr. Tahir Ahmad

Atta-Ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan
2022

Evaluation of Antidiabetic Potential of Whole Camel Milk and Camel Whey Protein in Streptozotocin Induced Diabetic Rats

By: Zainab Sohail
Registration No: NUST000003208609

A Thesis submitted in partial fulfilment of the requirement for the degree of
MS Industrial Biotechnology

Supervised by:
Dr. Tahir Ahmad

Atta-Ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan
2022

FORM TH-4

National University of Sciences & Technology

MS THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by: **Zainab Sohail Reg No: NUST00000328609**

Titled: **Evaluation of Antidiabetic Potential of Whole Camel Milk and Camel Whey Protein in Streptozotocin Induced Diabetic Rats** be accepted in partial fulfillment of the requirements for the award of **MS Degree in Biosciences** degree with (A grade).

Examination Committee Members

1. Name: **Dr. Abdur Rehman** Signature: _____

Dr. Abdur Rahman
Assistant Professor
Atta-Ur-Rahman School of Applied Biosciences (ASAB)
NUST, Islamabad

2. Name: **Dr. Salik Javed Kakar** Signature: _____

Associate Professor
DR. SALIK J. KAKAR
MBBS, MSc (UK), PhD (UK)
Dept. of Healthcare Biotechnology
ASAB, NUST, Islamabad

3. Name: **Dr. Yasir Waheed (External)** Signature: _____

Supervisor's name: **Dr. Tahir Ahmad** Signature: _____

Dr. Tahir Ahmad
Associate Professor
Dept. of Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Date: _____

Dr. Amjad Ali, PhD
Head of Department (HoD)
Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Head of Department

28-2-23

Date

COUNTERSINGED

Date: 28-2-23

Dr. Hussnain A. Janjua
Principal
Atta-ur-Rahman School of Applied Biosciences (ASAB)
NUST, Islamabad

Dean/Principal

THESIS ACCEPTANCE CERTIFICATE

Certified that final contents and form of MS/MPhil thesis entitled **"Evaluation of Antidiabetic Potential of Whole Camel Milk and Camel Whey Protein in Streptozotocin Induced Diabetic Rats."** composed by Zainab Sohail, (Registration No. 00000328609), of ASAB has been verified by undersigned, observed total in all regards according to NUST Status/Regulations, is free of plagiarism, blunders and botches and is acknowledged as partial fulfilment for grant of MS/MPhil degree. It is additionally ensured that fundamental revisions as called attention to by GEC individuals from the researcher have likewise been joined in the said proposal.

Dr. Tahir Ahmad
Associate Professor
Deptt of Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Signature: _____

Supervisor: Dr. Tahir Ahmad

Date: 14/3/23

Dr. Amjad Ali, PhD
Head of Department (HoD)
Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Signature (HOD): _____ 14/20/23

Date: _____

Dr. Hussnain A. Janjua
Principal
Atta-ur-Rahman School of Applied Biosciences (ASAB)
NUST Islamabad

Signature (Dean/Principal): _____

Date: 14/3/23

CERTIFICATE FOR PLAGIARISM

It is to confirm that MS thesis entitled **"Evaluation of Antidiabetic Potential of Whole Camel Milk and Camel Whey Protein in Streptozotocin Induced Diabetic Rats."** by Ms. Zainab Sohail; Registration No. 00000328609 has been examined by me. I undertake that,

a) Thesis has significant new work/information when contrasted with as of now somewhere else. No Sentence, table, condition, chart, passage, or segment has been replicated exact from past work with the exception of when put under quotes and appropriately referred to.

b) The work introduced is unique and own work if the creator for example there is no copyright infringement. No thought, results or expressions of others have been introduced as creator's own work.

c) The postulation has been actually looked at utilizing Turnitin duplicate of the creativity report appended and found inside the cutoff points according to HEC plagiarism strategy and guidance gave now and again.

Dr. Tahir Ahmad
Associate Professor
Dept. of Industrial Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST Islamabad

(Supervisor)

Dr. Tahir Ahmad

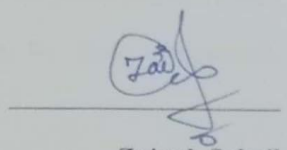
Associate Professor

Industrial Biotechnology

ASAB, NUST

DECLARATION

I, Zainab Sohail, declare that all of the work presented in this thesis is my own. I confirm that any information obtained from other sources has been mentioned in the thesis. The work presented here was completed while I was a postgraduate student at NUST Atta-ur-Rahman School of Applied Biosciences, working under Dr. Tahir Ahmad's supervision.



Zainab Sohail

00000328609

ACKNOWLEDGEMENT

Nothing except Almighty **"ALLAH"** the most benign, compassionate, and generous is deserving of love. All praise is due to Him. All praise to **HIS HOLY PROPHET HAZRAT MUHAMMAD (PEACE BE UPON HIM),** who taught me the proper road and enabled me to understand my maker's unity. As envisioned, this study succeeds due to the generosity of various businesses and individuals who deserve public acknowledgement for their compassion, assistance, and support throughout the examination.

To begin, I would like to convey my heartfelt appreciation to my meritorious supervisor, **Dr. Tahir Ahmad.** He has been a constant source of encouragement since I began working on this project. I am eternally grateful to him for his masterful supervision, gifted exhortation, liberal analysis, and considerate demeanor, without which it would have been impossible to complete this examination task. I wish to acknowledge my deep sense of profound gratitude to GEC members, **Dr. Salik Javaid Kakar**, **Dr. Abdur Rahman** and **Dr. Bashir Ahmad** for their valuable suggestions and continuous encouragement throughout the course of research.

I would acknowledge the well wishes and support of my colleagues and friends who remained along my side throughout this journey. Especially Shaheer Shafiq, Fareeha Iqbal, Ayesha Farooq, Kousain Saif and Iqra Mahnoor.

My acknowledgement would be incomplete without mentioning my Parents (**Sohail Sarwar and Shagufta Chaudhary**) and siblings (**M**. **Abdullah Bin Sohail and Ishmal Sohail**) their golden heartbeats with golden sentiments exhibited a prolonged patient for my study, their hands always raised in prayers for my success. I consider myself the luckiest to have such a supportive family, standing behind me with their love and support. I extend deep emotions of appreciation, gratitude, and indebtedness for their guidance.

I would like to extend my deepest thanks to the **Faizan Sethi** who motivated me the most after my parents and helped me in my MS journey. His faith in me brought the courage to rise up. I am grateful for his kind and never-ending support during all my research and personal problems.

May Allah bless all of them with long, happy and peaceful lives, **Ameen.**

Zainab Sohail

DEDICATION

This thesis is dedicated to

ZAINAB SOHAIL

For her hard working and never giving up attitude.

Table of Contents

List of Table

List of Figures

LIST OF ABBREVIATIONS

T2DM	Type 2 Diabetes Mellitus
IR	Insulin Resistance
CVD	Cardiovascular Disease
ROS	Reactive Oxygen Species
CM	Camel Milk
CWP	Camel Whey Protein
Lf	Lactoferrin
α-La	α-Lactalbumin
SA	Serum Albumin
GI	Glycemic Index
H&E	Hematoxylin and Eosin
β-Lg	β-Lactoglobulin
Igs	Immunoglobulins
HBG	High Blood Glucose
INSR	Insulin Receptor
PIK3	Phosphoinositol-3-Phosphate
AGE	Advanced Glycation End-product
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
STZ	Streptozotocin

Abstract

Diabetes mellitus (DM) is a set of chronic illness characterized by high blood glucose level and misfunctioning of pancreatic β-cells. Insulin is a protein hormone used as pharmacological strategy for the treatment of T2DM, but regular use of insulin makes the body resistant to the defined dose, and it has to be increased with time. It has been used intraperitoneal and subcutaneously by many years to reduce blood glucose level. Camel milk (CM) and its product, camel whey protein (CWP), has been thought to have antidiabetic property. In this study, camel milk and camel whey protein has been used for the treatment of type 2 diabetes. SEM and FTIR were performed for the characterization of camel whey protein. In in-vivo analysis, streptozotocin was induced in normal wister albino rats. On induction, they showed a hyperglycemic effect with $P<0.0001$ for all groups. To analyze the effect of camel milk and camel whey protein, the lipid profile, liver, and kidney function tests were performed that showed significant effect on diabetes. the treatments showed the significant reduction in the blood glucose, ALT, ALP, creatinine, uric acid, TCHO, TG, LDLC and MDA levels with all P-values of <0.0001 except uric acid with P-value 0.0008. Similarly, increase in albumin, HDLC, SOD and CAT was observed with P-value <0.0001 (except for albumin, CM=0.0044, CWP=<0.0004). Also, the histopathological analysis of the renal and hepatic tissue showed the revert effect by using CM and CWP. The results of current study show that CM and CWP has a strong hypoglycemic effect due to unique components present in CM and CWP.

Chapter 1

Introduction

Introduction

1.1 Background

One of the most growing health issues all over the world is the Type 2 Diabetes Mellitus (T2DM). It is characterized by the elevated blood glucose level (hyperglycemia) that is caused by the dysfunctioning of β-cells that aggravates insulin resistance (IR) (Kilari *et al.,* 2021). Complications associated with the T2DM are the oxidative stress, hyperlipidemia, organ dysfunction and the cardiovascular diseases (CVD) (Sayed *et al.,* 2017). In T2DM, increase in blood glucose levels (BGL) is related to the increase in reactive oxygen species (ROS) that leads to oxidative stress (Asmat *et al.,* 2016). Also, it imbalances the normal body functioning by affecting the liver enzymes, kidney enzymes and lipid profile (Kilari *et al.,* 2021). Therefore, to manage these circumstances, the normalization of the blood glucose levels will be the first priority.

Various treatments associated with the management of T2DM consists of pharmacological and non-pharmacological strategies. In addition to improving lifestyle choices, pharmacological strategies that uses specific BGL lowering medications is essential for maintaining glycemic control (Khursheed *et al.,* 2019). Pharmacological strategies include the insulin secretagogues, insulin sensitizers, and α-glucosidase inhibitors (Grossman *et al.,* 2018). Insulin, the most commonly used as the antihyperglycemic agent for the treatment of T2DM (Khursheed *et al.,* 2019). It is a hormonal protein that is found to be effective towards T2DM but it requires to be injected in an accurate amount as up or low amount may be life-threatening (Davis-Richardson & Triplett, 2015). Secondly, once someone starts insulin, he should have to take it permanently and its dose increases over time as the body starts to become resistant over

the defined dose (Abiola *et al.,* 2016). Whereas non-pharmacological strategies include exercise, diet and weight loss. However, people will always want the medication with minimal or negligible side effects (Grossman *et al.,* 2018). To prevent and treat T2DM, the American Diabetes Association (ADA) has mostly recommended nutritional measures (Abiola *et al.,* 2016). One of the most intriguing areas of research and innovation in the food industry is functional foods, and their potential application to the treatment of diabetes is particularly intriguing (Venkatakrishnan *et al.,* 2019).

The existence of physiologically active peptides derived from food proteins, among other food components, is strongly supported by scientific literature, which has considerable positive effects on human health, particularly diabetes (Nongonierma *et al.,* 2018). For this objective, milk proteins are found to have to biologically active peptides that have the potential to combat against many diseases. To more precision, camel milk is found to be more beneficial toward many diseases because of unique properties. Camel milk (CM) is found to have low cholesterol, low glucose, high insulin content, high mineral contents, high amount of vitamin C and high amount of iron containing protein (Agrawal *et al.,* 2004). It also contains large number of polyunsaturated acids and volatile compounds that are found to be essential for human nutrition. Camel milk is found to be effective towards jaundice, tuberculosis, asthma, pile, dropsy, and diabetes (Brezovečki *et al.,* 2015).

CM and its product-whey protein (WP) is thought to contain insulinotropic properties that makes insulin-like proteins to get stored in the stomach and efficiently absorb in the bloodstream to reach the target (Shori, 2015). The unique property of CM is that it doesn’t form coagulum in the acidic environment and has a good buffering capacity

(Shori, 2015). It contains roughly 52 micro unit/ml of insulin-like protein compared to cow milk's 16.32 micro unit/ml with plays the important role in insulin interaction with the receptor. High amount of zinc in CM also plays an important role in insulin secretory activity in β-cells (Malik *et al.,* 2012). So, it has been a common practice there to recommend camel's milk for the general treatment of diabetes which predicts that along with all other medicinal benefits of camel milk has also hypoglycemic effects.

Moreover, the camel whey protein (CWP) produced from the CM has also been reported to have antidiabetic effect but its potential towards T2DM was underreported. It is well known that CWP include more physiologically active proteins than their bovine counterparts. All of the essential and non-essential amino acids are present in CWP, which is also a great source of the branched-chain amino acids and glutamine required for cell growth. Other immunomodulatory proteins found in camel whey include lactoferrin (Lf), serum albumin (SA), α-lactalbumin (α-La), and peptidoglycan. These proteins occur naturally and are a component of the primary sequence of WP. In a streptozotocin (STZ)-induced type 2 diabetic rats, CWP supplementation has been shown to have positive antidiabetic effects by reducing free radicals, increasing antioxidant levels, and coordinating redox state, so restoring blood glucose and insulin levels. Whey increases the body's antioxidant activity, fights fatigue and inflammation, improves healing, enhances stamina, and may prevent infections owing to the immune system-stimulating and naturally antibacterial properties of its components.

Low glycemic index (GI) diets are found to be effective towards T2DM. Thus, the addition of WP to the diet is found to lower the GI (Jakubowicz & Froy, 2013). CWP is known to contain insulinotropic properties as compared to camel casein (Pal & Ellis,

2010). The insulinotropic response of CWP may be mediated by the high concentration of essential amino acids produced after digestion. The most likely amino acids have been identified as leucine, isoleucine, valine, lysine, and threonine in particular are known to increase the insulin secretion. Therefore, it is found to lower the BGL, improves lipid profile, renal and hepatic parameters (Jakubowicz & Froy, 2013).

1.2 Problem Statement

With the aforementioned studies in perspective, the current study was created to explore the effect of whole camel milk and camel whey protein as the use of insulin has many of the disadvantages discussed before.

1.3 Aims and Objectives

i. Preparation and characterization of camel whey protein from the whole camel milk.
ii. Induction of Streptozotocin in wister albino rats and the treatment with the whole camel milk and camel whey protein.
iii. Comparative analysis of whole camel milk and camel whey protein by biochemical analysis of different parameter.
iv. Determining the effect through histopathological examination using Hematoxylin and Eosin (H&E) stain.

Chapter 2

Review of Literature

Review of Literature

2.1 Dromedary Camels: Taxonomy & Geographical Distribution

Any member of the family Camelidae, including Old World Camels (OWC) and New World Camels (NWC), are referred to as camels. The very first ancestor of Camelid family was about to found in Northern America back to 40 million years ago (Sazmand *et al.*, 2019). The family Camelidae is categorized into three genera: genus Camelus, genus Lama and genus Vicugna. The three species of camels are the dromedary camels (94%), Bactrian camels (4%) and the Wild Bactrian camels (endangered). One of them, the one-humped dromedary camels are native to hot, dry, and semi-arid regions of Northern and Eastern Africa, Western Asia, and Australia (Khalafalla *et al.*, 2015). Dromedaries' distinctive physiological characteristics, including their circulatory and respiratory systems, water economy mechanism, heat tolerance, etc., makes them to live a week even without water and food (Sazmand *et al.*, 2019).

The dromedary camels were highly domesticated to the South Arabian Paninsula for the milk production, meat, hides and transportation. Later were introduced due to the spice trade to the Iran, Pakistan, India, North and Horn of Africa (Bahbahani & Almathen, 2022). They provide people with highly nutritional meat and milk. In the whole thesis, the term “camel” will refer to the “dromedary camel”.

2.2 Milk Production and Camels

The global camel population is estimated to be 35 million by 2021 and plays an important role as a milk producer in different areas of the world i.e., Africa (North and East), Asia. Camels produce milk for 8 to 18 months, reaching their peak output in the second or third

month of lactation. They have the longer period of lactation as they have higher potential of milk production genetically. The dromedary camels (one-humped) are the beloved companions and produces milk even at high temperatures and scantiness of food and water. These dromedary camels can produce up to 10 liters of milk a day and are considered to be the best milk produces with the annual production of about 4179 liters (Faraz *et al.,* 2018). But the yield may vary with the change in various factors that include genetic variations between different species of camels, feed, management system, water availability, age of camel and lactation period (Bs, 2018).

Due to their exceptional capacity to sustain their average daily milk supply over an extended length of time, dromedaries offer a great deal of potential as milking animals (Bs, 2018). The majority of dromedary camels (single humped), as well as a few herds of Bactrian camels (double humped), are located in Pakistan's northern regions and produces 15-20 liters of milk per day when they are fed and drunk properly. Camel milk is a good source of minerals, vitamins, and fats, particularly vitamin C. Because it is high in phosphorus, CM is superior to the milk of other domestic species in many ways. CM has a longer shelf life as a result of the reason for having more protein (Faraz *et al.,* 2013).

2.3 Composition of CM

CM is a complete diet with all the important nutritional components present in it. It incorporates a relatively strong protection system versus milk from other species. Cow milk and camel milk have different compositions, levels of protein, and structures (Akindykova *et al.,* 2019). CM is considered to be most important source of proteins. CM is opaque white in appearance with the sweet and salty in taste and sours slowly when

placed at room temperature compared to the other milk types. The pH of CM ranges from 6.3 to 6.7 with an average pH of 6.4 comparatively lower than the cow's milk. The CM is composed of various nutrients including proteins, fats lactose, ash, vitamins, unsaturated fatty acids, low levels of cholesterol (Mohammadabadi, 2019). The major composition of CM is relatively similar to that of bovine milk but shows the low number of total solids about 11.8% than the bovine milk that is about 12.33%. Also, the CM contains low amount of fats that becomes beneficial when consumed by humans. The compositional factors of the milk are influenced by various factors i.e., environment, geographical area, analytical methods, nutrition, age of mammal, lactation period etc. (Brezovečki *et al.*, 2015).

Due to its smaller milk-fat globules and hypoallergenic qualities, CM is mostly recognized for its improved digestion in the human gastrointestinal tract. CM doesn't contain β-lactoglobulin (β-Lg). The major component of whey protein present in CM are α-La which is only 25% in other types of milk. The CM contains low amount of lactose that's why its easily digestible and can be consumed easily by lactose intolerant people. It contains higher levels of monounsaturated fatty acids (MUFA), vitamins and minerals compared to cow's milk (Rahmeh *et al.*, 2019).

Table 1: The chemical composition and its ranges in camel milk

Chemical composition and its ranges in CM	
Water	86-91%
Fats	1.8-4.3%
Protein	2.0-3.2%
Lactose	3.3-5.4%
Total solids	7.7-12.1%
Ash	0.8-1.0%
Vitamin C	3-5 times higher than bovine milk and 1.5 times higher than human milk
Vitamin A	Twice of cow's milk
Vitamin D	5ug/day by 2 cups of milk uptake
Riboflavin	0.5mg/day
Zinc	55% more than other types of milk.

2.4 Properties of CM

CM is renowned not only because of its physiochemical properties. In earliest times, due to significant health benefits of CM and its fermented derivatives, it is known to consumed much by the people:

i. CM has a reputation for helping young children's developing bones.

ii. It has a potential to improve β-cells function in the pancreas due to the immunoregulatory effect.

iii. It also reduces the demand for insulin to the body.

iv. Also, In the treatment of liver diseases, viruses that cause diarrhoea, TB, and gastrointestinal ulcers.

v. It performs the activity of inhibition of angiotensin I-converting enzyme that is the major regulator of blood pressure (BP).

vi. Jaundice, ailments of the lungs and spleen, asthma, anaemia, autism, edoema, milk allergies, and autoimmune dermatological diseases can all be treated with CM.

vii. CM also provides strong innate immunity that refers to the natural resistance towards infectious diseases.

viii. Many beneficial chemicals and antimicrobials are thought to be abundant in CM.

ix. It is thought to be a potential source of new bioactive chemicals and probiotics (Rahmeh *et al.,* 2019).

2.5 Components of CM

According to literature, the CM contains two major proteins: Casein protein (CP) and whey protein (WP). These two proteins have their own important properties and functions in the body. It contains high number of amino acids except glycine, threonine, lysine and valine. The amount of protein present in the CM is about 2.0-3.2%. The overall content of the proteins present in the milk varies with the change in season and breed. This protein content is found to be lower in August and higher in December and January (Zibaee *et al.,* 2015).

2.5.1 Casein Protein (CP)

The main component of CM is found to be casein that accounts for about 61-88% of the total protein content. The components of camel casein (CNs) are similar to those of bovine casein: αS1-CN, αS2-CN, β-CN and k-CN. The primary casein in CM is β-CN that is about 65% of the total casein present in camel milk that makes it better digestible (Mati *et al.,* 2017). CM contains casein micelles with an average diameter of 260–300 nm as opposed to 130 nm in cow milk, which is twice as large. Presence of amino acids in the milk makes effective for the growth and development of young bones (Brezovečki *et al.,* 2015). Its amino acid composition seems to be similar to that of cow's milk except for the glycine and cysteine, that is found to be lower in camel's milk.

2.5.2 Whey Protein (WP)

WP is 20-25% of the total milk protein and contains a number of biologically active ingredients i.e., α-lactalbumin (α-La), serum albumin (SA), lysozyme, lactoferrin (Lf), peptidoglycan recognition protein, lactoperoxidase and immunoglobulins (Ig). It also contains all the necessary ingredients necessary for the growth of the cell. Unlike bovine milk, CM is deficient in β-lactoglobulin (Jansson *et al.,* 2020). But contains relatively two to three times higher amount of Lf and Ig. It also contains bioactive peptides that plays the role in many physiological processes (Mudgil *et al.,* 2022).

The bioactive peptides in whey protein bind to receptors in the intestinal lumen prior to absorption or in target organs after bloodstream absorption to carry out their function. Peptide transporters can carry larger peptides, while smaller peptides with fewer than four residues can pass through intercellular junctions and enter the bloodstream. Their sensitivity to brush boundary peptidases controls the rate of transport. Many bioactive

peptides have been discovered, including those with immunomodulatory and antibacterial properties as well as those that inhibit the angiotensin converting enzyme. But more research needs be done to find bioactive peptides that have metabolic functions (Jakubowicz & Froy, 2013).

