NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

INVESTIGATION OF THE EFFECT OF PROTOCATECHUIC ACID ON LIVER TISSUE IN EXPERIMENTAL DIABETIC RATS LIGHT MICROSCOPY AND BIOCHEMICAL STUDY

YOSSIRA M.S SWESE

MASTER THESIS

HISTOLOGY AND EMBRYOLOGY DEPARTMENT

THESIS SUPERVISOR

Prof. Dr. AYSEL KÜKNER

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ACCEPTANCE/APPROVAL

NEAR EAST UNİVERSİTY

DIRECTORATE OF HEALTH SCIENCES INSTITUTE

This work has been adopted as a master thesis in the program of

Histology and Embryology by the jury.

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This thesis has been approved by the above jury members in accordance with the relevant articles of the NEU postgraduate education, training and examination regulations and has been accepted by the decision of the board of the Institute.

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DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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COMPLIANCE AND APPROVAL

Her master thesis “Investigation of the effect of protocatechuic acid on liver tissue in experimental diabetic rats. Light microscopy and biochemical study”
was written in accordance with the NEU Postgraduate thesis proposal and thesis writing directive.

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DEDICATION

This thesis is dedicated to my loving family. I love you all. Thank you.

ACKNOWLEDGEMENT

First, it is with great pleasure to express my sincere appreciation to my supervisor Prof. Dr. Aysel Kukner. My deepest appreciations go to my loving family for striving with me to make this journey an intriguing one. To my friends, colleagues and all who helped me. Thanks for making me who I am.

Yossira M.S SWES

Investigation of the effect of protocatechuic acid on liver tissue in experimental diabetic rats. Light microscopy and biochemical study

Yossira M.S SWESE

Supervisor: Aysel KÜKNER

Department of Histology and Embryology

ABSTRACT

Aim: The main purpose of this study is to investigate the effects of Protocatechuic acid (PCA) on the liver in rats with diabetes caused by streptozotocin, biochemically and histologically.

Materials and Methods: Wistar Albino rats were used to analyze the antidiabetic activity of PCA. A total of four groups were created, six rats in each group. Group I, without any treatment, Group II, in which diabetes was created with a single dose of Streptozotocin (60 mg/ kg), Group III given PCA (20 mg/ kg) for 3 weeks, and Group IV administered 20 mg/ kg PCA to rats with diabetes. At the end of the experiment, blood and liver tissue samples were taken from the rats. Biochemical levels of liver enzymes were examined. HE, PAS and Masson trichrome staining were applied to the sections prepared in the tissues. Statistical analysis was carried out using Kruskal Wallis test and Mann-Whitney U test to determine the differences between the groups.

Results: Compared to the control group, hepatocytes around the portal areas in the diabetic group liver tissue were stained openly. It was observed that glycogen decreased, vacuolization in hepatocytes. Congestion and Kupffer cell increase were evident in sinusoids. It was observed that inflammation and vacuolization decreased in the diabetic group treated with PCA. No connective tissue increases or fibrosis was observed in all groups. Biochemically, in the diabetic group, AST, ALT and ALP enzyme levels were found to be very high and decreased in PCA-treated groups.

Conclusion: In STZ-induced diabetic rats, PCA reduced inflammation and vacuolization of the liver and increased enzyme levels.

Key words: Diabetes mellitus, liver, Protocatechuic acid

ÖZET

Amaç: Bu çalışmanın temel amacı, streptozotosin ile diyabet oluşturulmuş ratlarda Protocatechuic asitin (PCA) karaciğer üzerindeki etkilerinin biyokimyasal ve histolojik olarak incelenmesidir.

Materyal ve Metot: PCA'nın antidiyabetik aktivitesini analiz etmek için, Wistar Albino sıçanlar kullanıldı. Her grupta 6 denek olmak üzere toplam 4 grup oluşturuldu; Herhangi bir işlem yapılmayan Grup I, tek doz Streptozotosin (60 mg / kg) ile diyabet oluşturulan Grup II, 3 hafta boyunca PCA (20 mg / kg) verilen Grup III, ile diyabet oluşturulmuş sıçanlara 20 mg/kg PCA uygulanan Grup IV. Deney süresi sonunda deneklerden kan ve karaciğer doku örnekleri alındı. Biyokimyasal olarak karaciğer enzimlerinin düzeyleri bakıldı. Dokularda hazırlanan kesitlere HE, PAS ve Masson trikrom boyamaları uygulandı. Gruplar arasındaki farklılıkları belirlemek için Kruskal Wallis testi ve Mann-Whitney U testi kullanılarak istatistiksel analiz yapıldı.

Bulgular: Kontrol grubu ile karşılaştırıldığında diyabetik sıçan karaciğer dokusunda, portal alan çevresindeki hepatositlerin açık boyanmıştı. Hepatositlerde glikojenin azaldığı vakuolizasyon olduğu görüldü. Sinüzoidlerde konjesyon, Kupffer hücre artışı belirgindi. PCA ile tedavi edilen diyabetik grupta inflamasyonun, vakuolizasyonun azaldığı görüldü. Tüm gruplarda bağ doku artışı, fibrozis görülmedi. Biyokimyasal olarak diyabetik grupta AST, ALT ve ALP enzim düzeylerinin çok arttığı, PCA ile tedavi edilen gruplarda azaldığı saptandı.

Tartışma: STZ ile indüklenen diyabetik sıçanlarda, PCA karaciğerde inflamasyonu ve vakuolizasyonu ve artmış olan enzim düzeylerini **azaltmıştır.**

Anahtar Kelimeler: Diabetus Mellitus, karaciğer, Protocatechuic acid

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ABBREVIATIONS

BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxyl Toluene
BHQ	Butyl Hydro Quinone
BMP	Bone Morphogenetic Protein
C3G	Cynidin-3-O-β-Glucoside
CAPE	Caffeic Acid Phenethyl Ester
CV	Central vein (Cv)
Chd	Common hepatic ducts
Cbd	Common Bile Duct
Cd	Cystic duct
CTGF	Connective Transforming Growth Factor
DHBV	Duck Hepatitis B Virus
DM	Diabetes Mellitus
FGF	Fibroblast Growth Factor
FOXA2	Fork Head box Protein A2
H-PCA	Hibiscus protocatechuic acid
HBV	Hepatitis B Virus
Hc	Hemopoietic cell
Hd	Hepatic ducts
HHEX	Haematopoietically-expressed Homeobox Protein
HSCs	Hepatic Stellate Cells
HPLC	High Performance Liquid Chromatography
GSH	Glutathione
INFγ	Interferon Gamma
LDH	Lactate Dehydrogenase),
LPS	Lipopolysaccharide
MDA	Malondialdehyde
MHC	Major Histocompatibility Complex
MMPs	Matrix Metalloproteinases
mRNA	Messenger RNA
NK	Natural Killer
OC1	Onecut 1
PA	Portal Area
PCA	Protocatechuic acid
PDGF	Platelet-Derived Growth Factor
PPAR-γ	Peristome Proliferative-Activated-Receptor- γ
ROS	Reactive Oxygen Species
STZ	Streptozotocin
STS	Solanum torvum Swartz

TGF β	Transforming Growth Factor Beta
Th	T helper
TNF	Tumor necrosis factor
UV	Ultra Violet
V	Vein
VSMCs	Vascular Smooth Muscular Cells
WHO	World Health Organization
Wnt13	Wingless-Type 13

1. INTRODUCTION

1.1 Introduction

The metabolic disorder termed diabetes mellitus (DM) is described by hyperglycemias and interruption of carbohydrate and glycogenolysis and gluconeogenesis fat and protein metabolism. According to WHO, number of people increased from 171 million to 300 million in 2030 (wild et al 2004). There is insulin-dependent DM (type I) and non-insulin-dependent (type II). Ailments such as vision impairment, kidney dysfunction and nerve damage are often followed by the former (type I), whereas the latte (type II) is distinguished by peripheral tolerance to insulin and reduced insulin secretions contributing to a cardiovascular system disease (Almatar et al 2017).