In addition, As caseins are broken down enzymatically, whey may contain low-molecular-weight molecules and protease-peptone components. As precursors to the potent intracellular antioxidant glutathione and as components of one-carbon metabolism, sulphur amino acids in WP are essential as antioxidants. WP contains all 20 amino acids as well as nine amino acids necessary for human health (Birsen Bulut Solak & Nihat Akin, 2012).

The whey to casein ratio is higher in CM but its ratio is lower than human milk. Due to the fact, the CM coagulum is softer than the bovine milk (Bs, 2018). CW is white in appearance due to the high concentration of small size casein micelles and fat globules and lesser amount of riboflavin. The proteins present in CM at sensitive to high temperature because it doesn't contain β-Lg. But WP portion of the CM is thermally more stable to the high temperature as compared to the bovine milk. Also, the whey of the camel milk is relatively more prone to acidity than the bovine milk (Brezovečki *et al.*, 2015).

2.6 Functional and Biological Properties of CWP

The absence of β-Lg in CW makes it thermal stable while lyophilization compared to the bovine milk. The presence of large number of α-La in the CW makes it sensitive to solubility due to the change in pH. The SA present in the camel whey is less sensitive to the heat compared to the other types of whey. The α-La and SA are the two components

of CW that gives the animal the foul properties. The solubility of camel whey is less due to the absence of α-Lg and small amounts of k-casein. At 65°C, the camel milk loses the Ig activity whereas lysozymes and Lf are stable at this temperature. At the isoelectric point (4.5 pH), camel milk whey becomes less heat stable due to decreased solubility brought on by a reduction in the electrostatic repulsion between proteins (Elkot, 2019).

Due to its immune system-boosting and natural antibacterial qualities, whey also raises the body's antioxidant activity, fights fatigue and inflammation, speeds up healing, improves endurance, and may prevent infections. It also plays a vital role in the growth of cells due to the presence of essential and non-essential amino acids (Sayed *et al.,* 2017). WPs feature the immune-enhancing properties because of unique biologically active components. It is believed that WPs works by internally converting the amino acid cysteine to glutathione, a powerful intracellular antioxidant (Allam *et al.,* 2015).

WP is known to have many biological properties i.e., antimicrobial and antiviral activity, Immunomodulatory Behavior, anticarcinogenic properties, cardiovascular health, antidiabetic properties, physical performance, weight management and bone health (Birsen Bulut Solak & Nihat Akin, 2012). The iron-binding ability of α-La, β-Lg, Lf, lactoperoxidase, SA, and lysozyme is responsible for the antimicrobial activity of whey products (Tovar Jiménez *et al.,* 2012). The presence of various bioactive components in CWP has been linked to these possible health advantages.

2.7 Whey Protein and Diabetes

2.7.1 Diabetes Mellitus

Diabetes mellitus (DM), like osteoporosis, Cushing's syndrome, is a set of metabolic illnesses characterized by high blood glucose levels (HBG) and an insufficient amount of

insulin being made by the pancreas inside the body or acting as intended (Asmat *et al.,* 2016). It is a huge global public health problem affecting over 400 million individuals (Khursheed *et al.,* 2019). This metabolic condition develops into chronic, life-threatening microvascular, macrovascular, retinopathic and neuropathic consequences over time (Padhi *et al.,* 2020). The prime manifestation of this endocrinological disorder is the hyperglycemia in blood (Deepthi B *et al.,* 2017)

2.7.2 Classification of DM

DM is classified into four main categories based on the etiology and presentation of disease that are as follow:

i. Type 1 Diabetes Mellitus(T1DM),
ii. Type 2 Diabetes Mellitus (T2DM),
iii. Gestational Diabetes Mellitus (GDM)
iv. and the type of diabetes caused or connected to specific diseases, syndromes, or situations (Secondary Diabetes) (Banday *et al.,* 2020)

2.7.2.1 Type 1 Diabetes Mellitus

It is commonly known as the Insulin Dependent Diabetes Mellitus (IDDM) marked by the damage of β-cells being mediated by the T-cells resulting in high level of glucose in the blood (Banday *et al.,* 2020).

2.7.2.2 Type 2 Diabetes Mellitus

It is heterogenous disorder distinguished by a lack of insulin need to avert ketoacidosis. This happens due to the malfunctioning of the β-cells that causes IR (Ozougwu, 2013).

2.7.2.3 Gestational Diabetes Mellitus

GDM is a pregnancy's most frequent medical problem characterized by the increase in blood glucose levels while pregnancy that disappears after the childbirth (Alfadhli, 2015).

2.7.2.4 Other Specific Types

It refers the genetic based diabetes or due to the association of any disease or drug. It is caused due to any medical issue like cystic fibrosis, PCOS, pancreatic cancer etc. (Skyler *et al.,* 2017).

2.8 Type 2 Diabetes Mellitus

T2DM is a severe metabolic illness that is challenging to treat because it results from insulin resistance (IR) caused by pancreatic β-cell malfunction (Zaccardi *et al.,* 2016). At least 90% of all instances of diabetes mellitus are type 2 diabetes, which is the most common kind of diabetes. Changes in emerging countries toward a Western lifestyle (high food with little exercise) are directly responsible for this rise in T2DM. In addition to environmental variables like obesity, overeating, stress, lack of exercise, and ageing, T2DM is a heterogeneous condition resulting from the interaction of genetic variables linked to decreased insulin production, insulin resistance, and environmental factors. (Ozougwu, 2013).

T2DM is the condition that develops because of malfunctioning of β-cells when the cells get unable to produce enough insulin frequently due to the rise in IR (Kharroubi, 2015). Ectopic fat deposition in the liver and muscle leads to the development of IR. The aggregation of fat in the pancreas results in depreciation of the activity of β-cells, inflammation of islets and ultimately to the death of β-cells (Brooks-Worrell *et al.,* 2014).

The major risk factor of T2DM is obesity and the significant decrease in weight leads to the improved insulin activity in the liver, muscle and pancreas (Skyler *et al.,* 2017).

2.9 Epidemiology of T2DM

2.9.1 T2DM Globally

The increase in the rate of T2DM is becoming a major health concern globally. It has been recognized as a 21st century epidemic by United Nations (UN) (Kotwas *et al.,* 2021). According to World Health Organization, around 462 million people worldwide suffer from T2DM in 2017. Out of which, 4.4% were the 15-49 years aged people, 15% were of 50-69 aged, 22% were of 70 aged. According to literature, approximately 1 million deaths occur each year because of T2DM, the 9th leading cause of death (Khan *et al.,* 2020).

In 2014, 8.5% of people who were 18 or older had diabetes. In 2019, 1.5 million people died directly from diabetes; 48% of these fatalities occurred in people under the age of 70. Diabetes also contributed to 460 000 deaths from kidney disease, and high blood sugar is responsible for roughly 20% of fatalities from cardiovascular disease. Between 2000 and 2019, the age-standardized death rates for diabetes increased by 3%. In lower-middle income countries, the mortality rate from diabetes increased by 13%. (Vos *et al.,* 2020).

2.9.2 T2DM in Pakistan

This attention getting issue with many other complications has been reported to have a huge infelicitous with the rate of 16.98% T2DM and 10.91% prediabetes all over the Pakistan with the prevalence of T2DM 26.3% and prediabetes 14.4% (Aamir *et al.,* 2020). 11.77% of Pakistan's population currently has T2DM. Males were 11.20% more

likely to have it than females, whereas females were 9.19% more likely. The prevalence of men is 16.2% in Sindh province and women is 12.14, compared to 11.70% and 9.83% respectively in Punjab province. In Baluchistan, men make up 13.3% of the population while women make up 8.9%; in Khyber Pakhtunkhwa (KPK), men make up 9.2% of the population while women make up 11.60%. In Pakistan, the prevalence of type 2 diabetes is 10.34% in rural areas compared to 14.81% in urban areas. 11.77% of Pakistan's population has type 2 diabetes. The illness is more likely to affect men than women and is more common in metropolitan areas than rural areas (Meo, 2016)

2.10 Pathophysiology of DM

Several hormones work together to keep the body's level of glucose in equilibrium. Though, the regulation of glucose metabolism is mostly regulated by two hormones, glucagon, and insulin (Padhi *et al.*, 2020) Various genetic and environmental factors are involved in causing hyperglycemia through the progressive loss of β-cells (Skyler *et al.*, 2017). A person's blood sugar levels rise after eating, which causes the production of more insulin, increasing the quantity of glucose that is transported, adhered to, and stored in their muscles and fat cells (Asmat *et al.*, 2016). In addition to the retention of glucose, Insulin also prevents the release of glucagon and lowering blood fatty acids concentration, which reduces the amount of glucose that is produced by the liver (Asmat *et al.*, 2016). Intracellular hypoglycemia and extracellular hyperglycemia are the outcomes of insufficient insulin production or insulin sensitivity in the body. The extracellular hyperglycemia results in hyperglycemic catatonic state and osmotic endogeneity, while As a result of the intracellular hypoglycemia, glucogenesis and

gluconeogenesis are triggered, which reduce protein synthesis and gamma globulin production and break down lipids (resulting in diabetic ketoacidosis) (Ozougwu, 2013).

2.11 Mechanisms Associated with the Pathophysiology of T2DM

2.11.1 Insulin Secretion

2.11.1.1 Physiology of β-cells

β-cells are found in the islets of pancreas and are responsible for the production, storing and releasing of insulin hormone in the body. At the early stage, it is synthesized as pre-proinsulin in the β-cells. This pre-proinsulin with the help of various proteins is converted to pro-insulin in the endoplasmic reticulum (ER). In the next step, this pro-insulin is transported to the Golgi apparatus when it is converted to the functional insulin and C-peptide.

Release of insulin from the granules is triggered in response to high levels of glucose in the body. When the glucose levels get high in the body, the glucose transporter GLUT-2 takes the glucose into the β-cells. These GLUT-2 plays a role as a glucose sensor in the β-cells. As the glucose enters the β-cells, the intracellular ATP-to-ADP ratio increases, that triggers the closing of ATP-dependent K^+ channels and opening of Ca^{+2} channels, leading to release of Ca^{+2} into the cell. By inducing the primary and fusing of secretory insulin-containing granules to the plasma membrane, an increase in intracellular Ca^{+2} induces insulin exocytosis. The RY receptors (RYR) can additionally amplify this rise in Ca+2, which leads to an even greater rise in the body's production of insulin. There are also some other types of signals like cAMP, ATP and purinoreceptors (P2X, P2Y). All these signals amplify the Ca^{+2} concentrations in the body that in result enhances the release of β-cells.

2.11.1.2 β-cells Dysfunction

The dysfunctioning of β-cells occurs majorly due to the death of β-cells as wells as the integrity of islets of pancreas. The increase level of glucose leads to the more production of insulin and islet amyloid polypeptides (IAAP) in the cells leads to the amass of misfolded insulin and IAAP thus induces the stress on endoplasmic reticulum (ER). The physiological mobilization of ER Ca^{+2} is altered, proapoptotic signals are favored, proinsulin mRNA is promoted to degrade, and interleukin is released, attracting macrophages, and escalating local islet inflammation. As the cell fails to produce insulin precursors or the insulin, there will be the insulin secretory dysfunction and leads to the substratum of T2DM (Galicia-Garcia *et al.,* 2020).

2.11.2 Insulin Resistance

Insulin resistance (IR) is the impaired response to the circulating hormone insulin to the blood glucose (Roden *et al.,* 2017). The main conditions of insulin impairment are:

i. Reduced β-cell production of insulin.
ii. Insulin rivals in the blood.
iii. Impaired insulin response in target tissues.

IR is a major factor in the emergence of T2DM and is the cause of β-cell failure (Roden *et al.,* 2017). The major factors responsible for the IR are the secreted molecules or hormones i.e., catecholamines, glucocorticoids etc. for the lipolysis, glycogenolysis, muscle catabolism and gluconeogenesis. The excess release of these hormones in the body may induce IR in the body.

The three primary extra-pancreatic insulin-sensitive organs that are crucial to the above processes are:

i. Skeletal Muscle

ii. Adipose Tissue

iii. Liver

2.11.2.1 Skeletal Muscle

The most significant extra-pancreatic component in the progression of T2DM is thought to be skeletal muscle IR. Muscles stores glucose in the form of glycogen in response to insulin binding to Insulin receptors (INSR) resulting in the activation of GLUT-4 (glucose transporter) to allow uptake of glucose into the muscle cells. Mutations in any of the phosphorylation site on binding of insulin to INSR can mediate lessen insulin secretion (Galicia-Garcia *et al.,* 2020).

Mutations in IRS-1, IRS-2 or phosphoinositide-3-kinase (PI3K) also diminishes the effect of insulin action on muscular tissues. Some environmental factors like less or more than normal physical exercise, obesity, more immune cell infiltration and increase in the release of proinflammatory molecules results in the paracrine effects of IR (Galicia-Garcia *et al.,* 2020).

2.11.2.2 Adipose Tissue

Adipose tissues are involved in maintain the homeostasis at the systemic level. In the tissues, insulin acts as stimulator for glucose uptake and synthesis of triglycerides preventing the hydrolysis of triglycerides and enhancing the removal of FFA and glycerol from bloodstream. Under normal conditions, glucose is stored as triacylglycerols in the form of lipid droplets.

IR to adipose tissue occurs due to the impaired response of insulin that results in the impaired mechanisms like lipolysis, increase in glucose uptake and increase levels of

FFA release into the plasma. These released FFA accumulate in different organs and impairs the insulin signaling causing hyperglycemia (Galicia-Garcia *et al.,* 2020).

2.11.2.3 Liver

In the liver, IR lowers the creation of glycogen, does not stop the generation of glucose, promotes lipogenesis, and increases the production of pro-inflammatory proteins including CRP. Verily, the aberrant proinflammatory protein production in addition to oxidative stress leads to liver's impaired reaction to insulin (Galicia-Garcia *et al.,* 2020).

2.12 Prolonged Diabetes due to Pathological Conditions

The most common pathological conditions that enhances the effect of T2DM are:

2.12.1 Nutritional Factors

The Standard American diet is a high caloric diet that contain high levels of proteins, fats and carbohydrates that tends to increase circulating blood lipid profile markers and sugar levels in the body. As a result, the levels of reactive oxygen species (ROS) rise, which causes an abnormal synthesis of inflammatory chemicals. This increase in the levels of ROS becomes the pathogenesis of T2DM and IR. This oxidative environment in result lead to mitochondrial dysfunction, ER stress, NADPH oxidase activation and superoxide production. These impairments activate the major pathways leading to diabetes (Galicia-Garcia *et al.,* 2020).

2.12.2 Physical Activity

In case of reduced physical activity and sedentary lifestyle leads to the release of proinflammatory molecule like IL-6, CRP, TNF-α and IL-1 into the blood that causes metabolic inflammation. Certainly, IL-1 promotes apoptosis by inactivating the β-cells. Routine exercise and more physical activity substantially decrease the body weight and

increase in the release of anti-inflammatory molecules. It helps in improving the levels of inflammatory molecules and the T2DM.

2.12.3 Metabolic Memory

Metabolic memory refers to the consistency of the diabetic complications even after the cure of T2DM. it has a major effect on T2DM. the mechanism under metabolic memory involves miRNA deregulation, low-grade inflammation and ROS production. miRNA are the small non-coding non-mature sequences that through different modifications get mature and plays a role in β-cell physiology. The deregulation of miRNA effects the β-cells resulting into growth of T2DM. Low grade inflammation also known as low level of inflammation mediates the metabolic memory. Some factors that are responsible for the low-grade inflammation are the age, obesity, low physical exercise and dietary style. These factors stimulate the increment in T2DM by triggering the inflammatory effect towards IR. Hyperglycemia persuades the ROS production in the mitochondria that rests in the body even after the glycemia is controlled. Due to the increase in the ROS production, the advanced glycation end-product (AGE) rises and damages the respiratory chain and the mitochondrial DNA (mit. DNA). Through the receptor binding of AGEs or ROS, which can alter the extracellular matrix's composition and structure, this metabolic imbalance activates inflammatory processes. Endothelial dysfunction and subsequent atherosclerosis may result from these structural alterations.

2.12.4 Mitochondrial Dysfunction

A growing body of research links mitochondrial dysfunction to the onset of T2DM, age-related IR, and T2DM comorbidities. One perspective relating mitochondrial malfunction to IR is the buildup of ROS in the mitochondria (Galicia-Garcia *et al.,* 2020). Since

impaired mitochondrial activity promotes the ectopic deposition of fat in muscles and adipose tissues, it may be linked to insulin resistance and T2DM. The deterioration in mitochondrial oxidative activity and ATP production both are signs of a degradation in mitochondrial function (Wondmkun, 2020). The increased intramyocellular and intrahepatic lipid content lowers the density of mitochondria thus leading to the IR (Saini, 2010).

2.13 Mechanism of Camel Whey towards Diabetes

2.13.1 Stomach

In the stomach, increase in the pyloric contraction and slow gastric emptying due to the intake in WP helps in lowering postprandial glycemia in type 2 diabetic patients. Whey has interdependent effects on postprandial glycemia, incretin hormone secretion, and stomach emptying. The GLP-1 is not only involved in causing the insulinotropic effect but also slows gastric emptying, reduces calorie intake and enhances postprandial glycemia by exerting glucagon-static actions. The secretions of GLP-1 diminish with the increase of type 2 diabetes. The intact GLP-1 secretion in type 2 diabetic patients can be justified by using dietary strategy to increase endogenous GLP-1 secretion (Marathe *et al.,* 2013). Also, the nutritional interactions with the small intestine can result in feedback that slows stomach emptying by stimulating pyloric contractions and suppressing antral motility. Intraduodenal administration of whey reduces isolated pyloric pressure waves and enhances antral and duodenal waves in healthy young and older individuals. In addition, these alterations in antro-pyloric motility in response to nutrient administration in healthy young participants seem to be independently correlated with subsequent

energy intake. Antropyloroduodenal motility in connection to the effects of intraduodenal WP infusion on hunger and subsequent ad libitum calorie intake (Mignone, 2015).

2.13.2 Intestine

GIP and GLP-2 are the two incretin hormones that carry out insulinotropic effect by the strong expression of G-protein coupled receptors on β-cells. Both the hormones are dependent on the increase in blood glucose levels. In health and type 2 diabetes, incretin hormones are crucial for protein-stimulated insulin release (Campbell & Drucker, 2013). The intake of WP has a significant effect on the increase response of GIP than the intake of essential amino acids. The WP is reported to simulate the release of incretin hormones GLP-1 and GIP (Salehi *et al.,* 2012). WP can increase incretin hormone secretion and protein-stimulated insulin release, so it makes sense to consider it as a potential therapeutic agent for the treatment of T2DM (Mignone, 2015).

Dipeptidyl Peptidase-IV (DPP-IV) is involved in the degradation of the incretin hormones to the inactive metabolites. WP seemed to be involved in the inhibition of DPP-IV activity. Inhibition of the activity of DPP-IV results in the increase in incretin hormones GIP and GLP-1 activity thereby seems to be the beneficial for the treatment of T2DM (Mignone, 2015).

The enzyme alpha glucosidase hydrolyzes starch and disaccharides at the small intestine brush border to improve glucose absorption(Mignone, 2015).

At the small intestinal brush border, the enzyme alpha glucosidase hydrolyzes starch and disaccharides to allow glucose absorption. The intake of WP is found to be the potent inhibitor of alpha-glucosidase thus used in managing blood glucose levels in type 2 diabetic patients (Mignone, 2015).

2.13.3 Pancreas

Type 2 diabetic patients are thought to have the loss of the incretin effect. It has been hypothesized that the loss of incretin effect plays a major role in the pathogenesis of diabetes. Now this deficiency in incretin effect may be due to the adverse effects on islet function as well as impaired incretin hormone production (Holst & Ørskov, 2004). Also, the amino acids have the major effect on the functioning of β-cells. Ingestion of proteins along with the carbohydrates enhances the effect of insulin secretion. WP is an abundant source of branched chain amino acids leucine, valine, and isoleucine, which are known to have powerful insulinotropic effects. Intake of proteins either intravenously or orally, results in the increase in the insulin secretion and decrease in the levels of blood glucose. At least in part, the insulinotropic effects of whey are due to the direct stimulation of β-cells by amino acids. Amino acids have a strong effect on insulin secretion in type 2 diabetic patients. In this case, ingestion of WP breaks down into amino acids that plays a potent role in the secretion of insulin (Manders *et al.*, 2014).

2.13.4 Brain

The gut-brain axis plays a critical role in modulating the effectiveness of therapies that have had a significant impact on treating T2DM and obesity. This system is intricately engaged in regulating glucose homeostasis and hunger (Richards *et al.*, 2021). The macronutrient content of the diet can be modulated to reduce energy expenditure and appetite. Intake of WP has been demonstrated in short-term trials to increase satiety and decrease food consumption at the next meal. Following whey consumption, an increase in plasma amino acid concentrations may influence appetite through vagal feedback and direct hypothalamic suppression of hunger. Leucine, lysine, tryptophan, isoleucine, and

threonine concentrations are higher in whey as compared to soy or casein, which results in a larger suppression of appetite. Additionally, tryptophan is converted into serotonin, which is known to affect appetite (Mignone, 2015).

Chapter 3

Materials and Methods

Materials and Methods

3.1 Materials

3.1.1 Collection of Camel Milk

Fresh pure Camel milk samples were collected on daily basis from the nearby herds of camels. Milk was collected from the healthy camels and was kept in the sterile bottles and was transported to the laboratory. This milk was used for the preparation of camel whey proteins and for the feeding to the rats.

3.1.2 Drugs and Chemical Reagents

Streptozotocin and the other chemicals were purchased from Sigma Aldrich and was prepared by dissolving in 0.1M (pH 4.5) citrate buffer. It was administered immediately to the rats through intraperitoneal (IP) route. Biochemical analyses are performed by using diagnostic kits purchased from Bio Research and CHEMELEX S.A Diagnostic reagents. Mixtard 30/70 insulin injection were purchased from the Shaheen Chemist, Saddar.