The liver is the main internal biochemical, exocrine and endocrine system. This involves bile development, nutritional digestion, detoxification, glycogen-storage regulation and maintenance of blood homeostasis via the secretion of coagulation factors and serum proteins such as albumin (Joshi et al, 2009). Hepatocytes constitute 55-65% that is two thirds of the cell mass of an adult liver. A variety of essential roles of hepatocytes, include protein production, detoxification and lipid, carbohydrate metabolism.

Protocatechuic acid (PCA) a polyphenol present in most edible plants (Liu, 2004). It is readily available in fruits such berries, grapes and in vegetables like onions and most grain foods possess PCA like brown rice. Anthocyanins and flavonoids when they are metabolised in the human body by microbes they produce PCA via the shikimic acid pathway (Masella et al, 2012). PCA has been studied for its pharmacological effects and past research has shown that it possesses antidiabetic, anti-inflammatory, anticancer and analgesic properties (Khan et al. 2015). The beneficial effects of PCA cannot be argued that's why we wanted to study its effects on diabetic rats.

Different approaches in treatment and prevention for bettering DM is something that has been commonly regarded, for instance, the existence and dietetic reformation (such as, low-sodium consumption, fruit- and vegetable-enriched utilisation). This however are difficult to follow as most individuals may not have will or time to do so. The development of more potent and safer drugs may be the easiest way to improve the damage caused by diabetes. The benefits of more natural products from plant materials is the best solution as they will not cause any

problems within affected individuals. To evaluate the effect of Protocatechuic acid on the liver tissue in streptozotocin induced diabetic rats.

1.2 Significance of Study

Diabetes causes liver disease by affecting the functioning of the liver enzymes, hepatocellular structures and often causing carcinomas. The significance of this study is to evaluate whether PCA has the ability to restore liver function tests and repair the damaged cells caused by diabetes. The results will be highly significant in that if PCA is able to improve the function of the liver in diabetic rats it can be given to diabetic patients in order to combat their disease. The research of natural chemicals from plants which can be able to complement traditional medicine is highly significant in that it can aide pharmacologists to develop new drug regimens which are safe and less toxic.

1.3 Study Limitation

In this study, we could not use some special and immunological pigments to obtain results and other readings, as well as difficulties we faced while taking the textile sectors because they need accuracy and experience in taking them. Additionally, due to global pandemic of COVID_19, this research was limited since the access of the laboratories to revaluate the samples became impossible. Only the initial results were used.

2. GENERAL INFORMATION

2.1 Anatomy of the Liver

In the right hypochondrium is where the liver is located (Jamieson, 2006). It is under the diaphragm and is surrounded by other organs like pancreas, gall bladder, intestines and is just on top of the stomach (Figure 2.1). It has cone-shape like appearance and is protected within the rib cage (Hargen-Ansert, 2012).

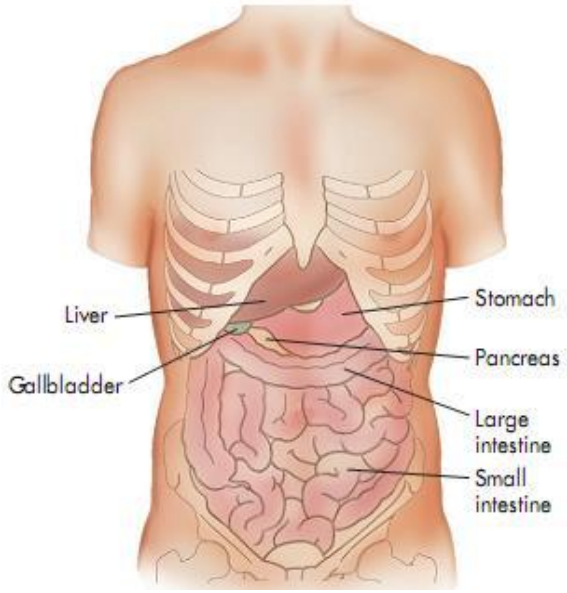


Figure 2.1: Shows AP view of the abdomen (Adopted from Hagen-Ansert, 2012)

2.1.1 Lobes of the Liver

1. Right Lobe: In terms of volume it is the largest to all surfaces of the liver. The falciform ligament divides it from the left lobe (Blumgart and Belghiti, 2007). The ligamentum venosum divides it superiorly resulting in having the caudate lobe and quadrate lobe which are posterior and anteriorly located.