3.1.3 Experimental Model Design

The experiment was conducted using Wistar albino male and female rats, aged 8-12 weeks, weighing 180-250g for both male and female rats. The rats were housed in cages with a 12-hour light/dark cycle at a regulated temperature of 25°C. The animals were fed standard chow and provided with drinking water on a daily basis. Rats were acclimatized to laboratory conditions for one week prior to the experiment's start. The trial lasted 5 weeks in total. Wistar female rats were obtained from the Atta-ur-Rahman School of Applied Biosciences (ASAB) of the National University of Sciences and Technology

(NUST), Islamabad, Pakistan. Wister male rats were purchased from the University of Veterinary and Animal Sciences, Lahore, Pakistan. They were transported through special care to the assigned place.

3.1.4 Ethical Approval

All the animal procedures and experiments were conducted after the approval from the Institutional Review Board (Ref. IRB No. 09-2021-01/03) and all of the experimental procedures were according to the guidelines described by the laboratory animal house (LAH), ASAB, NUST (Annex A attached).

3.1.5 Animal Feed and Water

The rat's feed was prepared on fresh basis that contained 4% fibers, 23% protein, 4-5% fats and 11-12% carbohydrates.

3.2 Methods

3.2.1 Preparation of Camel Whey Proteins

Whey protein was prepared from the fresh camel milk. Milk was first centrifuged at 5000×g at 4˚C for 20 mints to remove the milk fatty layer. The resulting skimmed milk was collected by centrifugation will be acidified by using 1N HCl to pH 4.5 at room temperature. The acidified milk was centrifuged at 5000×g at 20˚C for 20 mints to precipitate out casein portion of the milk. The resultant whey was centrifuged again to flush out any casein component left behind. The resulted camel whey protein was saturated with 70% ammonium sulfate and incubated at 4˚C overnight. The solution was centrifuge at 5000×g at 20˚C for 20 mins to precipitate the CWP. The precipitated CWP was dialyzed using a porous membrane with a molecular weight cut-off (MWCO) of 6000–8000 kDa against 20 liters of distilled water for 48 hours. The prepared dialysate

containing the undenatured CWP was placed at -80˚C and then was lyophilized and store at 4˚C until use.

3.2.2 Characterization of Prepared Undenatured Camel Whey Proteins

To determine the production of the CWP and their overall topography, a series of characterization tests on the CWP were conducted.

3.2.2.1. Scanning Electron Microscopy

SEM is used to determine the morphology and size of a product. Each sample was fixed overnight on a 6x6 mm slide using a 1:10 dilution. The following day, after mounting the dried samples onto a conducting surface attached to the glass slide via carbon tape, they were sputtered with gold. The slides were then inspected under a microscope at various magnifications to determine the CWP's shape and size.

3.2.2.2. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR (Agilent ATR-FTIR) was performed between range 450 cm^{-1} to 4500 cm^{-1} at normal room temperature to detect the range of functional groups present in a protein sample presenting the molecular compounds. The absorbance and transmittance on different wavelength ranges help in the formation of a wave like pattern that is unique to the respective bond and can help in identifying various bonds.

3.2.2.3. Spectrophotometric Analysis

The protein determination was carried out with BioRad method.

i. Reagent

BioRad solution: Dissolve 0.1 g Brilliant blue G-250 (Sigma Chemical Company) in 50 ml of 95% ethanol. To this added 100 ml of phosphoric acid (85%) and mixed the

contents. The volume of solution was adjusted and filtered through Whatman No. 1 filter paper. The solution was prepared fresh and stored in cold cabinet.

Protein standards: Dissolved 0.15 g bovine serum albumin (BSA) in 100 ml distilled water. Protein standard solution containing 10 µg-100 µg protein ml^{-1} were made by appropriate dilution of stock solution.

ii. **Sample preparation:** Fresh CWP sample weighing 0.2 g was crushed in a mortar. Then 10 ml of distilled water was added to the sample and the contents were mixed and then centrifuged at 5000 rpm for 15 minutes. Decanted the supernatant and 200µl aliquot was used for estimation of protein.

iii. **Preparation of Standard Curve:** A series of various dilution was prepared in the eppendorph tubes and the measured distilled water and the BioRad reagent was added to each tube containing protein solution and absorbance was taken at 595nm wavelength.

3.2.3 Induction of Diabetes

Streptozotocin was dissolved in a citrate buffer concentration of 0.1M (pH 4.5). Diabetes was produced intraperitoneally with a single dose of STZ (40mg/kg body weight). Instead of Streptozotocin, a non-diabetic control group is injected with freshly prepared citrate buffer. These injections were given for two days and were allowed to rest for 3 days (fed on normal feed and water). Then, the blood glucose levels were determined by checking the blood glucose level using glucometer. The rats with the blood glucose levels above 200mg/dl were considered to be diabetic and used for further experimentation.

3.2.4 Experimental Design

A total of 20 rats (10 males and 10 females) n=4 (2 males, 2 females) normal, and n=16 (8 males, 8 females) diabetic rats were divided into the 10 groups (5 males, 5 females). Each group consists of the 2 rats. Nature of each group taken in this study is group 1 is healthy control animals (C) chow with normal feed and water. Group 2 contains diabetic negative control rats (D) chow with normal feed and water. Group 3, insulin treated (D+I) contains positive control diabetic rats treated with market available insulin (0.9/g) of the body weight of rats subcutaneously for 6 weeks. Group 4 was given raw camel milk (CM) (50mg/kg body weight) orally for 6 weeks and Group 5 was supplemented with the prepared undenatured camel whey proteins (CWP) (100 mg/kg/body weight dissolved in 250 µl/day) orally for 6 weeks.

3.2.5 Estimation of Body Weight

Rat's body weights were evaluated before induction of diabetes and then after a week on the same day and time throughout the experiment by using a digital balance. Throughout the trial, signs of abnormalities in body weight were observed.

3.2.6 Blood Glucose Test

The blood glucose level of the rats was monitored on weekly basis by using Easygluco glucometer. The glucose levels were checked in the morning. Blood samples for glucose testing of rats were taken by pricking the tails.

3.2.7 Blood Sampling

At the end of experiment, the experimental animals were fasted for 12 hours, water was not restricted. Blood samples were collected by direct heart puncture in clotting blood tubes and organs are stored in 10% formalin and stored at -80°C for further analysis.

Blood samples were centrifuge at 4000×rpm for 5 minutes and serum were separated and stored at -20℃ for biochemical analysis.

3.3 Biochemical Analysis

3.3.1 Blood Glucose Test

Glucose is the key source of energy in human body. Body converts carbohydrates into glucose. If the blood glucose levels are high, it will be considered diabetic. Blood glucose level is a measure of hypoglycemia, hyperglycemia, pancreatic mis functionality, hyperthyroidism and Cushing syndrome. Glucose was measured in serum samples using Glucose (SL), GOD-PAP CS008 (Bioresearch diagnostic kit). Measurement was taken according to manufacturer's instructions by using Chemistry Analyzer (CHEMREADER Smart-N SE250).

3.3.2 Lipid Profile

Diabetic dyslipidemia mostly occurs in diabetic patients as well as in cardiovascular diseases. One of the most prevalent secondary causes of hyperlipidemia is Type 2 Diabetes. Cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and triglycerides are the components in lipid profile. These are measured in serum using Cholesterol (SL), CHOD-PAP CS005, LDL Cholesterol CS011 and Triglyceride (SL), GPO-PAPCS016 (Bioreasearch Diagnostic Kits) respectively. Measurements were taken according to manufacturer's instructions by using Chemistry Analyzer (CHEMREADER Smart-N SE250).

3.3.3 Liver Function Tests

Liver enzymes are proteins that help your body's chemical reactions move faster. Producing bile and compounds that help your blood coagulate, breaking down food and

pollutants, and combating illness are all examples of chemical reactions. Some of the most common liver enzymes are Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Gamma-glutamyl transferase (GGT). Alkaline phosphatase (ALP), Alanine amino transferase (ALT), Albumin (ALB) and Bilirubin (BIL) were measured in serum using Alkaline Phosphatase (SL), DGKC, (CZ001), ALT/GPT (SL), UV IFCC, (CZ003), Albumin, BCG, (CS001) (Bioresearch diagnostic kits) and BILIRUBIN T&D-DMSO DMSO, Colorimetric (CHEMELEX S.A Diagnostic reagents, ref #30157). Measurements were taken according to manufacturer's instructions by using Chemistry Analyzer (CHEMREADER Smart-N SE250).

3.3.4 Renal Functions Tests

Creatinine and uric acid were measured in serum using Creatinine (SL), KINTEIC, (CS006) and Uric Acid (SL), URICASE-PAP (CS018) (Bioresearch diagnostic kits British Columbia), respectively. Measurements were taken according to manufacturer's instructions by using Chemistry Analyzer (CHEMREADER Smart-N SE250).

3.4 Antioxidant Enzyme Activities in Liver Tissue

There are various enzyme systems that catalyze free radical and reactive oxygen species neutralization processes. Among these enzymes are:

i. Superoxide dismutase
ii. Catalases
iii. Lipid peroxidation MDA

These are the body's natural defensive systems against free radical-induced cell damage. Antioxidant enzymes such as catalase and superoxide dismutase (SOD) metabolize oxidatively damaging intermediates. These enzymes also require co-factors like

selenium, iron, copper, zinc, and manganese for maximum catalytic activity. It's been suggested that if you don't get enough of these trace minerals in your diet, your antioxidant defence mechanisms will be less effective. Consumption and absorption of these critical trace elements may decrease as people age.

3.4.1 Tissue Lysate

Tissue homogenate was made by dissolving 500 mg of liver tissue in 1ml of 0.1M cold phosphate buffer (PBS) pH 7.4. In a chilly jacket, all tissues were homogenized with a pestle and mortar. The homogenates were transferred to tubes without increasing the heat, and then centrifuged for 20 minutes at 4°C at 4,000 rpm. Following this technique, the generated supernatants were kept at -80°C and employed in lipid peroxidation and antioxidant enzyme assays such as SOD and CAT.

3.4.2 Lipid peroxidation (MDA)

Lipid peroxidation assay (also named as TBARS) basically tells about the oxidative degradation of lipids. Oxidative stress results in generation of free radicles that takes electrons from lipids (especially from lipids of membrane), this ultimately damages the cell. In order to measure the oxidative stress, quantification of lipid peroxidation is an important assay which is performed by Satoh method:

i. 0.5ml of tissue homogenate with 1.5ml of 10% TCA solution.
ii. Incubate the mixture solution at room temperature for 10 minutes.
iii. Add 1.5ml of supernatant of above solution into 2ml of 0.67% TBA solution.
iv. Boiling in water bath for 30 minutes
v. Cooling it for 20 minutes on ice.
vi. Add 1.2 ml of N-butanol and centrifuge for 5 minutes at 2000rpm at 4°C.

vii. Record absorbance at 532nm.

Calculation

Concentration (mM)= Change in Abs/155 x sample volume

3.4.3 Catalase (CAT)

Catalase is an anti-oxidant enzyme that protects the cell against reactive oxygen species (ROS) that are produced as a result of oxidative stress. This enzyme is primarily responsible for catalyzing the synthesis of water and oxygen from hydrogen peroxide H2O2. Catalase is present in the tissue sample is degraded, resulting in the formation of water and oxygen molecules. Catalase assay was carried out by Aebi method;

i. Reaction mixture containing 0.1ml tissue homogenate with 0.85ml of potassium phosphate buffer (0.05M, pH 7.0); incubate at room temperature for 10 minutes.
ii. Add 0.05ml of H2O2 (30mM, pH 7.0).
iii. Record the decrease in absorbance at 240nm for 3 minutes.

Calculations

Catalase concentration (M): Change in absorbance/$36mM^{-1}cm^{-1}$

3.4.4 Superoxide dismutase (SOD)

Superoxide dismutase (SODs) assumes a basic part in the body's cell reinforcement safeguard against oxidative pressure. The compound is a viable treatment for sicknesses brought about by responsive oxygen species. The capacity of oxygen radical to communicate with NBT and diminish the yellow tetrazolium inside the gel to a blue hasten is the premise of this measure. Grass makes a reasonable region (colorless groups) in regions where it is dynamic, rivaling NBT for oxygen radical. SOD was done by following procedure:

i. Reaction mixture containing 0.5ml of potassium phosphate buffer (0.1M, pH. 7.8), 0.2ml of 0.5M EDTA (pH. 7.8 adjust with NaoH), 0.1ml of 13mM methionine, 0.1ml of 20uM of riboflavin, 0.1ml of 750uM of NBT with 50ul of enzyme extract (Tissue lysate).

ii. Measure absorbance at 560nm.

Calculation

Control OD-Treated OD/Control OD*100= X% of inhibition

X% of inhibition is equal to 1/50*X =Y unit

Y unit in 50ul of enzyme extract

1000ul of enzyme extract contain SOD units= n value

So 1ml of enzyme extract from 100mg tissue = n value/ 100mg= SOD units/mg

3.5 Histopathological Examination

The histopathological examination of the desired tissue specimen was performed through the given optimized protocol:

i. Fresh specimen stored in 10% formalin on ice.

ii. Heat Paraffin Wax and store it at 65 to 70°C.

iii. Cut the tissue to a thickness of 3 - 4 mm.

iv. Put it in cassette and label it with pencil.

v. Dehydrate it in Ethanol soln. of 50, 70, 90 and 100% for 20 minutes

   a. 50% ethanol for 20 minutes

   b. 70% ethanol for 20 minutes

   c. 90% ethanol for 20 minutes

   d. 100% ethanol for 20 minutes

e. 100% ethanol for 20 minutes

vi. Clear it with two changes of Xylene for 30 minutes each.

vii. Infiltrate in Paraffin Wax for 2 hours in paraffin 1 and 15 min in paraffin 2.

viii. Pour the wax in mold and place tissue in proper orientation.9. Allow it to solidify and cool it at room temperature.

ix. Trim the tissue to remove excess paraffin.

x. Place in Freezer for further processing.

xi. Apply mordant on slide and incubate at 45°C for 10 min.

xii. Set the water bath at 55° C.

xiii. Switch on microtome and adjust the position of cassette.

xiv. Cut few sections to make tissue appear from the wax, and place in ice

xv. After 10 min, adjust it again and take ribbons.

xvi. Place in water bath and quickly take the tissue over the slide. Remove excess with a tissue paper.

xvii. Place it to dry in incubator at 45°C for 20 minutes.

xviii. Deparaffinize in 3 changes of Xylene for 2min each.

xix. Rehydrate in 100% of ethanol in 3 changes for 2min each then in 95% and 70% for 2 min each.

xx. Wash with Distilled water for 2 min.

xxi. Dip in Hematoxylin Solution for 2-3 min.

xxii. Wash with water for 5 minutes at room temperature

xxiii. Counter stain with eosin for 3-5 minutes.

xxiv. Dehydrate with 95% ethanol (dip 20 times in it) then place in 95% for 2 min.

xxv. Place slides in 2 changes of absolute alcohol for 2 min.

xxvi. Place in 3 changes of Xylene for 2 minutes in each.

xxvii. Dry the slides and mount the cover slips after applying mounting media (dppx).

xxviii. Observe under Microscope.

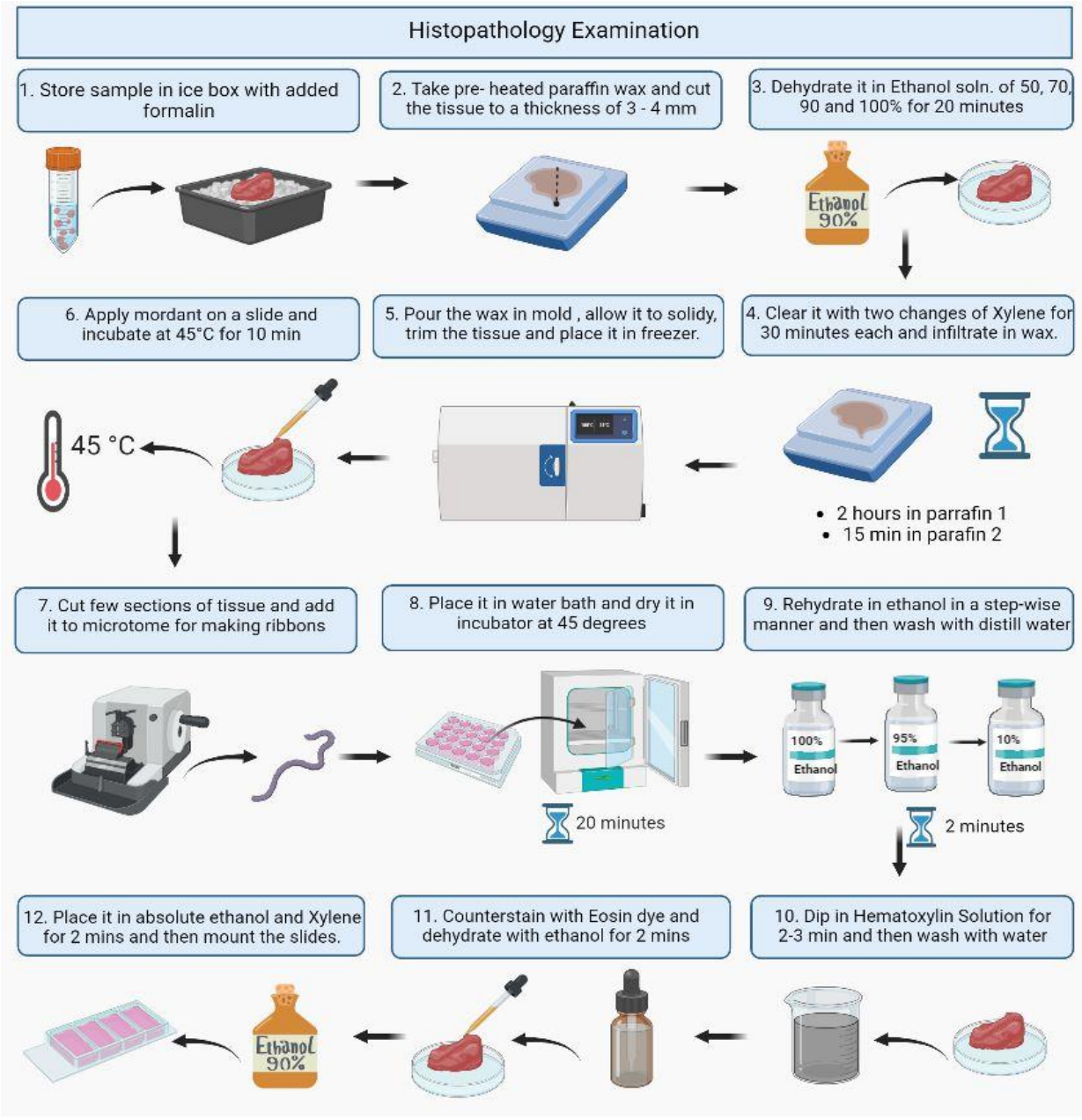


Figure 1 An overview to the protocol of the histopathological examination of the desired specimen.

3.6 Statistical Analysis

All the data were analyzed using GraphPad Prism version 8.0.1 software. The significant values among different groups were determined by using ONE-WAY and TWO-WAY ANOVA and significant value considered as when P value is less than 0.05.

Chapter 4

Results

Results

4.1 Whey Protein

Whey protein was prepared from the camel milk by reacting it with the HCl and ammonium sulfate and then dialyzing the obtained pallet through 6000-8000 kDa porous membrane. The obtained dialysate was freeze-dried. Figure 2 below shows the appearance of powdered whey proteins prepared from the raw camel milk.

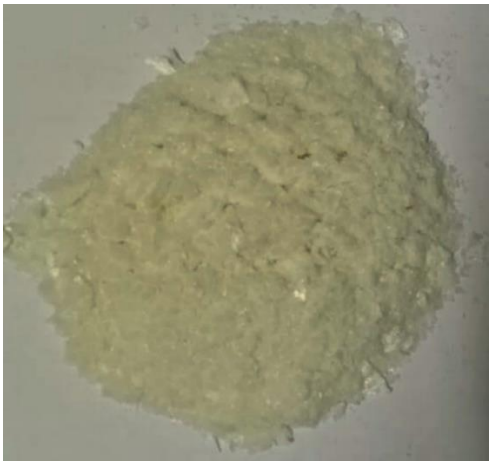


Figure 2 The prepared camel whey protein from the camel milk.

4.2 Fourier Transform Infrared Spectroscopy

To analyze the specific functional groups, present in the samples, the FTIR analysis of the prepared whey samples was performed. The stretches or bends obtained tells about the specific functional groups present on the surface of sample. Figure shows the spectrum obtained after analysis. The observed bonds in the spectra were the C-Cl, C-N, C-O, N=O, C=N, O-H, C-H, N-H and C=C. the figure 3 shows the FTIR spectra of the whey protein sample.

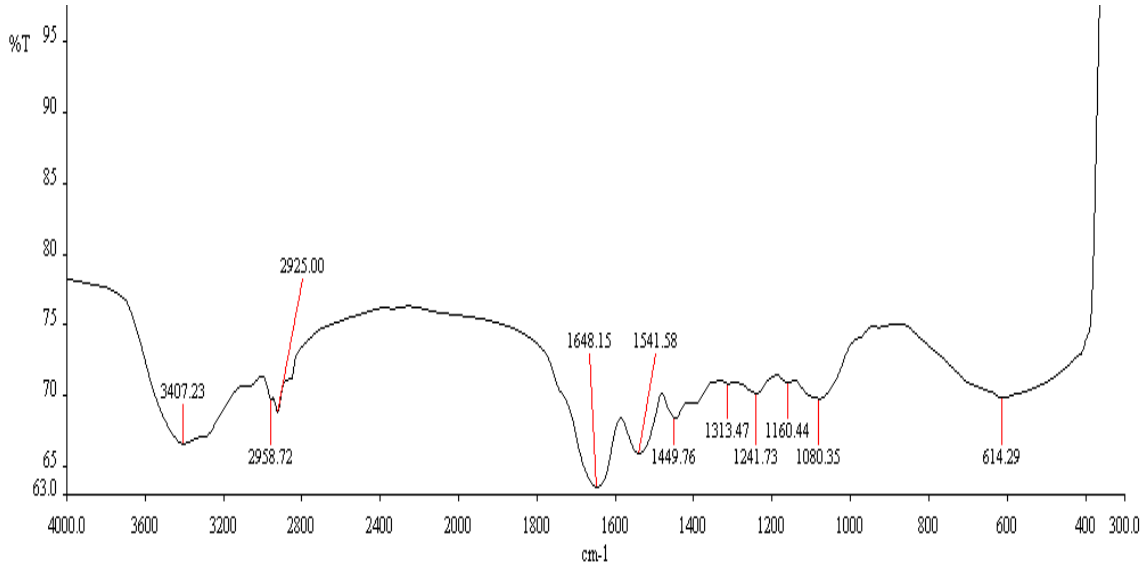


Figure 3 The FTIR spectrum of camel whey protein sample.

4.3 Scanning Electron Microscopy

Scanning electron microscopic images of the whey protein gave an idea about the clear morphology of the powder. However, the problem encountered here was that in order to go through the SEM the material has to be dried completely in order to sputter it with gold and fix it on the glass slides. If it fails to dry completely and any of the moisture remain in there, then the machine fails to give the quality results. Hence the SEM of fresh samples wasn't possible. Figure 4 below shows the images of the samples under the SEM. The obtained SEM micrograph is showing the irregular continuous cavity like structures with the continuity of smooth surfaces on the surface of protein.

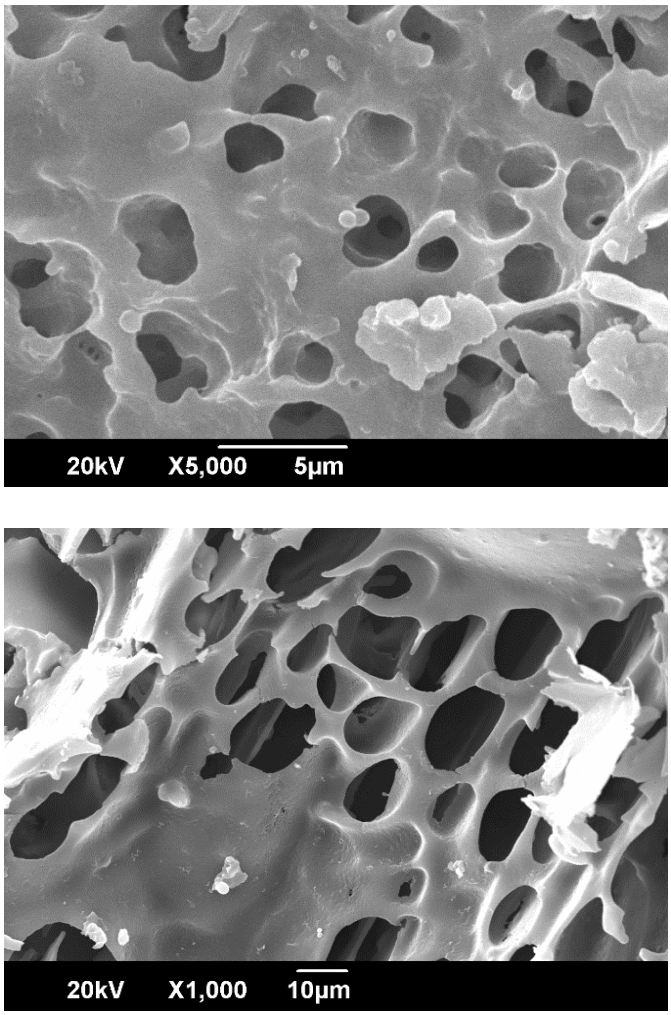


Figure 4 SEM images of the camel whey protein sample at different resolutions.

In-vivo Experimental Results

4.4 Effect of CM and CWP on Body Weight of Diabetic Rats

The Body weight is the most common factor that gets affected by the diabetes. the body weight of all the groups diabetic, non-diabetic and the treated groups are shown in fig. the induction of STZ (40mg/kg) for the consecutive two days to all the groups except the control group, that was on normal feed and water, resulted in the remarkable change in the body weight of both the male and female wister rats (P=0.001). These rats were then

given the treatment for the six weeks. Treatment with the insulin resulted in the gain of weight but this change was not much significant. On the other hand, the raw camel milk treatment to the diabetic rats resulted in the significant weight gain to both the male (P=0.0015) and female P=0.0034) rats. The treatment with the camel whey protein was much more significant in both the male (P<0.0001) and female (P=0.0002) rats. By applying one way ANOVA, the effect was observed to both the gender separately and by applying two-way ANOVA, the combined effect of both the male and female rats was analyzed.

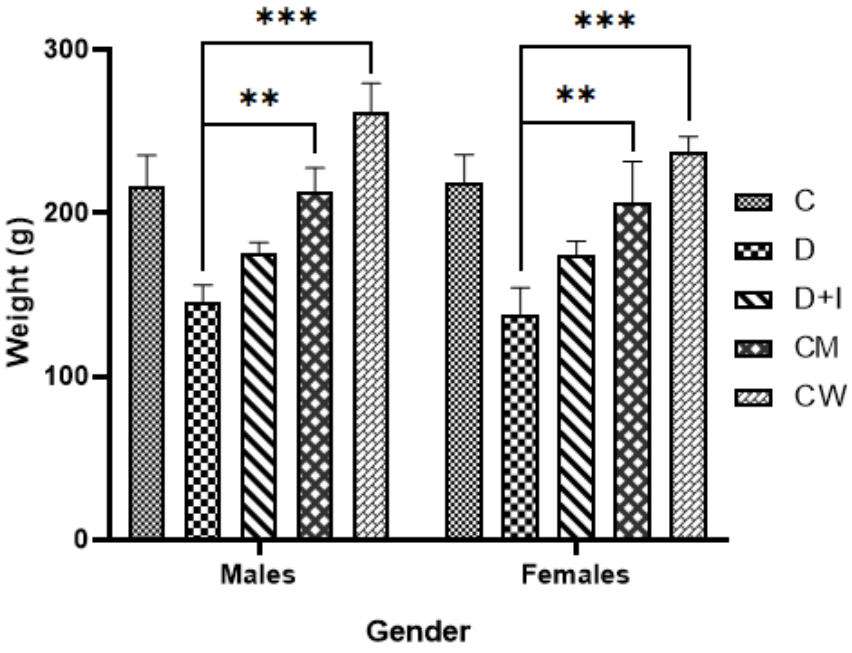


Figure 5 The results show the significant increase in the blood glucose levels of both the male and female diabetic rats with raw CM and CWP (P<0.0001).

4.5 Effect of CM and CWP on Blood Glucose of Diabetic Rats

The treatment was given to experimental groups till the normalization in the blood glucose levels. The blood glucose levels of the male and female treated groups were compared with diabetic group (D). There was significant effect observed in all the treated groups of both the males and females ($P<0.0001$). In males, comparative to the CM there was more effect of CW on diabetes and in case of females, the change in the blood glucose levels was more effective towards CWP. By applying two-way ANOVA, the diabetic group (D) was compared to the raw camel milk (CM) and camel whey protein (CWP) in both the male and female rats.

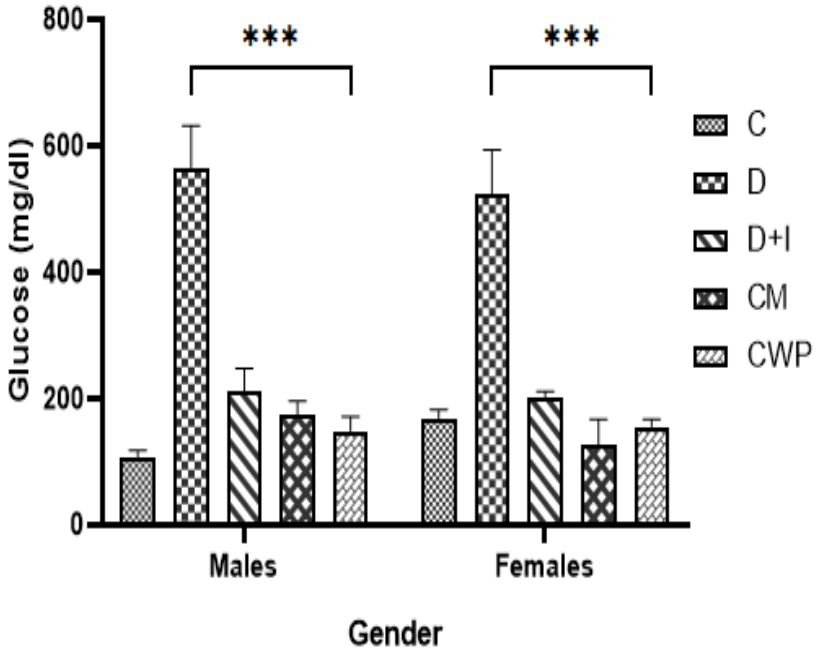


Figure 6 The results show the significant increase in the blood glucose levels of both the male and female diabetic rats with raw CM and CWP ($P<0.0001$).

4.6 Effect of CM and CWP on Biochemical Parameters of Diabetic Rats

4.6.1 Liver Function Tests

4.6.1.1 Alkaline Phosphatase (ALP)

ALP levels increase as a result of liver injury or bone damage, its raised levels indicate cholestatic liver disease. With normal values ranging from 30-120 U/L, The ALP levels usually increase several folds in diabetic beings. There was the significant elevation in the ALP levels observed when the STZ was compared with the control group (P<0001) due to the damage of liver and kidney and the insulin resistance. By apply one-way ANOVA, the individual analysis was performed for both the male and female rats. In males, the obtained P-value for CM and CWP was <0.0001 when compared to the diabetic group while in females, D+I showed less significant results (P=0.0029) as compared to CM and CWP (P<0.0001, P<0.0001) respectively. The figure given below is showing the effect of ALP on diabetes.

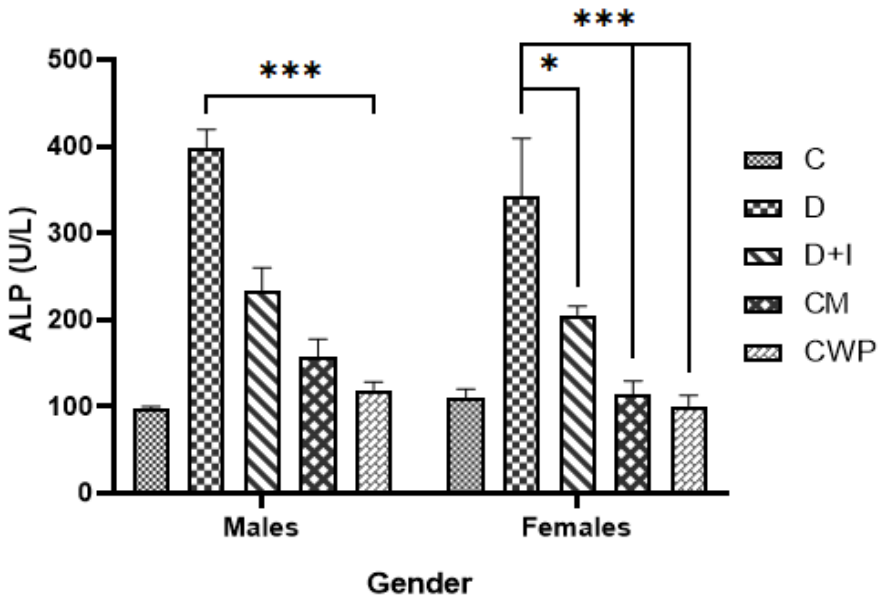


Figure 7 The results show the significant decrease in the ALP levels of both the male and female diabetic rats with raw CM and CWP (P<0.0001).

4.6.1.2 Alanine Aminotransferase (ALT)

Diabetes causes an increase in ALT levels because insulin resistance triggers lipolysis, which leads to a buildup of non-esterified fatty acids. It is well recognized that the hepatocytes are directly harmful from this increased fat deposition in the liver. This causes the ALT to rise. The significant increase in the ALT levels of the diabetic group was observed when compared to the control group ($P<0.0001$). On treatment, the decrease in the levels of ALT were observed when D+I, CM and CWP groups of both the males and females were compared to the diabetic (D) group ($P<0.0001$). One-way ANOVA was performed to do analysis of males and females separately and for the combined effect, two-way ANOVA was performed. The figure given below is showing the effect of ALP on diabetes.

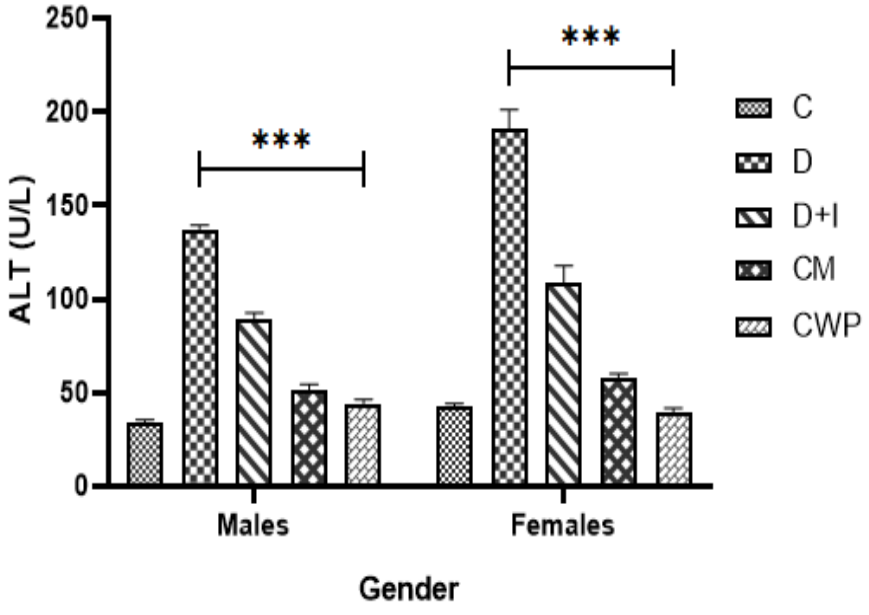


Figure 8 The results show the significant decrease in the ALT levels of both the male and female diabetic rats with raw CM and CWP ($P<0.0001$).

4.6.1.3 Albumin

The albumin accounts for the 80% of the total protein mass. And alteration in the levels results in the liver dysfunction. The induction of diabetes to the normal male and female rats resulted in the significant decrease in the albumin levels (P<0.0001). When treated with the insulin resulted in the increase in albumin levels but the results were not significant in both the males and females (males, P=0.8934, females P=0.9942). On the other hand, the raw camel milk treatment to the diabetic rats resulted in the less significant increase in the albumin levels to both the male (P=0.0083) and female (P=0.0338) rats. The treatment with the camel whey protein was much more significant in both the male (P<0.0004) and female (P=0.0003) rats. By applying one way ANOVA, the effect was observed to both the gender separately and by applying two-way ANOVA, the combined effect of both the male and female rats was analyzed. Hence the CM and CWP improves the diabetic effects as the CM and CWP contains have the insulin like properties that enhances albumin production in the body.

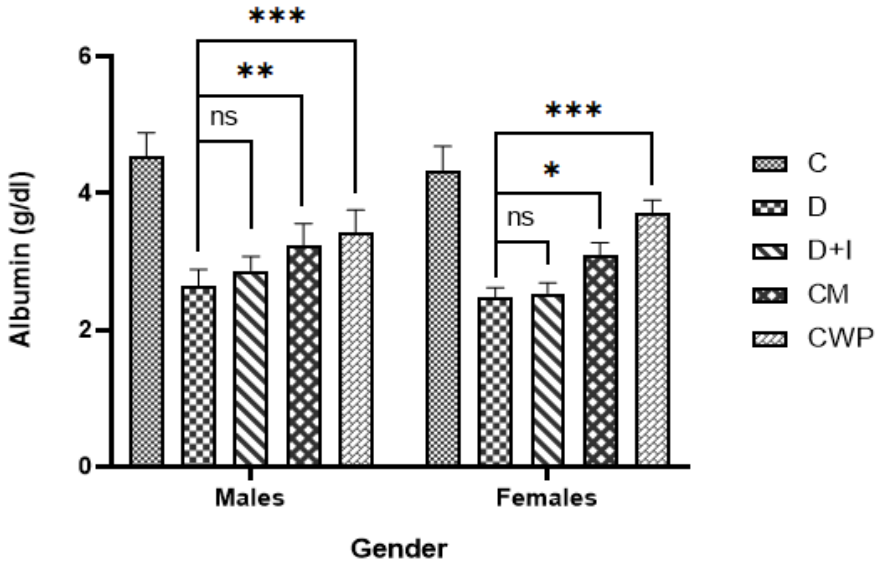


Figure 9 The results show the significant increase in albumin levels of both the male and female diabetic rats with raw CM (P=0.0044) and CWP (P<0.0004).

4.6.2 Renal Function Tests

4.6.2.1 Creatinine

The rising levels of the creatinine associated with the damaging of kidney. As the kidney failure progresses due to type 2 diabetes, the levels of creatinine in the blood increases. During the whole study, there was the significant elevation in the creatinine levels observed when the STZ was compared with the control group (P<0001) due to the severe damage of kidney and the insulin resistance. By apply one-way ANOVA, the individual analysis was performed for both the male and female rats. In males, the obtained P-value for CM and CWP was <0.0001 when compared to the diabetic group while in females, D+I showed less significant results (P=0.0086) as compared to CM and CWP (P<0.0001, P<0.0001) respectively. The figure given below is showing the effect of creatinine on diabetes.

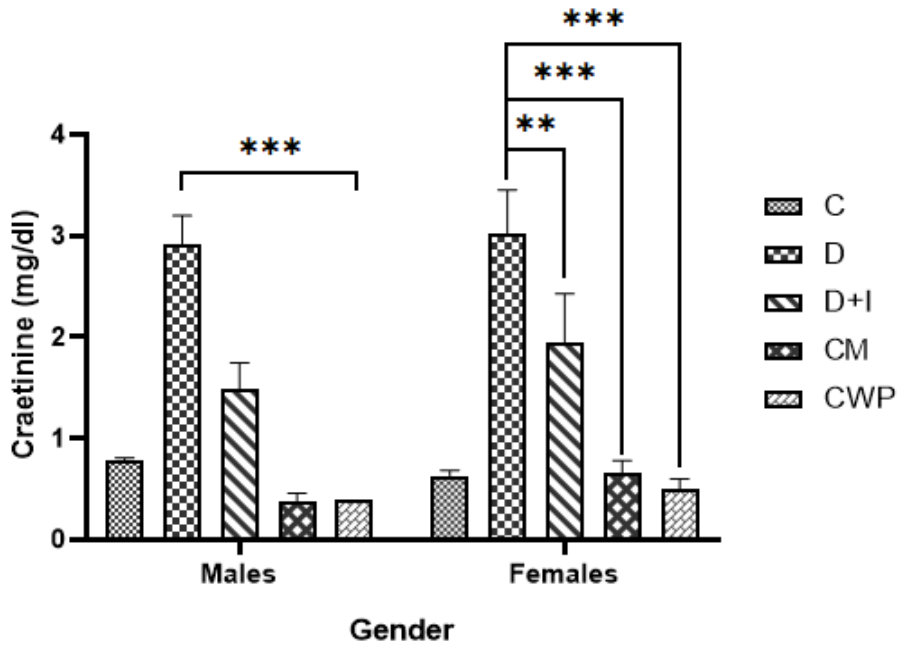


Figure 10 The results show the significant decrease in the creatinine levels of both the male and female diabetic rats with raw CM and CWP (P<0.0001).

4.6.2.2 Uric Acid

The incidence to the increase in uric acid levels is much more in diabetic male and female rats. This happens because of the increase in insulin resistance in diabetes. During the study, the significant increase in the levels of uric acid has been observed when compared to the control group. On treatment of diabetic rats with the CM and CWP, the uric acid levels became lowered comparative to the diabetic group (P=0.0044, P=0.0015). The results were analyzed by applying two-way ANOVA. The below figure is presenting the uric acid levels in diabetic, no-diabetic and treated groups.

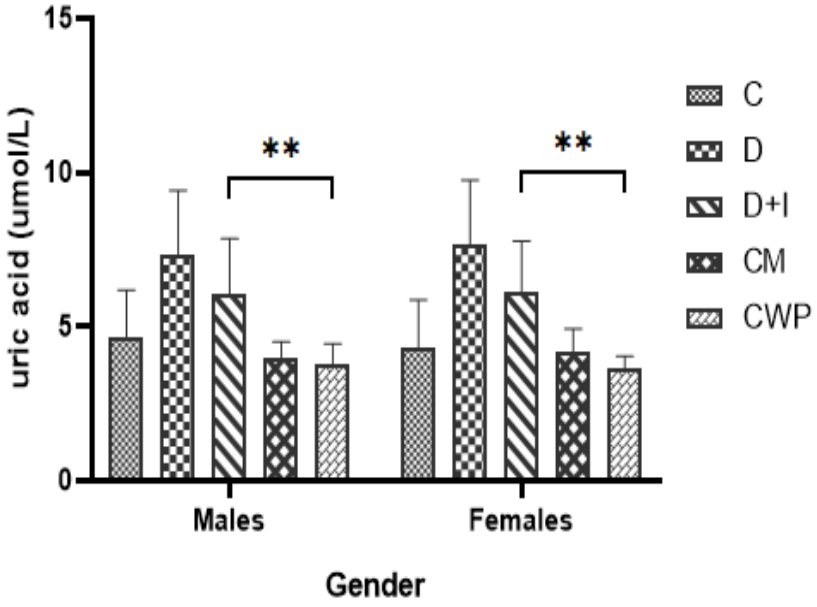


Figure 11 The results show the significant decrease in the uric acid levels of both the male and female diabetic rats with raw CM and CWP (P=0.0008).

4.6.3 Lipid Profile

4.6.3.1 Total Cholesterol

The elevated total cholesterol levels were observed to be significant in the diabetic rats when compared to the control group (P<0.0001). With normal values <200mg/dl, the total cholesterol levels usually increase several folds in diabetics. By apply one-way ANOVA, the individual analysis was performed for both the male and female rats. In males, the obtained P-value for CM and CWP was <0.0001 when compared to the diabetic group while in females, D+I showed less significant results (P=0.0006) as compared to CM and CWP (P<0.0001, P<0.0001) respectively. The figure given below is showing the effect of CM and CWP on total cholesterol of diabetes.