2. Left Lobe: it's the smaller part of the two lobes and is on the left side of the falciform ligament. The upper portion nearly touches the diaphragm where it is moulded into upper left quadrant (Jamieson, 2006).

3. Caudate Lobe: located on the posterosuperior and is different anatomically from the other lobes (Figure 2.2). between inferior vena cava and hepatic veins, there is a direct connection that enable the circulation of blood from right to left veins (Kogure *et al*, 2007).

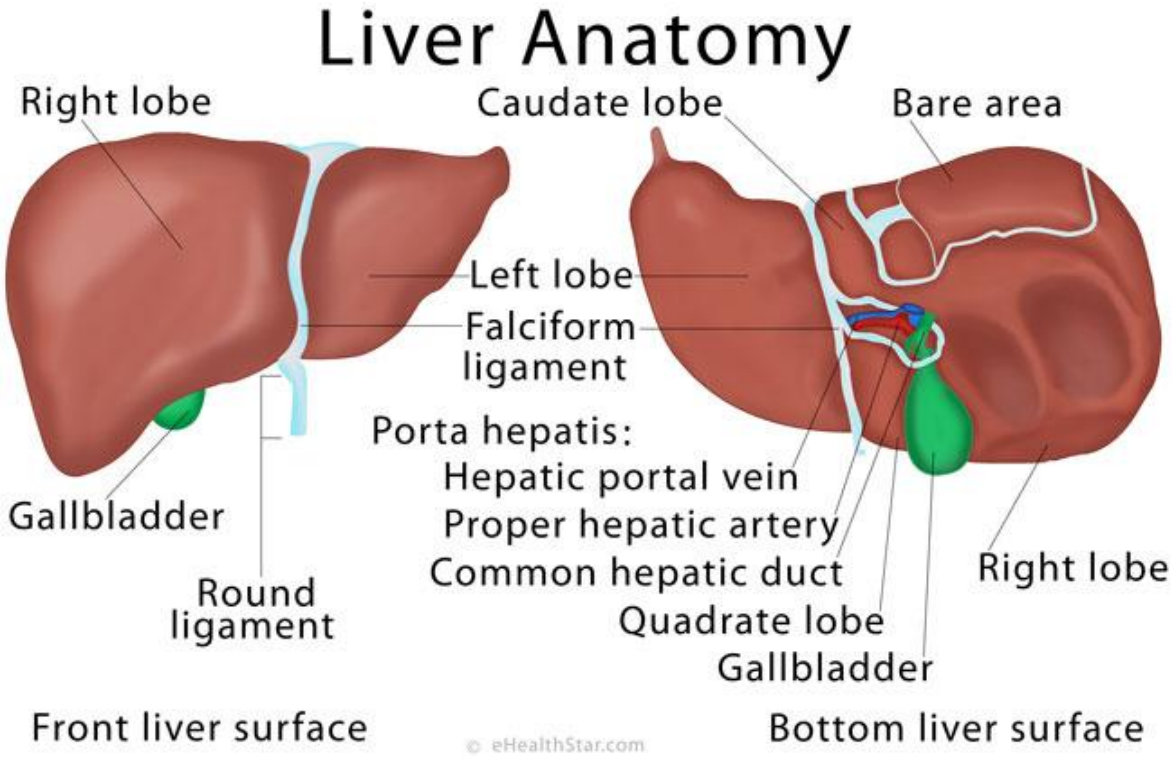


Figure 2.2. Liver anatomy anterior and inferior surface (Adopted from Encyclopedia Britannica, 2010).

Ligaments and fissures: Glisson's capsule, main lobar fissure, falciform ligament, ligamentum teres (round ligament), and ligamentum venosum. These ligaments and fissures appear echogenic or hyperechoic due to fat and collagen. This capsule completely surrounds the liver (Kogure *et al*, 2007).

2.1.2 Vascular Supply Portal Venous System

The portal venous system is responsible for carrying nutrients and contaminants that are absorbed in the intenstinal mucosa (Figure 2.3). Transcending from the right side the main portal vein meets the porta hepatis in a posterior direction resulting in two branches being formed that is the left and right portal veins (Sutherland *et al*, 2002). The right portal vein is the larger than the left portal vein. Three components make up the hepatic vein: middle, right

and left. The portal veins carry blood to liver from the stomach. The hepatic arteries carry oxygenated blood from the aorta to the liver (Sutherland *et al*, 2002).

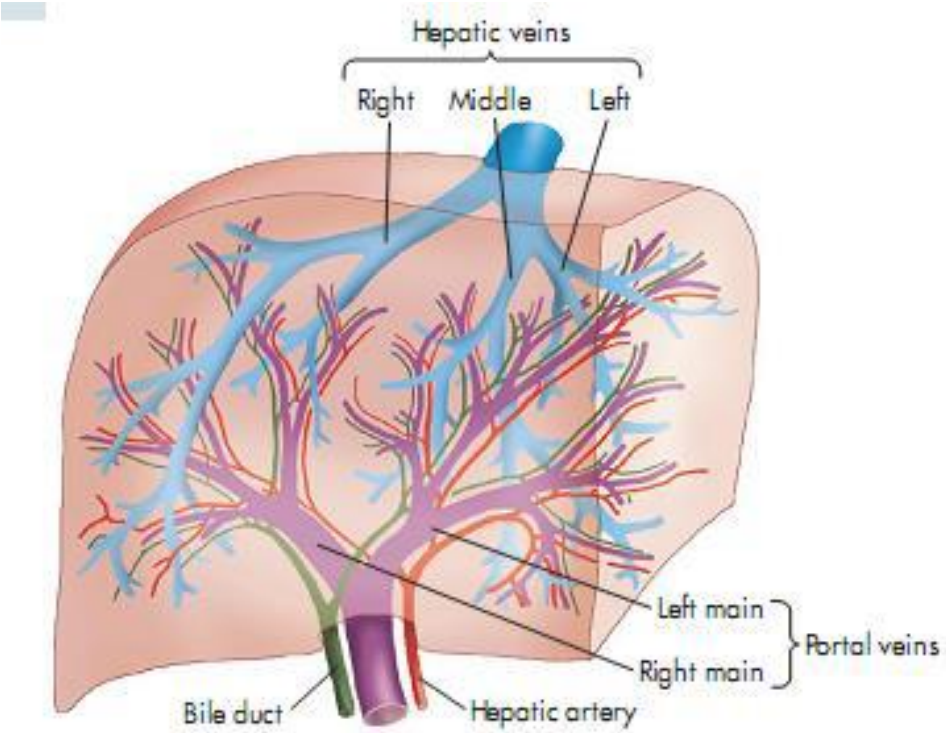


Figure 2.3: Shows Vascular system of the liver (Adopted from Hagen-Ansert, 2012)

2.2 Development of the Liver

During early improvement of the embryo a development called embryonic folding continues until we have the mesoderm and endoderm cells. The endoderm cells form the liver and these express proteins known as Foxa proteins which are important in liver development (Datta, 2010). Following gastrulation, the liver bud is initially and fundamentally structured with hepatoblasts. This term Hepatoblasts are regarded as the stem cells that eventually transform to cholangocytes or in other word hepatocytes, additionally, these cells are line bile ducts. The significant and principal epithelial cells available in liver are extracted from endoderm (Behbahan et al, 2011). Notwithstanding, these cells make to just around 66% of the liver volume. The staying 33% comprises of an assortment of cells got basically from the mesoderm, including vascular cell types: Kupffer cells, stellate cells, fibroblasts, and leukocytes (Figure 2.4).