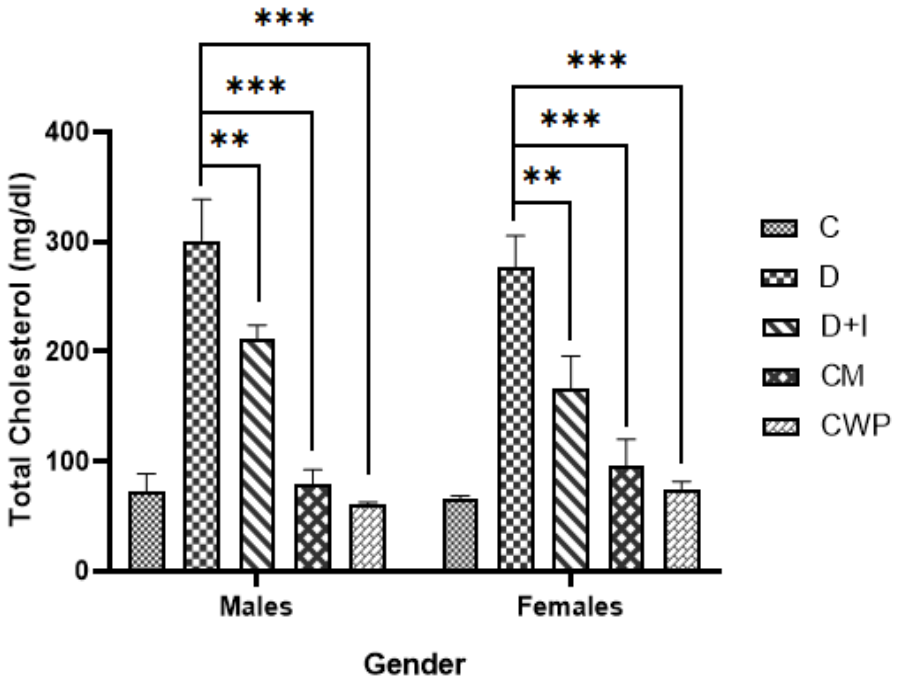


Figure 12 The results show the significant decrease in the total cholesterol levels of both the male and female diabetic rats with raw CM and CWP (P<0.0001).

4.6.3.2 Triglycerides

Figure shows the TG levels for all diabetic, non-diabetic, and treated groups. Numerous interrelated lipid and lipoprotein abnormalities in the blood, such as low HDL cholesterol, a high prevalence of small, thick LDL particles, and excessive levels of fatty substances are linked to insulin resistance and type 2 diabetes. Keeping in notice the normal range of TG (<150mg/dl), the treatment was given to the diabetic rats and the significant change was observed. On the induction of diabetes with the STZ, there was the significant increase in the TG levels (P<0.0001). When the CM was given to the diabetic rats, the significant decline in male (P<0.0001) and female(P=0.0003) rats was observed. And when the treatment was given using CWP, the more significant results were observed in both the male (P<0.0001) and female (P<0.0001) rats.

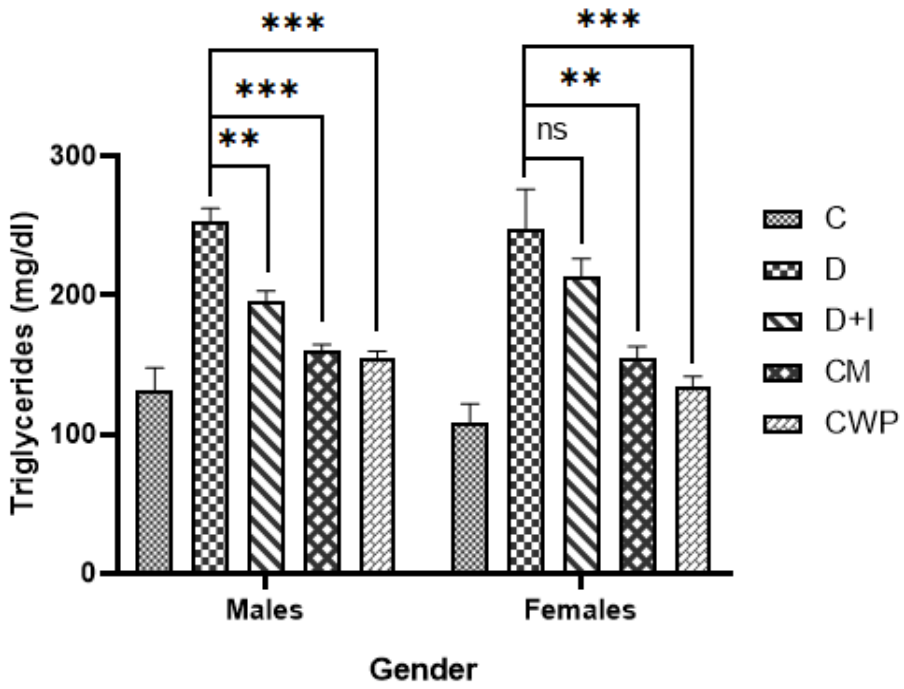


Figure 13 The results show the significant decrease in the triglyceride levels of both the male and female diabetic rats with raw CM and CWP (P<0.0001).

4.6.3.3 Low Density Lipoprotein Cholesterol (LDLC)

LDLC refers to the bad cholesterol in the body that should be low in the normal circumstances. The normal range for the LDLC in the body is estimated to be less than 70mg/dl. There are many different diseases associated with the increase in LDLC levels, one of which is the T2DM. in the experiment, the significant increase in the diabetic levels ($P<0.0001$) was observed when compared to the normal control. On treatment with the CM and CWP, a significant decline in the LDLC levels was observed in both the male ($P<0.0001$) and female ($P<0.0001$) rats was observed. The figure below represented a comparison of diabetic, non-diabetic and treated groups.

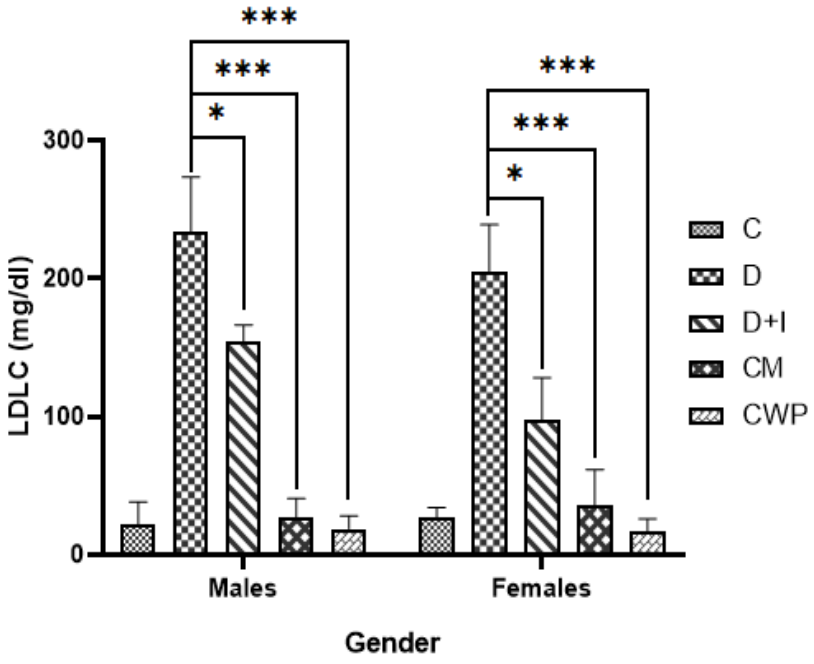


Figure 14 The results show the significant decrease in the LDLC levels of both the male and female diabetic rats with raw camel milk and camel whey protein ($P<0.0001$).

4.6.3.4 High Density Lipoprotein Cholesterol (HDLC)

HDLC refers to the good cholesterol whose normal range should be more than 60mg/dl in the body. It is associated with the circulation of cholesterol in the body. In case of diabetes, its levels gradually become low because of the disruption of the beta cell. Its reduction leads to the atherosclerosis in the T2DM patient. In the present study, on the induction of diabetes by STZ, the HDLC became low in the male and female wister rats ($P<0.0001$). On treatment with the CM and CWP to both the male and female diabetic rats, the significant increase in the HDLC levels was observed. By applying one-way ANOVA individually to both the males and females, the comparison among the diabetic, non-diabetic and treated group was performed. In males, on treatment of T2DM, significant increase was observed with the CM ($P<0.0001$) and CWP ($P<0.0001$). In females, on treatment of T2DM, compared to the males, less significant results were observed with the CM ($P=0.0032$) and CWP ($P=0.0008$).

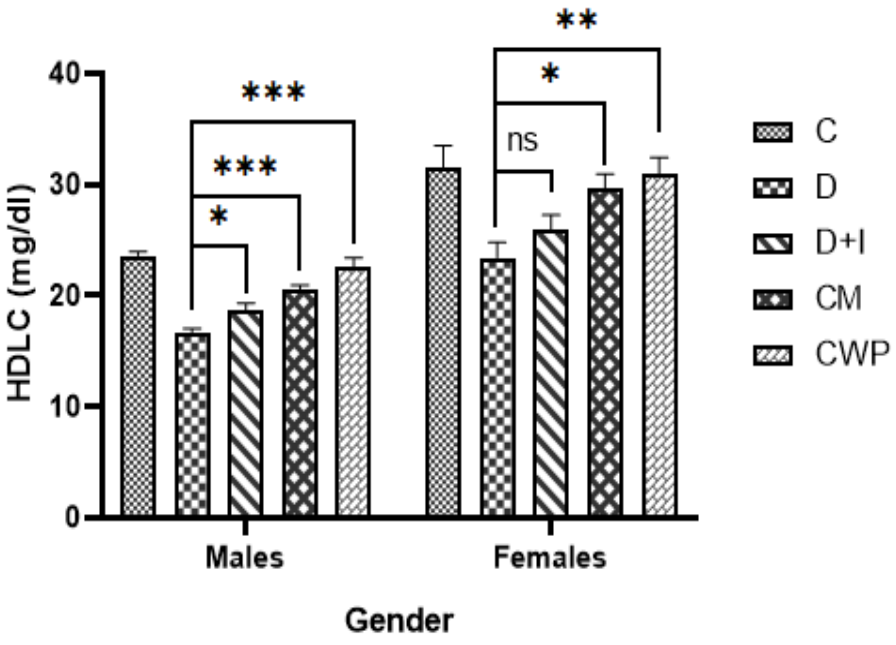


Figure 15 The results show the significant increase in the HDLC levels of both the male and female diabetic rats with raw CM and CWP ($P<0.0001$).

4.7 Change in Antioxidant levels in Liver by Induction of STZ and treatment by CM and CWP

4.7.1 Superoxide Dismutase Activity

By converting the percentage of superoxide, the main ROS in oxygen metabolism, to molecular oxygen and peroxide, superoxide dismutase offers first line defence against ROS-mediated cell harm. So, we can state that SODs dismutate superoxide into other molecules that are less harmful. This helps to prevent or improve liver injury. With the increase in T2DM, the liver damage results in the decrease in the SOD levels ($P<0.0001$). When treated with CM and CWP, the normalization of the SOD occurred. By applying one way ANOVA, individual analysis was performed for both the males and females wister rats. Both showed the same effect of CM ad CWP with the P-value <0.0001.

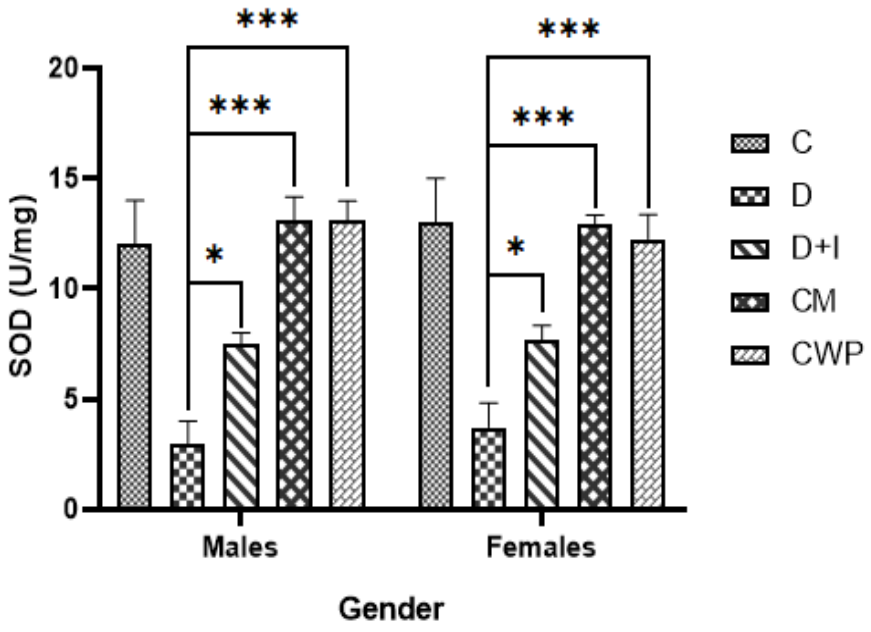


Figure 16 The result shows the effect of CM and CWP on the SOD levels of liver tissue in diabetic rats ($P<0.0001$).

4.7.2 Malonaldehyde Activity

Malonaldehyde activity involves the lipid peroxidation of polyunsaturated fatty acids. The oxidative breakdown of lipids known as lipid peroxidation occurs when free radicals are produced. So, the increase level of MDA causes increase in reactive oxygen species and relatively decrease in the antioxidant levels of enzymes. On study, the significant increase in the MDA levels of the diabetic group was observed when compared to the control group ($P<0.0001$). On treatment, the decrease in the levels of MDA was observed when D+I, CM and CWP groups of both the males and females were compared to the diabetic (D) group ($P<0.0001$). One-way ANOVA was performed to do analysis of males and females separately and for the combined effect, two-way ANOVA was performed. The figure given below is showing the effect of CM and CWP on MDA of diabetes.

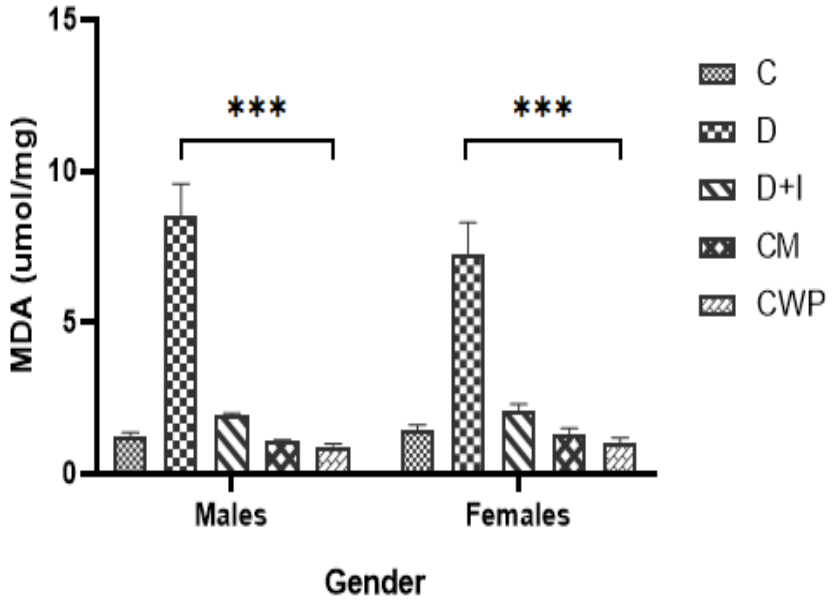


Figure 17 The results show the significant decrease in the MDA levels of both the male and female diabetic rats with raw camel milk and camel whey protein ($P<0.0001$).

4.7.3 Catalase Activity

Catalase activity is also associated with the oxidative stress. It is an enzyme responsible for the breakdown of hydrogen peroxide. By using the Aebi approach, liver enzymatic reactions are used to gauge the levels of catalase enzyme in the therapy groups. On induction of STZ to the normal male and female rats, the significant increase happened to the diabetic (D) group ($P<0.0001$). On treatment, effect was compared to the D+I, CM and CWP group and significant results were observed. The analyze the results, one way ANOVA was applied, and the individual analysis was performed. In males, on comparison to the diabetic group, the results obtained had the P-value 0.0028 for CM and 0.0007 for CWP. In females, on comparison, the obtained P-value was the 0.0007 for CM and 0.0001 or CWP. The figure showing the respective effect is given below:

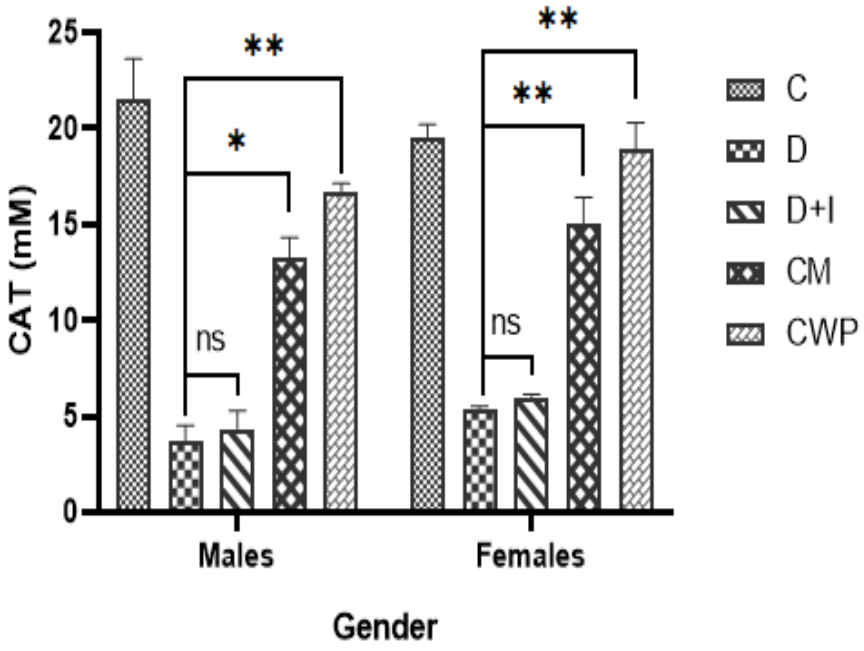


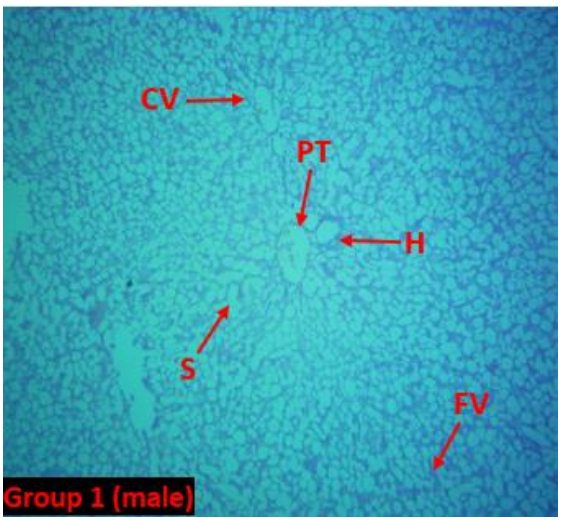
Figure 18 The results show the significant increase in the CAT levels of both the male and female diabetic rats with raw camel milk and camel whey protein ($P<0.0001$).

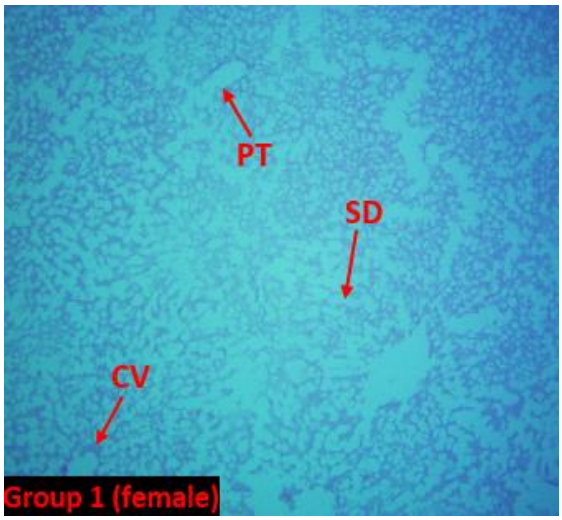
4.9 Histopathological Examination of Organs

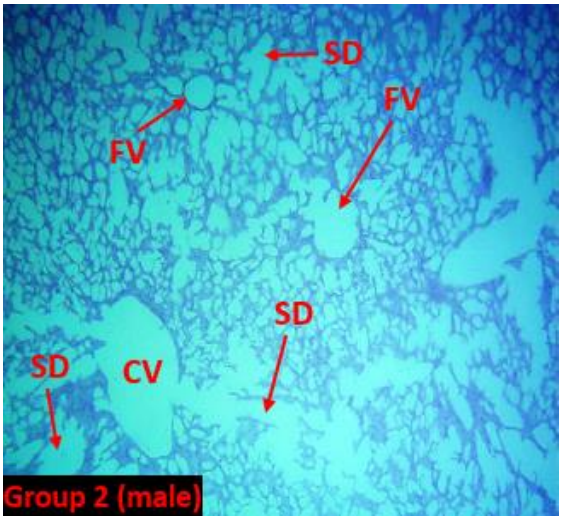
To determine the effect of diabetes and the effect of correction mechanism by CM and CWP on the body organs i.e., liver and kidney, the histopathological examination was performed using Hematoxylin and Eosin (H&E) stain to understand the prognosis of disease. In this histopathology staining scheme, the shape of cells, their structure and the arrangement of cells in the tissue was determined. All the steps of histopathological staining: Dehydration, Paraffinization, microtome cutting, staining and microscopic analysis, were performed in the same lab, ASAB, NUST.