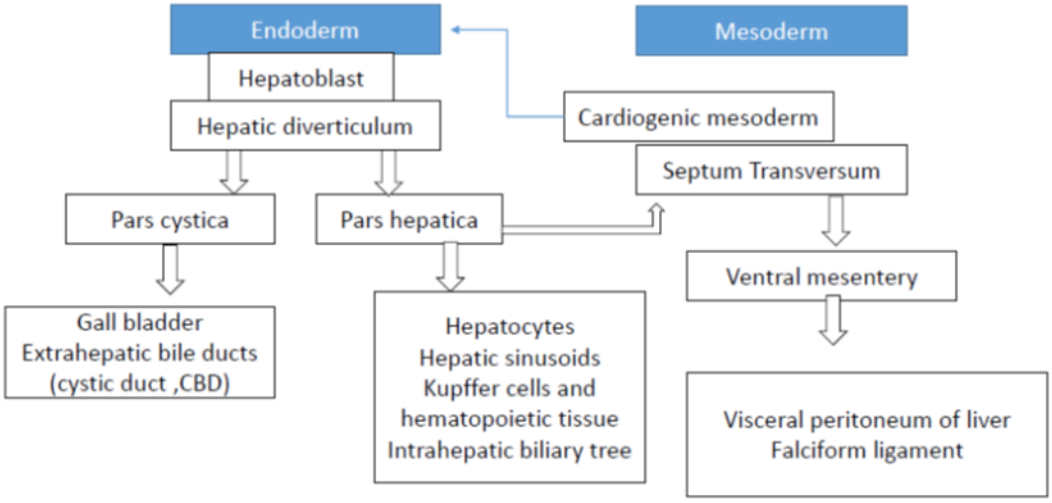


Figure 2.4: Flowchart of the liver development. Adopted from Sherlocks Diseases of the Liver and Biliary System 12th Edition.

2.2.1 Early stages in liver development

Consequently, for liver to develop, there is a need for a harmonised combination of the cells to come together in a form of an embryonic stratums. Development of the liver involves a specific arrangement of cells that is able to allow cells to carry out their metabolic, excretory and circulatory functions. For this to occur the cells should be able to various hepatic cell types. This process is therefore controlled controlled by many signalling molecules just like any biological system as well as transcription factors (Behbahan et al, 2011).

Specification occurs when hepatoblasts begin to differentiate (figure 2.5) as a result of receiving effector molecules like fibroblast growth factor (FGF), septum transversum mesenchyme (STM). Bud formation and expansion: The hepatoblasts start to expand into the endoderm basement membrane through penetration forming the liver bud (hepatic diverticulum). First and foremost the basement membrane separates the hepatic diverticulum from the septum transversum. Eventually the coating is vanished, and cells delaminate from the bud to attack the septum transversum as lines of hepatoblasts (Bort et al, 2006).

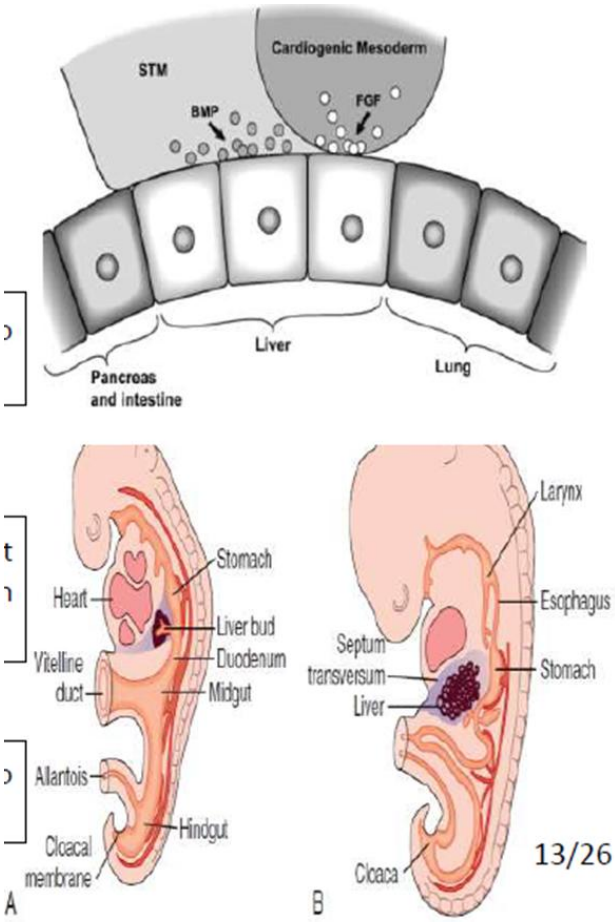


Figure 2.5 Liver development (A) day 25 (B) day 1 Adopted from (Behbahan et al, 2011). Adopted from Sherlocks Diseases of the Liver and Biliary System 12th Edition.

2.2.2 Development of liver and biliary passages

This process starts when the hepatic diverticulum starts to grow in-between layers of ventral mesentery forming distinct parts which are the cranial (pars hepatica) and caudal buds (pars cystica).

- Pars hepatica: this structure is known to give rise to the kupffer cells, hepatic sinusoids, hematopoietic tissue and the hepatocytes. When development starts the two sections that are equally same, however, regenerates to transform into something bigger with the organ filling up a significant portion of the abdominal cavity (Sahni and Sodhi, 2000).
- Pars cystica: the stem structure develops into cystic duct and the upper part into the gall bladder. The bile duct is formed from the duodenum which is connected to the

hepatic and the cystic ducts. Both sides of the branches in tube form of he hepatica. The left and right hepatic ducts come from the left and right branches of the pars hepatica (Behbahan et al, 2011).

2.2.3 Formation of the capsule and ligaments of the liver

When the growing pars hepatica penetrates the septum transversum the mesodermal part which is lies between the anterior abdominal wall and the liver develops into the falciform ligament. The omentum is formed from the duodenum and stomach. The surface mesodermal layer differentiates into peritoneal covered by a capsule.

2.2.4 Vascular development

Three major venomous system are seen during early developments in the embryo. Two extraembryonic venous systems develop which are the umbilical (placental) veins and the omphalomesenteric (vitelline). The remaining system which is the intraembryonic encompasses the cardinal veins which is responsible for passage of blood to the heart of the embryo (Behbahan et al, 2011). Therefore these systems will then merge into the sinus venosus, a cavity that is combined into the heart.

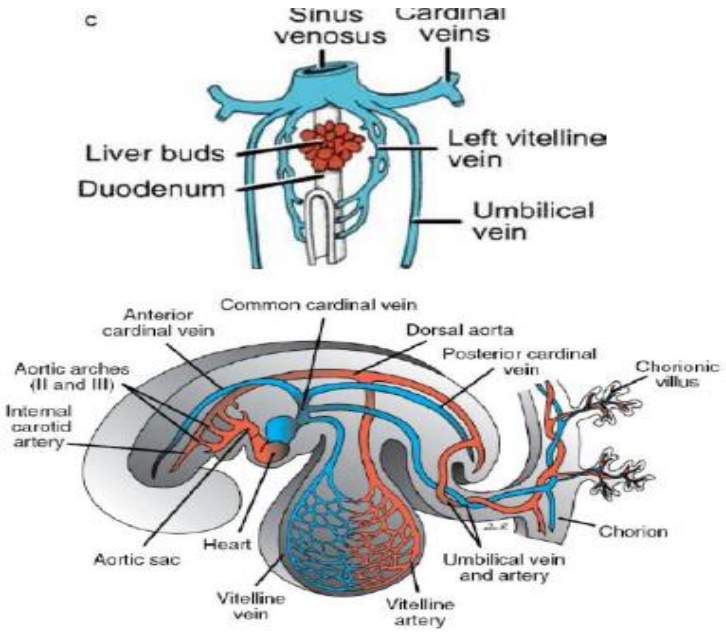


Figure 2.6 Main intraembryonic and extraembryonic arteries (red) and veins (blue) in a 4 mm embryo (end of forth week). Adopted from Sherlocks Diseases of the Liver and Biliary System 12th Edition.

2.2.5 Development of Vitelline and umbilical veins

The vitelline veins form a plexus around the duodenum just before they enter the sinus venosus. The sinusoids form as a result of the interruption of growing veins by the septum initiating the right and left shunting. The inferior vena cava which is the hepatocardiac portion develops as a result of the enlargement of the right vitelline due to blood flowing from the left to the right side of the liver (Data, 2010). The portal vein is formed from the vast network that surrounds the duodenum resulting in a single vessel. The superior mesenteric vein derives from the right vitelline vein and becomes the hepatic veins (figure 2.8).