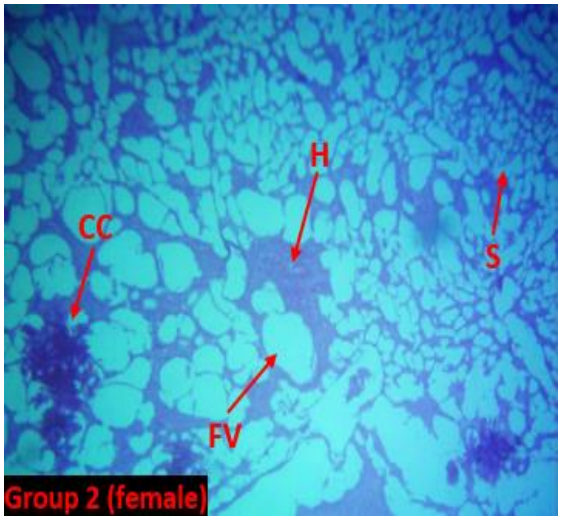
4.8.1 Histopathology of Liver

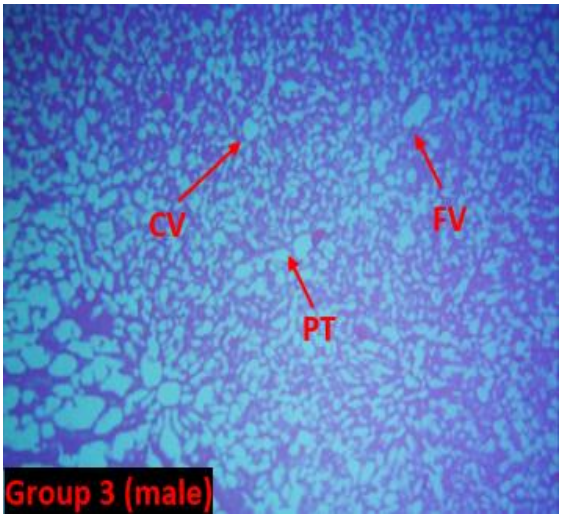
The points to be assessed in the liver microscopic analysis were the central vein that is surrounded by the hepatocytes in form of hexagonal arrangement. The sinusoids are the empty spaces between the hepatocytes that consists of Kupffer cells. There also lies the portal triad, the comprises three structures continued with it i.e., hepatic arteriole, portal vein and bile duct. the figure representing the structures found in the diabetic, non-diabetic and treated groups are given below.

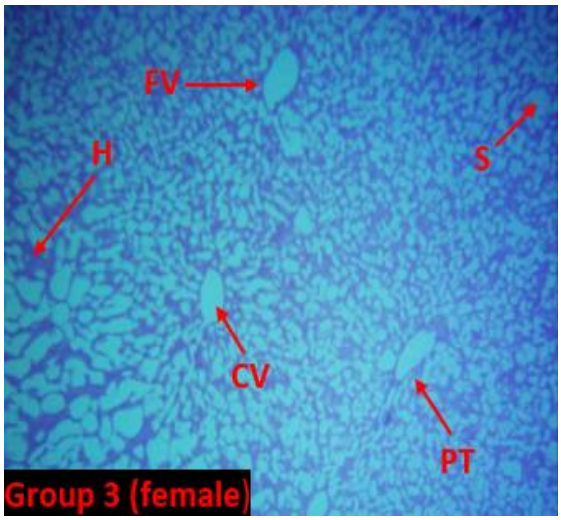


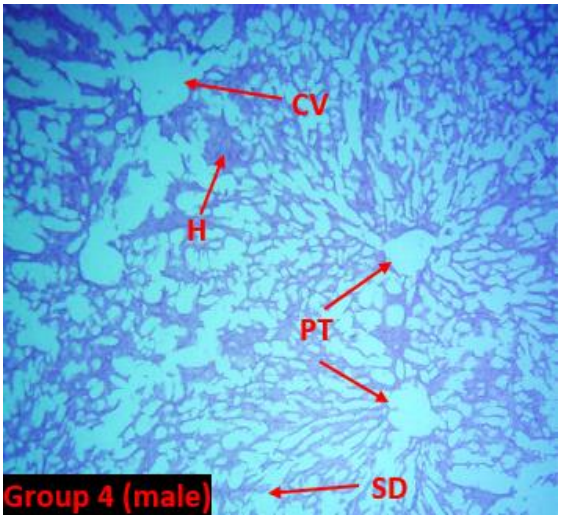


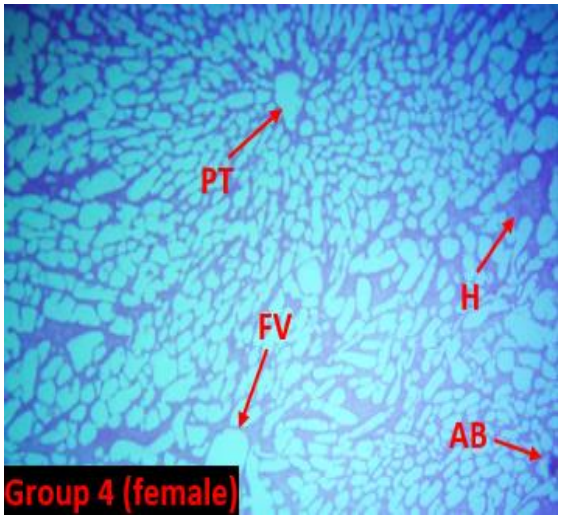

SD
FV
FV
SD
SD
CV
Group 2 (male)


H
CC
S
FV
Group 2 (female)


CV
FV
PT
Group 3 (male)


FV
H
S
CV
PT
Group 3 (female)


CV
H
PT
SD
Group 4 (male)


PT
H
FV
AB
Group 4 (female)

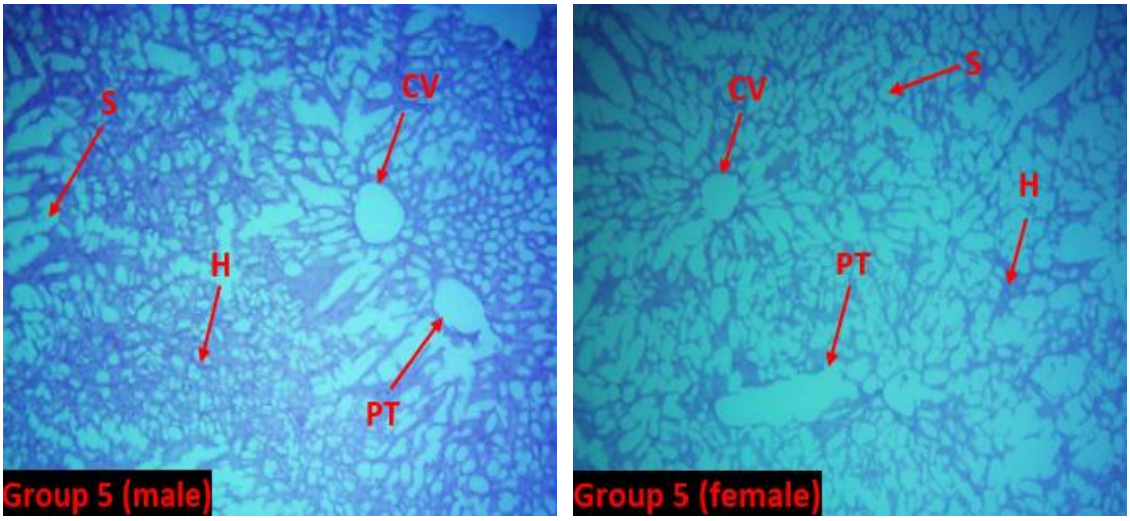
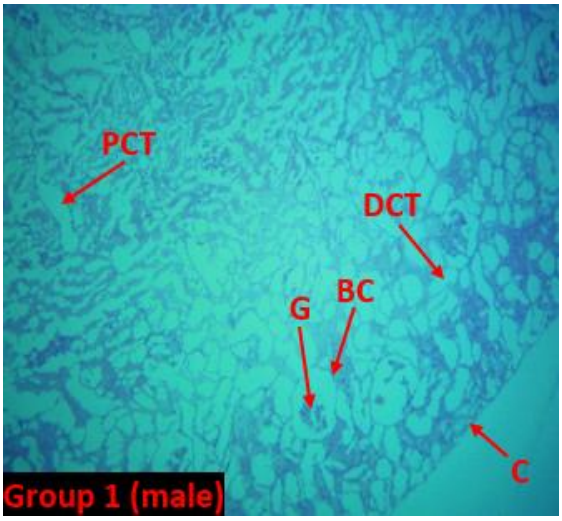
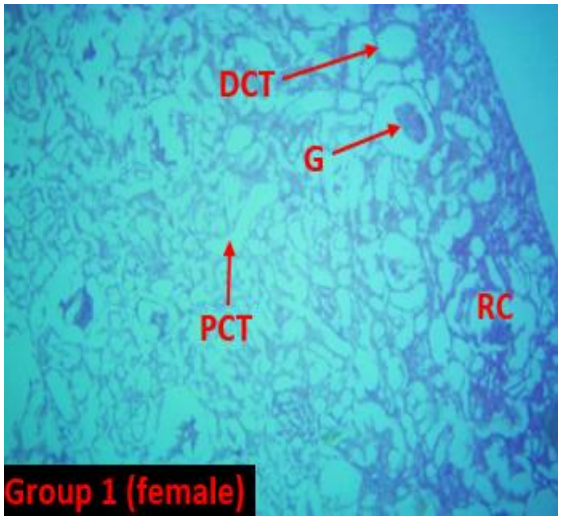


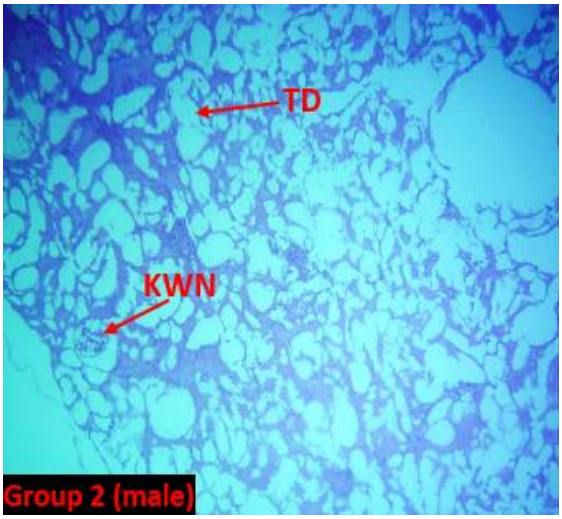
Figure 19 The images show the histopathological analysis of liver tissue of all diabetic, non-diabetic and treated rats at 10X. Group 1 presenting the normal (N) rat's tissue. Group 2 presenting the negative control (D) rat's tissue. Group 3 presenting the positive control (D+I) rat's tissue. Group presenting the camel milk (CM) treated group rat's tissue. Group 5 presenting the camel whey protein (CWP) treated group rat's tissue.

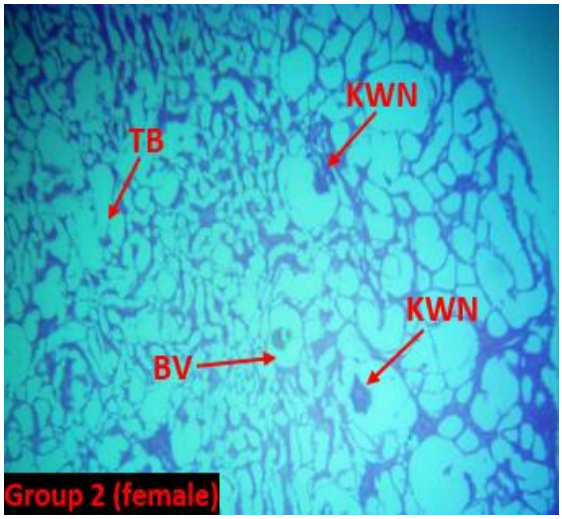
2.8.2 Histopathology of Kidney

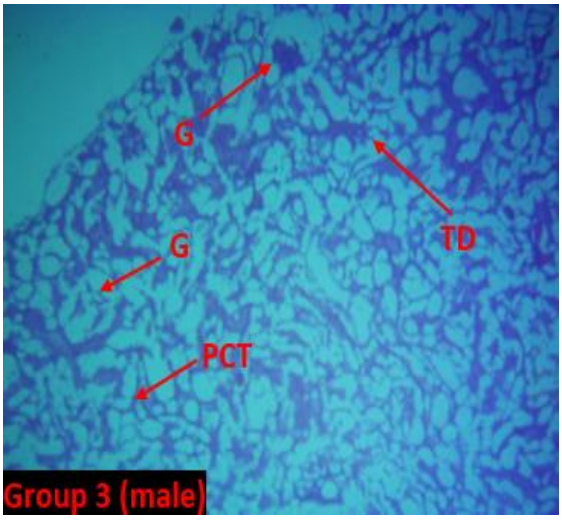
The microscopic analysis of the kidney involves the identification of its structures and the cell arrangement. The points that were observed were the outer cortex, inner medulla, corticomedullary region, glomerulus, Bowman's capsule, Bowman's fluid, Convoluted Tubules, tubular dilation, apoptic bodies, Kimmelstiel wilson nodules. The images presenting the comparison of diabetic, non-diabetic and the treated tissue structures are represented in the figure.

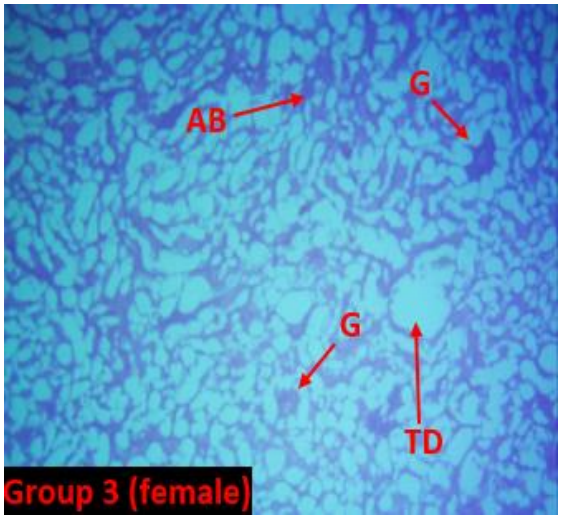

PCT
DCT
G
BC
C
Group 1 (male)


DCT
G
PCT
RC
Group 1 (female)


TD
KWN
Group 2 (male)


KWN
TB
KWN
BV
Group 2 (female)


G
TD
G
PCT
Group 3 (male)


AB
G
G
TD
Group 3 (female)

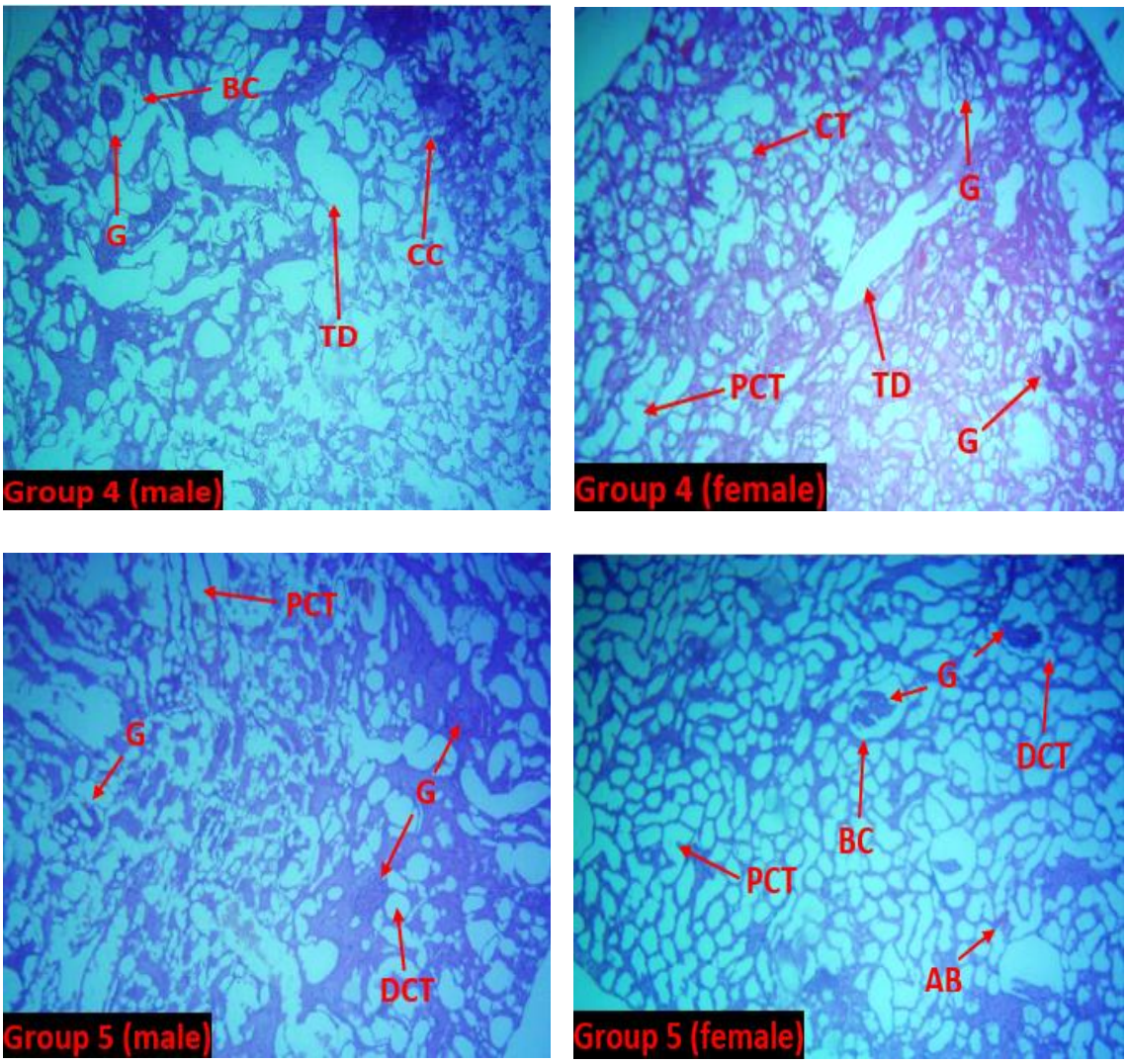


Figure 20 The images show the histopathological analysis of kidney tissue of all diabetic, non-diabetic and treated rats at 10X. Group 1 presenting the normal (N) rat's tissue. Group 2 presenting the negative control (D) rat's tissue. Group 3 presenting the positive control (D+I) rat's tissue. Group presenting the camel milk (CM) treated group rat's tissue. Group 5 presenting the camel whey protein (CWP) treated group rat's tissue.

Chapter 5

Discussion

Discussion

Traditional medicine is frequently employed as a kind of public health care (Mansour *et al.*, 2017) and the Arab nomads are known to use variety of naturally available products. A number of articles are available that documents the benefits of camel milk and its products (Brezovečki *et al.,* 2015; Kilari *et al.,* 2021; Nongonierma *et al.,* 2018; Venkatakrishnan *et al.,* 2019). The current study has been designed by focusing the most prevalent disease, Type 2 Diabetes. In this study, the effect of camel milk (CM) and the camel whey protein (CWP) has been evaluated on the STZ-induced diabetic rats. The results showed the effect of CM and CWP on the serum glucose levels (Kilari *et al.,* 2021), body weight (Mirmiran *et al.,* 2017), lipid profile, renal and hepatic enzymes (Kilari *et al.,* 2021) and antioxidant activity (Asmat *et al.,* 2016) on diabetic rats. This has been done on the basis that the CM and CWP protein contains insulin like proteins (Shori, 2015) and various proteinaceous components (Sayed *et al.,* 2017) that are protected from the proteases in the acidic environment and absorbs directly into the intestine and helps in lowering the blood glucose levels and effects significantly on the different body parameters (Galicia-Garcia *et al.,* 2020). Due to the CM stability in the acidic environment and the good buffering capacity, it is considered to have good and unique properties. It contains roughly 52 micro unit/ml of insulin-like protein compared to cow milk's 16.32 micro unit/ml with plays the important role in insulin interaction with the receptor (Shori, 2015). Renal and hepatic disorders are strongly correlated with diabetes (Alsahli & Gerich, 2015). CM and CWP protects the liver and kidney from failure by reinstating the blood glucose levels (Mirmiran *et al.,* 2017). Diabetes also creates the oxidative stress environment by decreasing the SOD and CAT levels and by

increasing the MDA levels (Saleh *et al.,* 2016). Due to the distinctive properties of the CM and CWP, it has been reported that these aid in normalizing the oxidative stress at the cellular levels also (Sayed *et al.,* 2017). Diabetes results in physio-metabolic abnormalities such as a decrease in body weight gain, an increase in food consumption, and an increase in urine volume due to the death of beta cells and interruption of insulin release. A prominent indicator of diabetes brought on by STZ is weight loss. It is possible that the diabetic rats' weight loss is the result of protein breakdown brought on by a lack of glucose as an energy source. Compared to normal rats, diabetic rats consume more food and have lower body weights, which suggests that they are polyphagic and lose weight through excessive tissue protein breakdown (Al-Attar & Alsalmi, 2019). The increased amounts of leucine, isoleucine, and valine in WP may have an anti-obesity impact by directing energy use toward muscle protein synthesis rather than toward fat formation (Ejtahed *et al.,* 2015)

The aim of our research is to prepare the camel whey protein from the camel milk and the treatment of the prepared CWP along with the camel milk towards the type 2 diabetes. The prepared camel whey protein was about 95% protein that consists of alpha lactalbumin, serum albumin, lysozyme, lactoferrin, peptidoglycan recognition protein, lactoperoxidase and immunoglobulins (Jansson *et al.,* 2020). The SEM analysis of the prepared CWP showed the irregular continuous cavity like structures with the continuity of smooth surfaces on the surface of protein. The results of SEM were consistent with the previous study (Ahmad *et al.,* 2019). The FTIR analysis of the CWP showed the presence of C-N, C-O, N=O, C=C, C=N, N-H at the specific frequency band predicting the presence of various proteins at the surface of CWP.

In the in-vivo model, the CM and CWP showed an effect on various biochemical parameters and the changes at the cellular level when compared to the diabetic group. In our findings, CM and CWP were observed to increase the body weight of rats that was decreased due to diabetes. The diabetic group treated with the CWP showed more significant increase in body weight compared to the CM group (Mirmiran *et al.,* 2017). Furthermore, diabetes causes the increase in blood glucose levels. CM and CWP both played a significant role in normalizing the blood glucose levels of the diabetic rats. These results were also consistent with the previous study (Kilari *et al.,* 2021).

Among the liver function tests, the significant rise in the ALT and ALP levels ($P<0.0001$) and decrease in the albumin levels ($P<0.0001$) was observed when diabetic control was compared to the normal control. In the treated group, the reversal of the parameters ALT and ALP levels ($P<0.0001$) and albumin ($P<0.0004$, $P=0.0003$) was observed when treated with CWP. The results were more significant with CWP as compared to CM. The various factors associated with the imbalances of liver enzymes includes the insulin resistance that results in glycogenolysis, elevated free fatty acids that causes hepatocytes toxicity, oxidative stress due to lipid peroxidation and the proinflammatory cytokines etc. all these factors result in hepatocellular disruption (Teshome *et al.,* n.d.) (Shibabaw *et al.,* 2019). The repairment of central vacuole of the liver with the appearance of tiny dak nuclei in the hepatocytes demonstrated the recovery of the liver tissue on treatment with the CM and CWP. CWP effect on the liver histopathology was much more effective than CM (Zuberu *et al.,* 2017).

Antioxidant activity of the liver enzymes both the CM and CWP was also reversed and showed significance. The antioxidant biomarkers analyzed were the SOD, MDA and

CAT. The MDA activity in the diabetic rats was suppressed due to the lipid peroxidation of PUFA that resulted in the increase in MDA levels. Treatment with the CM and CWP reversed the oxidative stress of the liver. MDA levels of the treated groups showed the significant effect by both CM and CWP ($P<0.0001$) when compared to the diabetic control. The observed results were in accordance with the previous study (Sayed *et al.*, 2017). the other two enzymes CAT and SOD was lowered when the diabetes was induced to the experimental rats. SOD is known to provide first line of defence against the ROS-mediated harm. SOD activity of the diabetic rats was normalized by the treatment with CM and CWP significantly. CAT enzyme in the body is responsible for the breakdown of hydrogen peroxide. The oxidative damage results in the decline of CAT levels in diabetic rats. Correction mechanism with the CM and CWP reverted the effect of the catalase enzyme to the normal level. In this case, the CWP ($P=0.0001$) as compared to the CM ($P=0.0007$) showed more significant result on the CAT levels. The results obtained during the testing were in accordance with the previous literature. These effects and reversals contribute to the pathological alterations of the immune organs(Sayed *et al.*, 2017).

Both the CM and CWP showed the significant decline in the TCHO, HDLC and TG ($P<0.0001$) and significantly increase in LDLC levels. In case of TCHO, both CM and CWP show the significant decrease ($P<0.0001$). In case of TG, CWP showed more significant result compared to CM. For LDLC, significant results were observed for both CM and CWP. In our study, it was observed that the CWP showed more significance to results when compared to CM. These effects are thought to be effective because of the significant cholesteryl esterase inhibition activity performed more effectively by the

CWPs. It is also because of the fact that the CWP significantly reduces the serum lipids ad lipoproteins. The results of our findings were consistent with the previous studies by performed by (Althwab *et al.,* 2020) (Ejtahed *et al.,* 2015)

When diagnosing kidney injury, serum creatinine and uric acid levels are employed as nephrotoxicity markers. CM and CWP are observed to reduce the creatinine levels ($P<0.0001$) in the body by acting on the insulin activity and reverse the insulin resistance effect. The uric acid also got reversed by treating the diabetic group with the CM and CWP. The observed P-value was the 0.0008. the results of creatinine and uric acid were consistent with the study done by the (Shaban *et al.,* 2022). Histopathological analysis of kidney showed the atrophy of glomerulus and the deformative effect renal corpuscle along with the loss of striped appearance in medullary region It also provides the positive effect at the cellular level by reducing sinusoid dilutions, repairing the glomerulus activity thus presenting the intact and normal tissue structure of the kidney (Arab *et al.,* 2021)(Abdel-Salam *et al.,* 2016).

Conclusion

Conclusion

CM and CWP are the traditional productions that contains many health-related properties. A number of articles are available that documents the benefits of camel milk and its products. Camel milk has been used long by the nomadic people because of its natural anti diabetic potential. In this study, the comparative effect of CM and CWP was evaluated. CWP were prepared from the milk and were analyzed through Spectrophotometric analysis, SEM and FTIR. In the in-vivo experiment, the results were evaluated on streptozotocin induced diabetic rats and has been shown that it has a strong hypoglycemic effect. The given treatment by using CM and CWP improved the lipid, renal and hepatic parameters. It also showed the improved and enhanced immunity of the body by decreasing the oxidative stress of the body. Also, the effect was confirmed by the histopathological analysis of the liver and kidney tissue whose normal structure determined the effect of camel milk and camel whey protein of diabetes.

References

References

Aamir, A. H., Ul-Haq, Z., Fazid, S., Shah, B. H., Raza, A., Jawa, A., Mahar, S. A., Ahmad, I., Qureshi, F. M., & Heald, A. H. (2020). Type 2 diabetes prevalence in Pakistan: what is driving this? Clues from subgroup analysis of normal weight individuals in diabetes prevalence survey of Pakistan. *Cardiovascular Endocrinology and Metabolism*, *9*(4), 159–164. https://doi.org/10.1097/XCE.0000000000000212

Abdel-Salam, B., Ebaid, H., Al-Tamimi, J., Alhazza, I., & Alhazza, I. M. (2016). Enhancement of Wound Healing by Un-denatured Camel Whey Proteins in Protein Malnourished Mice Fetal programming diseases View project Enhancement of Wound Healing by Un-denatured Camel Whey Proteins in Protein Malnourished Mice. In *Pakistan J. Zool. vol* (Vol. 48, Issue 1). https://www.researchgate.net/publication/289124352

Abiola, D., Sathyapalan, T., & Hepburn, D. (2016). Management of type 1 and type 2 diabetes requiring insulin. *Prescriber*, *27*(9), 50–57. https://doi.org/10.1002/psb.1500

Agrawal, R. P., Kochar, D. K., Sahani, M. S., Tuteja, F. C., & Ghorui, S. K. (2004). HYPOGLYCEMIC ACTIVITY OF CAMEL MILK IN STREPTOZOTOCIN INDUCED DIABETIC RATS. In *INT. J. DIAB. DEV. COUNTRIES* (Vol. 24).

Ahmad, M., Mudgil, P., & Maqsood, S. (2019). Camel whey protein microparticles for safe and efficient delivery of novel camel milk derived probiotics. *LWT*, *108*, 81–88. https://doi.org/10.1016/j.lwt.2019.03.008

Akindykova, A., Cakir-Kiefer, C., Baubekova, A., & Jurjanz, S. (2019). Isolation and characterization of camel milk proteins. *International Journal of Biology and Chemistry*, *12*(1), 5–10. https://doi.org/10.26577/ijbch-2019-i1-2ï

Al-Attar, A. M., & Alsalmi, F. A. (2019). Effect of Olea europaea leaves extract on streptozotocin induced diabetes in male albino rats. *Saudi Journal of Biological Sciences*, *26*(1), 118–128. https://doi.org/10.1016/j.sjbs.2017.03.002

Alfadhli, E. M. (2015). Gestational diabetes mellitus. *Saudi Medical Journal*, *36*(4), 399–406. https://doi.org/10.15537/smj.2015.4.10307

Allam, A., Ajarem, J., Ebaid, H., Al-Tamimi, J., Metwalli, A., Zohir, K., Rady, A., Alhazza, I. M., & Ibrahim, K. E. (2015). attachment Effect of STZ-Induced Diabetes on Spleen of Rats: Improvement by Camel Whey Proteins. *Pakistan J. Zool*, *47*(4), 1109–1116.

Alsahli, M., & Gerich, J. E. (2015). Hypoglycemia in patients with diabetes and renal disease. In *Journal of Clinical Medicine* (Vol. 4, Issue 5, pp. 948–964). MDPI. https://doi.org/10.3390/jcm4050948

Althwab, S. A., Mousa, H. M., Ashoush, I. S., & Hamad, E. M. (2020). *Original paper Reduction of Lipid Profile and Adipocyte Size in Rats Fed on High-Fat Diet Using Camel Milk and Whey Protein Mixture Impact of Macronutrient Modified High-energy Supplements on Metabolic Health View project Wild poisonous plants of Qassim Region (Saudi Arabia), and their effect on Human and animal Health View project*. https://doi.org/10.3136/fstr.26

Arab, H. H., Eid, A. H., Gad, A. M., Yahia, R., Mahmoud, A. M., & Kabel, A. M. (2021). Inhibition of oxidative stress and apoptosis by camel milk mitigates

cyclosporine-induced nephrotoxicity: Targeting Nrf2/HO-1 and AKT/eNOS/NO pathways. *Food Science and Nutrition*, *9*(6), 3177–3190. https://doi.org/10.1002/fsn3.2277

Asmat, U., Abad, K., & Ismail, K. (2016). Diabetes mellitus and oxidative stress—A concise review. In *Saudi Pharmaceutical Journal* (Vol. 24, Issue 5, pp. 547–553). Elsevier B.V. https://doi.org/10.1016/j.jsps.2015.03.013

Bahbahani, H., & Almathen, F. (2022). Homogeneity of Arabian Peninsula dromedary camel populations with signals of geographic distinction based on whole genome sequence data. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-021-04087-w

Banday, M. Z., Sameer, A. S., & Nissar, S. (2020). Pathophysiology of diabetes: An overview. *Avicenna Journal of Medicine*, *10*(04), 174–188. https://doi.org/10.4103/ajm.ajm_53_20

Birsen Bulut Solak, & Nihat Akin. (2012). Health Benefits of Whey Protein: A Review. *Journal of Food Science and Engineering*, *2*(3). https://doi.org/10.17265/2159-5828/2012.03.001

Brezovečki, A., Čagalj, M., Dermit, Z. F., Mikulec, N., Ljoljić, D. B., & Antunac, N. (2015a). Devino mlijeko i proizvodi od devinog mlijeka. *Mljekarstvo*, *65*(2), 81–90. https://doi.org/10.15567/mljekarstvo.2015.0202

Brezovečki, A., Čagalj, M., Dermit, Z. F., Mikulec, N., Ljoljić, D. B., & Antunac, N. (2015b). Devino mlijeko i proizvodi od devinog mlijeka. *Mljekarstvo*, *65*(2), 81–90. https://doi.org/10.15567/mljekarstvo.2015.0202

Brooks-Worrell, B. M., Boyko, E. J., & Palmer, J. P. (2014). Impact of islet autoimmunity on the progressive β-cell functional decline in type 2 diabetes. *Diabetes Care*, *37*(12), 3286–3293. https://doi.org/10.2337/dc14-0961

Bs, A. O. (2018a). *Effects of industrial processing methods on skimmed camel milk properties*.

Bs, A. O. (2018b). *Effects of industrial processing methods on skimmed camel milk properties*.

Campbell, J. E., & Drucker, D. J. (2013). Pharmacology, physiology, and mechanisms of incretin hormone action. In *Cell Metabolism* (Vol. 17, Issue 6, pp. 819–837). https://doi.org/10.1016/j.cmet.2013.04.008

Davis-Richardson, A. G., & Triplett, E. W. (2015). A model for the role of gut bacteria in the development of autoimmunity for type 1 diabetes. In *Diabetologia* (Vol. 58, Issue 7, pp. 1386–1393). Springer Verlag. https://doi.org/10.1007/s00125-015-3614-8

Deepthi B, Sowjanya K, Lidiya B, Rs, B., & Ps, B. (2017). *A Modern Review of Diabetes Mellitus: An Annihilatory Metabolic Disorder*. http://www.imedpub.com/

Ejtahed, H. S., Naslaji, A. N., Mirmiran, P., Yeganeh, M. Z., Hedayati, M., Azizi, F., & Movahedi, A. M. (2015). Effect of Camel Milk on Blood Sugar and Lipid Profile of Patients With Type 2 Diabetes: A Pilot Clinical Trial. *International Journal of Endocrinology and Metabolism*, *13*(1). https://doi.org/10.5812/ijem.21160

Elkot, W. F. (2019). *Characterization and Healthier Properties of Whey proteins of Camel Milk: A Review* (Vol. 25, Issue 2). http://

Faraz, A., A, W., MM, N., & RH, M. (2018). Milk Production Potential of Marecha Dromedary Camel in Desert Thal Punjab, Pakistan. *Journal of Fisheries & Livestock Production*, *06*(03). https://doi.org/10.4172/2332-2608.1000280

Faraz, A., Iqbal Mustafa, M., Lateef, M., Yaqoob, M., & Younas, M. (2013). Production potential of camel and its prospects in Pakistan. Production potential of camel and its prospects in Pakistan. In *Punjab Univ. J. Zool* (Vol. 28, Issue 2).

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. In *International Journal of Molecular Sciences* (Vol. 21, Issue 17, pp. 1–34). MDPI AG. https://doi.org/10.3390/ijms21176275

Grossman, L. D., Roscoe, R., & Shack, A. R. (2018). Complementary and Alternative Medicine for Diabetes. *Canadian Journal of Diabetes*, *42*, S154–S161. https://doi.org/10.1016/j.jcjd.2017.10.023

Holst, J. J., & Ørskov, C. (2004). *The Incretin Approach for Diabetes Treatment Modulation of Islet Hormone Release by GLP-1 Agonism THE INCRETIN EFFECT IN TYPE 2 DIABETES*. http://diabetesjournals.org/diabetes/article-pdf/53/suppl_3/S197/379436/zdb1120400s197.pdf

Jakubowicz, D., & Froy, O. (2013a). Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and Type 2 diabetes. In *Journal of Nutritional Biochemistry* (Vol. 24, Issue 1, pp. 1–5). https://doi.org/10.1016/j.jnutbio.2012.07.008

Jakubowicz, D., & Froy, O. (2013b). Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and Type 2 diabetes. In *Journal of*

Nutritional Biochemistry (Vol. 24, Issue 1, pp. 1–5). https://doi.org/10.1016/j.jnutbio.2012.07.008

Jansson, T., Nielsen, S. B., Petersen, M. A., & Lund, M. N. (2020). Temperature-dependency of unwanted aroma formation in reconstituted whey protein isolate solutions. *International Dairy Journal*, *104*. https://doi.org/10.1016/j.idairyj.2020.104653

Khalafalla, A. I., Hashim, W. M., Yousif, G. M., Majid, A. A., Kalafalla, A. I., Abdalla, H. S., Eldein, S., & Ahmed, S. (2015). *DROMEDARY CAMELS IN SUDAN, TYPES AND SUB TYPES, DISTRIBUTION AND MOVEMENT*. www.ijpra.com

Khan, M. A. B., Hashim, M. J., King, J. K., Govender, R. D., Mustafa, H., & Kaabi, J. al. (2020). Epidemiology of Type 2 diabetes - Global burden of disease and forecasted trends. *Journal of Epidemiology and Global Health*, *10*(1), 107–111. https://doi.org/10.2991/JEGH.K.191028.001

Kharroubi, A. T. (2015). Diabetes mellitus: The epidemic of the century. *World Journal of Diabetes*, *6*(6), 850. https://doi.org/10.4239/wjd.v6.i6.850

Khursheed, R., Singh, S. K., Wadhwa, S., Kapoor, B., Gulati, M., Kumar, R., Ramanunny, A. K., Awasthi, A., & Dua, K. (2019). Treatment strategies against diabetes: Success so far and challenges ahead. In *European Journal of Pharmacology* (Vol. 862). Elsevier B.V. https://doi.org/10.1016/j.ejphar.2019.172625

Kilari, B. P., Mudgil, P., Azimullah, S., Bansal, N., Ojha, S., & Maqsood, S. (2021). Effect of camel milk protein hydrolysates against hyperglycemia, hyperlipidemia, and associated oxidative stress in streptozotocin (STZ)-induced diabetic rats.

Journal of Dairy Science, *104*(2), 1304–1317. https://doi.org/10.3168/jds.2020-19412

Kotwas, A., Karakiewicz, B., Zabielska, P., Wieder-Huszla, S., & Jurczak, A. (2021). Epidemiological factors for type 2 diabetes mellitus: evidence from the Global Burden of Disease. *Archives of Public Health*, *79*(1). https://doi.org/10.1186/s13690-021-00632-1

Malik, A., Al-Senaidy, A., Skrzypczak-Jankun, E., & Jankun, J. (2012). A study of the anti-diabetic agents of camel milk. *International Journal of Molecular Medicine*, *30*(3), 585–592. https://doi.org/10.3892/ijmm.2012.1051

Manders, R. J. F., Hansen, D., Zorenc, A. H. G., Dendale, P., Kloek, J., Saris, W. H. M., & van Loon, L. J. C. (2014). Protein co-ingestion strongly increases postprandial insulin secretion in type 2 diabetes patients. *Journal of Medicinal Food*, *17*(7), 758–763. https://doi.org/10.1089/jmf.2012.0294

Mansour, A. A., Nassan, M. A., Saleh, O. M., & Soliman, M. M. (2017). PROTECTIVE EFFECT OF CAMEL MILK AS ANTI-DIABETIC SUPPLEMENT: BIOCHEMICAL, MOLECULAR AND IMMUNOHISTOCHEMICAL STUDY. *African Journal of Traditional, Complementary, and Alternative Medicines : AJTCAM*, *14*(4), 108–119. https://doi.org/10.21010/ajtcam.v14i4.13

Marathe, C. S., Rayner, C. K., Jones, K. L., & Horowitz, M. (2013). Relationships between gastric emptying, postprandial glycemia, and incretin hormones. In *Diabetes Care* (Vol. 36, Issue 5, pp. 1396–1405). https://doi.org/10.2337/dc12-1609

Mati, A., Senoussi-Ghezali, C., Si Ahmed Zennia, S., Almi-Sebbane, D., El-Hatmi, H., & Girardet, J. M. (2017). Dromedary camel milk proteins, a source of peptides having

biological activities – A review. In *International Dairy Journal* (Vol. 73, pp. 25–37). Elsevier Ltd. https://doi.org/10.1016/j.idairyj.2016.12.001

(Meo, S. (Zia, I. (Bukhari, I. (Arian, S. (2106). Type 2 diabetes mellitus in Pakistan: Current prevalence and future forecast. *J Pak Med Assoc*, *66*(12), 1637–1642.

Mignone, L. E. (2015a). Whey protein: The "whey" forward for treatment of type 2 diabetes? *World Journal of Diabetes*, *6*(14), 1274. https://doi.org/10.4239/wjd.v6.i14.1274

Mignone, L. E. (2015b). Whey protein: The "whey" forward for treatment of type 2 diabetes? *World Journal of Diabetes*, *6*(14), 1274. https://doi.org/10.4239/wjd.v6.i14.1274

Mirmiran, P., Ejtahed, H. S., Angoorani, P., Eslami, F., & Azizi, F. (2017a). Camel milk has beneficial effects on diabetes mellitus: A systematic review. In *International Journal of Endocrinology and Metabolism* (Vol. 15, Issue 2). Kowsar Medical Institute. https://doi.org/10.5812/ijem.42150

Mirmiran, P., Ejtahed, H. S., Angoorani, P., Eslami, F., & Azizi, F. (2017b). Camel milk has beneficial effects on diabetes mellitus: A systematic review. In *International Journal of Endocrinology and Metabolism* (Vol. 15, Issue 2). Kowsar Medical Institute. https://doi.org/10.5812/ijem.42150

Mohammadabadi, T. (n.d.). *Cronicon EC NUTRITION Review Article Camel Milk; A Superfood as a Treatment for Diabetes*.

Mudgil, P., AlMazroui, M., Redha, A. A., Kilari, B. P., Srikumar, S., & Maqsood, S. (2022). Cow and camel milk-derived whey and casein protein hydrolysates demonstrated effective antifungal properties against selected Candida species.

Journal of Dairy Science, *105*(3), 1878–1888. https://doi.org/10.3168/jds.2021-20944

Nongonierma, A. B., Cadamuro, C., le Gouic, A., Mudgil, P., Maqsood, S., & Fitzgerald, R. J. (2018). *Dipeptidyl peptidase IV (DPP-IV) inhibitory properties of a camel whey protein enriched hydrolysate preparation*.

Ozougwu, O. (2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *Journal of Physiology and Pathophysiology*, *4*(4), 46–57. https://doi.org/10.5897/jpap2013.0001

Padhi, S., Nayak, A. K., & Behera, A. (2020). Type II diabetes mellitus: a review on recent drug based therapeutics. In *Biomedicine and Pharmacotherapy* (Vol. 131). Elsevier Masson SAS. https://doi.org/10.1016/j.biopha.2020.110708

Pal, S., & Ellis, V. (2010). The acute effects of four protein meals on insulin, glucose, appetite and energy intake in lean men. *British Journal of Nutrition*, *104*(8), 1241–1248. https://doi.org/10.1017/S0007114510001911

Rahmeh, R., Alomirah, H., Akbar, A., & Sidhu, J. (n.d.). *Composition and Properties of Camel Milk*. www.intechopen.com

Richards, P., Thornberry, N. A., & Pinto, S. (2021). The gut–brain axis: Identifying new therapeutic approaches for type 2 diabetes, obesity, and related disorders. In *Molecular Metabolism* (Vol. 46). Elsevier GmbH. https://doi.org/10.1016/j.molmet.2021.101175

Roden, M., Petersen, K., & Shulman, G. (2017). *3 Insulin Resistance in Type 2 Diabetes*.

Saini, V. (2010). Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. *World Journal of Diabetes*, *1*(3), 68. https://doi.org/10.4239/wjd.v1.i3.68

Saleh, O. M., Awad, N. S., Soliman, M. M., Mansour, A. A., & Nassan, M. A. (2016). Insulin-mimetic activity of stevioside on diabetic rats: Biochemical, molecular and histopathological study. *African Journal of Traditional, Complementary and Alternative Medicines*, *13*(2), 156–163. https://doi.org/10.4314/ajtcam.v13i2.19

Salehi, A., Gunnerud, U., Muhammed, S. J., Östman, E., Holst, J. J., Björck, I., & Rorsman, P. (2012). *The insulinogenic effect of whey protein is partially mediated by a direct effect of amino acids and GIP on β-cells*. http://www.nutritionandmetabolism.com/content/9/1/48

Sayed, L. H., Badr, G., Omar, H. M., Abd El-Rahim, A. M., & Mahmoud, M. H. (2017a). Camel whey protein improves oxidative stress and histopathological alterations in lymphoid organs through Bcl-XL/Bax expression in a streptozotocin-induced type 1 diabetic mouse model. *Biomedicine and Pharmacotherapy*, *88*, 542–552. https://doi.org/10.1016/j.biopha.2017.01.076

Sayed, L. H., Badr, G., Omar, H. M., Abd El-Rahim, A. M., & Mahmoud, M. H. (2017b). Camel whey protein improves oxidative stress and histopathological alterations in lymphoid organs through Bcl-XL/Bax expression in a streptozotocin-induced type 1 diabetic mouse model. *Biomedicine and Pharmacotherapy*, *88*, 542–552. https://doi.org/10.1016/j.biopha.2017.01.076

Sazmand, A., Joachim, A., & Otranto, D. (2019). Zoonotic parasites of dromedary camels: So important, so ignored. In *Parasites and Vectors* (Vol. 12, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13071-019-3863-3

Shaban, A. M., Raslan, M., Qahl, S. H., Elsayed, K., Abdelhameed, M. S., Oyouni, A. A. A., Al-Amer, O. M., Hammouda, O., & El-Magd, M. A. (2022). Ameliorative

Effects of Camel Milk and Its Exosomes on Diabetic Nephropathy in Rats. *Membranes*, *12*(11), 1060. https://doi.org/10.3390/membranes12111060

Shibabaw, T., Dessie, G., Molla, M. D., Zerihun, M. F., & Ayelign, B. (2019). Assessment of liver marker enzymes and its association with type 2 diabetes mellitus in Northwest Ethiopia. *BMC Research Notes*, *12*(1). https://doi.org/10.1186/s13104-019-4742-x

Shori, A. B. (2015). Camel milk as a potential therapy for controlling diabetes and its complications: A review of in vivo studies. In *Journal of Food and Drug Analysis* (Vol. 23, Issue 4, pp. 609–618). Elsevier Taiwan LLC. https://doi.org/10.1016/j.jfda.2015.02.007

Skyler, J. S., Bakris, G. L., Bonifacio, E., Darsow, T., Eckel, R. H., Groop, L., Groop, P. H., Handelsman, Y., Insel, R. A., Mathieu, C., McElvaine, A. T., Palmer, J. P., Pugliese, A., Schatz, D. A., Sosenko, J. M., Wilding, J. P. H., & Ratner, R. E. (2017). Differentiation of diabetes by pathophysiology, natural history, and prognosis. In *Diabetes* (Vol. 66, Issue 2, pp. 241–255). American Diabetes Association Inc. https://doi.org/10.2337/db16-0806

Teshome, G., Ambachew, S., Fasil, A., & Abebe, M. (n.d.). *Prevalence of liver function test abnormality and associated factors in type 2 diabetes mellitus: a comparative cross-sectional study*.

Tovar Jiménez, X., Cuenca, A. A., Jurado, A. T., Corona, A. A., Rosario, C., & Urista, M. (2012). Traditional Methods for Whey Protein Isolation and Concentration: Effects on Nutritional Properties and Biological Activity Traditional Methods for

Whey Protein Isolation and Concentration: Effects on Nutritional Properties and Biological Activity Article. In *Chem. Soc* (Vol. 2012, Issue 4).

Venkatakrishnan, K., Chiu, H. F., & Wang, C. K. (2019). Popular functional foods and herbs for the management of type-2-diabetes mellitus: A comprehensive review with special reference to clinical trials and its proposed mechanism. In *Journal of Functional Foods* (Vol. 57, pp. 425–438). Elsevier Ltd. https://doi.org/10.1016/j.jff.2019.04.039

Vos, T., Lim, S. S., Abbafati, C., Abbas, K. M., Abbasi, M., Abbasifard, M., Abbasi-Kangevari, M., Abbastabar, H., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abolhassani, H., Aboyans, V., Abrams, E. M., Abreu, L. G., Abrigo, M. R. M., Abu-Raddad, L. J., Abushouk, A. I., … Murray, C. J. L. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *396*(10258), 1204–1222. https://doi.org/10.1016/S0140-6736(20)30925-9

Wondmkun, Y. T. (2020). Obesity, insulin resistance, and type 2 diabetes: Associations and therapeutic implications. In *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* (Vol. 13, pp. 3611–3616). Dove Medical Press Ltd. https://doi.org/10.2147/DMSO.S275898

Zaccardi, F., Webb, D. R., Yates, T., & Davies, M. J. (n.d.). *Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective*. https://doi.org/10.1136/postgradmedj

Zibaee, S., Hosseini, S. M. al-reza, Yousefi, M., Taghipour, A., Kiani, M. A., & Noras, M. R. (2015). Nutritional and Therapeutic Characteristics of Camel Milk in

Children: A Systematic Review. *Electronic Physician*, *7*(7), 1523–1528. https://doi.org/10.19082/1523

Zuberu, J., Saleh, M. I. A., Alhassan, A. W., Adamu, B. Y., Aliyu, M., & Iliya, B. T. (2017). Hepatoprotective effect of camel milk on poloxamer 407 induced hyperlipidaemic wistar rats. *Open Access Macedonian Journal of Medical Sciences*, *5*(7), 852–858. https://doi.org/10.3889/oamjms.2017.158

Annex A

Atta-Ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences & Technology (NUST)

Research Project Title: Streptozotocin Induced Antidiabetic Treatment in Rats by using Whole Camel Whey Protein and Purified Whey Protein

Name of Principal Investigator	Dr Tahir Ahmad Baig
Duration	2-3 Months
Name of School/Department	ASAB-NUST
IRB No.	09-2021-01/03

The project entitled above has been reviewed by the NUST Ethical Review Committee Meeting held on **September 27, 2021.** Keeping in view the following mentioned areas.

- Qualification and Expertise of the Principle Investigator.
- Proposed Goals of the Study
- Selection Criteria of the Subjects
- Informed Consent in local language if required
- Potential Problems
- Research Design and Methods
- Potential Benefits of the study
- Risks of the Study
- Assessment & Management of Risk
- Confidentiality & Conflict of Interest.

The Committee approves above entitled project on scale and criteria given below to be implemented before/during project execution.

- Safety measures of carcinogenic chemicals.
- Designated space/work place/rooms for the experimental work.
- Protection of other research students/lab staff/animals from chemical hazardous.

The Ethical Review Committee reserves the rights to re-review the project during the project execution to address the suggested guidelines.

Prof. Dr. Peter John
Deptt of Healthcare Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Prof. Dr. Peter John

President Ethical Review Committee

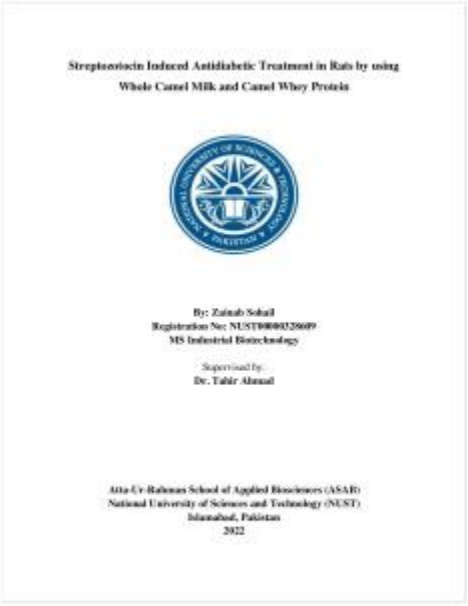
turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Turnitin 1
Assignment title:	Plagiarism Detection 1 Part 1 (Moodle TT)
Submission title:	Streptozotocin Induced Antidiabetic Treatment in Rats by us...
File name:	138194_Turnitin_1_Streptozotocin_Induced_Antidiabetic_Tre...
File size:	1.06M
Page count:	93
Word count:	16,537
Character count:	96,658
Submission date:	22-Nov-2022 11:43AM (UTC-0800)
Submission ID:	1893047193



Streptozotocin Induced Antidiabetic Treatment in Rats by using Whole Camel Milk and Camel Whey Protein

By: Zainab Sohail
Registration No: NUST000320689
MS Industrial Biotechnology

Supervised by:
Dr. Tahir Ahmad

Ata-Ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan
2022

Evaluation of Antidiabetic Potential of Whole Camel Milk and Camel Whey Protein in Streptozotocin Induced Diabetic Rats by Zainab Sohail

ORIGINALITY REPORT

17%	11%	9%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Higher Education Commission Pakistan Student Paper	2%
2	Submitted to Savitribai Phule Pune University Student Paper	1%
3	dmd.aspetjournals.org Internet Source	1%
4	Linda E Mignone. "Whey protein: The "whey" forward for treatment of type 2 diabetes?", World Journal of Diabetes, 2015 Publication	1%
5	www.mdpi.com Internet Source	1%
6	www.ncbi.nlm.nih.gov Internet Source	<1%
7	environhealthprevmed.biomedcentral.com Internet Source	<1%

8	Submitted to University of Melbourne Student Paper	<1%
9	Priti Mudgil, Sajid Maqsood. "Bioactive peptides derived from camel milk proteins", Elsevier BV, 2023 Publication	<1%
10	www.news-medical.net Internet Source	<1%
11	Submitted to United Colleges Group - UCG Student Paper	<1%
12	prr.hec.gov.pk Internet Source	<1%
13	worldwidescience.org Internet Source	<1%
14	lipidworld.biomedcentral.com Internet Source	<1%
15	pubmed.ncbi.nlm.nih.gov Internet Source	<1%
16	Submitted to Manchester Metropolitan University Student Paper	<1%
17	www.inabj.org Internet Source	<1%
18	Fernanda M. Ferreira, António J. Moreno, Raquel Seiça, Maria S. Santos, Carlos M.	<1%

	Palmeira. "Mitochondrial Bioenergetics, Diabetes, and Aging: Top-Down Analysis Using the Diabetic Goto-Kakizaki (GK) Rat as a Model", Toxicology Mechanisms and Methods, 2008 Publication	
19	Advances in Experimental Medicine and Biology, 2003. Publication	<1 %
20	Leila H. Sayed, Gamal Badr, Hossam M. Omar, Ali M. Abd El-Rahim, Mohamed H. Mahmoud. "Camel whey protein improves oxidative stress and histopathological alterations in lymphoid organs through Bcl-XL/Bax expression in a streptozotocin-induced type 1 diabetic mouse model", Biomedicine & Pharmacotherapy, 2017 Publication	<1 %
21	www.researchgate.net Internet Source	<1 %
22	Atef M. Al-Attar, Fawziah A. Alsalmi. "Effect of Olea europaea leaves extract on streptozotocin induced diabetes in male albino rats", Saudi Journal of Biological Sciences, 2017 Publication	<1 %
23	www.karger.com Internet Source	<1 %

24	Submitted to rtc Student Paper	<1%
25	researchspace.ukzn.ac.za Internet Source	<1%
26	Submitted to University of Cape Town Student Paper	<1%
27	Jafari, Mahvash, Maryam Salehi, Alireza Asgari, Sediq Ahmadi, Maryam Abbasnezhad, Reza Hajihoosani, and Mansoure Hajigholamali. "Effects of paraoxon on serum biochemical parameters and oxidative stress induction in various tissues of Wistar and Norway rats", Environmental Toxicology and Pharmacology, 2012. Publication	<1%
28	Raghvendar Singh, Gorakh Mal, Devendra Kumar, N. V. Patil, K. M. L. Pathak. "Camel Milk: An Important Natural Adjuvant", Agricultural Research, 2017 Publication	<1%
29	agnee.tezu.ernet.in:8082 Internet Source	<1%
30	patents.google.com Internet Source	<1%
31	A. Shipp, G. Lawrence, R. Gentry, T. McDonald, H. Bartow, J. Bounds, N. Macdonald, H.	<1%

	Clewell, B. Allen, C. Van Landingham. "Acrylamide: Review of Toxicity Data and Dose-Response Analyses for Cancer and Noncancer Effects", Critical Reviews in Toxicology, 2008 Publication	
32	nutritionandmetabolism.biomedcentral.com Internet Source	<1%
33	Submitted to Bond University Student Paper	<1%
34	eprints.usm.my Internet Source	<1%
35	Yohannes Tsegyie Wondmkun. " Obesity, Insulin Resistance, and Type 2 Diabetes: Associations and Therapeutic Implications ", Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 2020 Publication	<1%
36	wltpr.rivistateoria.it Internet Source	<1%
37	Submitted to Angeles University Foundation Student Paper	<1%
38	Submitted to Manipal Academy of Higher Education (MAHE)	<1%

	Student Paper	
39	www.science.gov Internet Source	<1%
40	A.A. Adedapo, I.O. Ogunmiluyi, O.O. Falayi, B.S. Ogunpolu et al. "The lyophilized aqueous leaf extract of Moringa oleifera blunts streptozocin-induced diabetes in rats through upregulation of GLUT 4 signaling pathway and anti-oxidant effect", Scientific African, 2020 Publication	<1%
41	Marie K Walsh, Rodney J Brown. "Use of amino acid analysis for estimating the individual concentrations of proteins in mixtures", Journal of Chromatography A, 2000 Publication	<1%
42	hdl.handle.net Internet Source	<1%
43	www.coursehero.com Internet Source	<1%
44	Yakubu OE. "Comparative Hepatoprotective and Haematological Effects of the Ethanol Extracts of the Leaves of Vitex Doniana and Senna Occidentalis in Alloxan-Induced Diabetes in Male Wistar Rats", Journal of Analytical & Pharmaceutical Research, 2017 Publication	<1%
	ijpar.com	

45	Internet Source	<1%
46	"Phytoremediation", Springer Science and Business Media LLC, 2023 Publication	<1%
47	repository.its.ac.id Internet Source	<1%
48	Manoj Mandal, - Rakibuzzaman, Begum Rokeya, Liaquat Ali, Zahid Hassan, Md Omar Faruque. "Anti-diabetic effect of Oyster Mushroom mediates through increased AMP-activated protein kinase (AMPK) and cyclic AMP-response element binding (CREB) protein in Type 2 Diabetic model Rats", Bangladesh Journal of Medical Science, 2018 Publication	<1%
49	Sepide Behrouz, Saeideh Saadat, Arghavan Memarzia, Hadi Sarir, Gert Folkerts, Mohammad Hossein Boskabady. "The Antioxidant, Anti-Inflammatory and Immunomodulatory Effects of Camel Milk", Frontiers in Immunology, 2022 Publication	<1%
50	Submitted to University of Greenwich Student Paper	<1%
51	repository.futminna.edu.ng:8080 Internet Source	<1%

52	techylib.com Internet Source	<1%
53	www.researchsquare.com Internet Source	<1%
54	A Aigbiremolen, R Ativie, M Aisuodionoe, O Odigie, J Igweh, M Egwaoje. "Effect of Aqueous Extract of Persea americana Seed on Blood Glucose in Alloxan-induced Diabetic Wistar Rats", Asian Journal of Medicine and Health, 2018 Publication	<1%
55	Submitted to Nelson Mandela Metropolitan University Student Paper	<1%
56	Submitted to Panjab University Student Paper	<1%
57	Submitted to RMIT University Student Paper	<1%
58	Submitted to Aspen University Student Paper	<1%
59	Submitted to Leeds Trinity and All Saints Student Paper	<1%
60	Submitted to University of Hertfordshire Student Paper	<1%
61	cardiab.biomedcentral.com Internet Source	

		<1%
62	journals.lww.com Internet Source	<1%
63	twas.assaf.org.za Internet Source	<1%
64	www.nature.com Internet Source	<1%
65	"International Handbook of the Demography of Obesity", Springer Science and Business Media LLC, 2022 Publication	<1%
66	Submitted to Curtin University of Technology Student Paper	<1%
67	Submitted to Universiti Sains Malaysia Student Paper	<1%
68	Submitted to University of Nicosia Student Paper	<1%
69	acikbilim.yok.gov.tr Internet Source	<1%
70	www.foodandnutritionjournal.org Internet Source	<1%
71	www.medcraveonline.com Internet Source	<1%

72	Isabella Jasmin Giambra, Lina Chianese, Pasquale Ferranti, Georg Erhardt. "Genomics and proteomics of deleted ovine CSN1S1□I", International Dairy Journal, 2010 Publication	<1 %
73	J. J. Holst, F. K. Knop, T. Vilsboll, T. Krarup, S. Madsbad. "Loss of Incretin Effect Is a Specific, Important, and Early Characteristic of Type 2 Diabetes", Diabetes Care, 2011 Publication	<1 %
74	academic.oup.com Internet Source	<1 %
75	www.frontiersin.org Internet Source	<1 %
76	www.tandfonline.com Internet Source	<1 %
77	Lehner, A.. "Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains", Journal of Cereal Science, 200805 Publication	<1 %
78	catalog.lib.kyushu-u.ac.jp Internet Source	<1 %
79	digitalcommons.wayne.edu Internet Source	<1 %

80	uzspace.unizulu.ac.za Internet Source	<1%
81	www.mmc.gov.bd Internet Source	<1%
82	www.pt.bme.hu Internet Source	<1%
83	Ann E. Weber. "Dipeptidyl Peptidase IV Inhibitors for the Treatment of Diabetes", Journal of Medicinal Chemistry, 2004 Publication	<1%
84	Campbell, G.M., S. Tiwari, C. Hofbauer, A.-K. Picke, M. Rauner, G. Huber, J.A. Peña, T. Damm, R. Barkmann, M.M. Morlock, L.C. Hofbauer, and C.-C. Glüer. "Effects of parathyroid hormone on cortical porosity, non-enzymatic glycation and bone tissue mechanics in rats with type 2 diabetes mellitus", Bone, 2016. Publication	<1%
85	Elizabeth Omodanisi, Yapo Aboua, Oluwafemi Oguntibeju. "Assessment of the Anti-Hyperglycaemic, Anti-Inflammatory and Antioxidant Activities of the Methanol Extract of Moringa Oleifera in Diabetes-Induced Nephrotoxic Male Wistar Rats", Molecules, 2017 Publication	<1%

86	Linda E. Watson, Tongzhi Wu, Michael Horowitz, Christopher K. Rayner. "Whey Protein and Diabetes", Elsevier BV, 2017 Publication	<1%
87	Shiro Iwagami, Yoshifumi Baba, Masayuki Watanabe, Hironobu Shigaki et al. "Pyrosequencing Assay to Measure LINE-1 Methylation Level in Esophageal Squamous Cell Carcinoma", Annals of Surgical Oncology, 2011 Publication	<1%
88	Submitted to University of Plymouth Student Paper	<1%
89	cris.maastrichtuniversity.nl Internet Source	<1%
90	docslib.org Internet Source	<1%
91	hi-aware.org Internet Source	<1%
92	krishikosh.egranth.ac.in Internet Source	<1%
93	paduaresearch.cab.unipd.it Internet Source	<1%
94	professional.diabetes.org Internet Source	<1%

95	pu.edu.pk Internet Source	<1 %
96	researchonline.ljmu.ac.uk Internet Source	<1 %
97	tesisenxarxa.net Internet Source	<1 %
98	www.grafiati.com Internet Source	<1 %
99	www.um.edu.mt Internet Source	<1 %
100	Bae, S.K.. "Pharmacokinetic changes of DA-7867, a new oxazolidinone, after intravenous and oral administration to rats with short-term and long-term diabetes mellitus induced by streptozotocin", European Journal of Pharmaceutical Sciences, 200506 Publication	<1 %
101	G. C. Major. "Recent developments in calcium-related obesity research", Obesity Reviews, 2/15/2008 Publication	<1 %
102	Gamal Badr, Nancy K Ramadan, Hanem S Abdel-Tawab, Samia F Ahmed, Mohamed H Mahmoud. "Camel whey protein protects lymphocytes from apoptosis via the PI3K/AKT, NF-κB, ATF-3 and HSP-70 signaling pathways	<1 %

	in heat-stressed male mice", Biochemistry and Cell Biology, 2017 Publication	
103	J. Peter Bercz, Merrel Robinson, Lucille M. Garner, Norbert P. Page, Greg R. Olson. "Repeated-Dose and Subchronic Toxicity Studies of 2,2,2-Trichloroethanol in Sprague-Dawley Rats", Journal of the American College of Toxicology, 2016 Publication	<1 %
104	Tetsuya Tsukamoto. "Mouse Strain Susceptibility to Diethylnitrosamine Induced Hepatocarcinogenesis Is Cell Autonomous Whereas Sex-susceptibility Is Due to the Micro-environment: Analysis with C3H <-> BALB/c Sexually Chimeric Mice", Cancer Science, 7/2000 Publication	<1 %
105	Submitted to University of Houston System Student Paper	<1 %
106	bmccomplementmedtherapies.biomedcentral.com Internet Source	<1 %
107	core.ac.uk Internet Source	<1 %
108	docksci.com Internet Source	<1 %
	psasir.upm.edu.my	

109	Internet Source	<1%
110	pure.port.ac.uk Internet Source	<1%
111	ujpr.org Internet Source	<1%
112	uokerbala.edu.iq Internet Source	<1%
113	www.hindawi.com Internet Source	<1%
114	www.projektakademien.se Internet Source	<1%
115	Benoit Cudennec, Rafik Balti, Rozenn Ravallec, Juliette Caron, Ali Bougatef, Pascal Dhulster, Naïma Nedjar. "In vitro evidence for gut hormone stimulation release and dipeptidyl-peptidase IV inhibitory activity of protein hydrolysate obtained from cuttlefish (Sepia officinalis) viscera", Food Research International, 2015 Publication	<1%
116	Prabhat Upadhyay, Diya Kalra, Aishwarya Shrikant Nilakhe, Vijay Aggrawal, Sarika Gupta. "PL02® alleviates joint Pain and Inflammation, and subchondral bone deterioration in the Monosodium	<1%

iodoacetate-induced Osteoarthritis rat model", Research Square Platform LLC, 2023
Publication

117 Sung Hye You, Beum Suk Kim, Soon Jun Hong, Chul-Min Ahn, Do-Sun Lim. "The Effects of Pioglitazone in Reducing Atherosclerosis Progression and Neointima Volume in Type 2 Diabetic Patients: Prospective Randomized Study With Volumetric Intravascular Ultrasonography Analysis", Korean Circulation Journal, 2010 <1%
Publication

118 Salma M Mohyeldin, Wael M Samy, Doaa Ragab, Doaa A Abdelmonsif, Rania G Aly, Nazik A Elgindy. "Precisely Fabricated Sulpiride-Loaded Nanolipospheres with Ameliorated Oral Bioavailability and Antidepressant Activity", International Journal of Nanomedicine, 2021 <1%
Publication

119 Shen-Shih Chiang. "Effect of bioactive compounds in lactobacilli-fermented soy skim milk on femoral bone microstructure of aging mice", Journal of the Science of Food and Agriculture, 01/30/2012 <1%
Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		