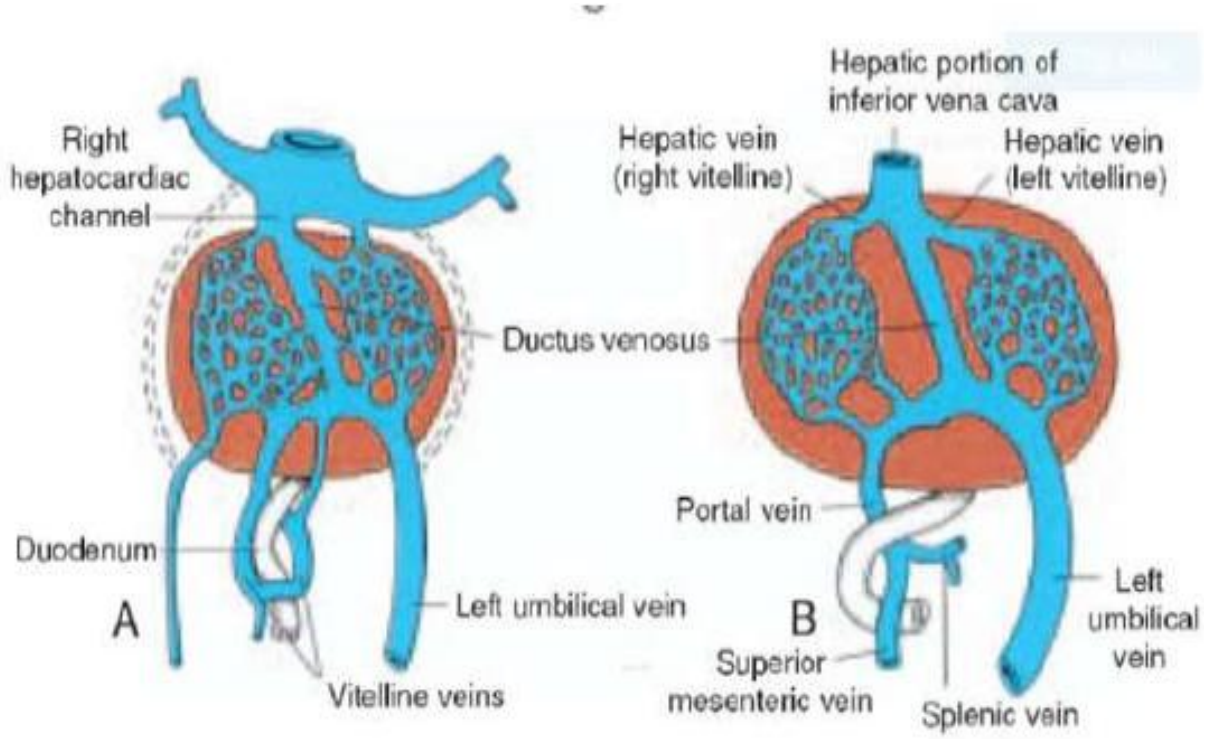


Figure 2.7 A. 2nd mnth B.3rd mnth. Note formation of the ductus venosus, portal vein, hepatic portion of IVC. The splenic & SMV enter the PV Adopted from Sherlocks Diseases of the Liver and Biliary System 12th Edition.

2.3 Physiology of the liver

2.3.1 Synthesis of amino acids

This incorporate amino acids and another entity in the procedures is known as transamination. The procedure has been regarded as transamination and the compound usage is typically regarded as transaminase or amino transferase (Jamieson, 2006). On the off chance that we have glutamic corrosive here in addition to pyruvic corrosive with what compound will yield alpha ketoglutarate corrosive and furthermore alanine. Alanine is the amino corrosive where the amino gathering is moved from the glutamic corrosive into the pyruvic corrosive creation alanine the protein utilized is known as alanine transaminase (ALT). in this manner, liver on its own can likewise utilize amino corrosive through a procedure known as oxidative deamination were, they basically deaminate an atom by expelling an amine bunch which is NH3 or NH2 on the grounds that it is an oxidation procedure the particle itself is oxidized thus hydrogen is evacuated (Kmiec, 2001).

2.3.2 Synthesis of proteins

- Albumin is basically among the few significant proteins available. Albumin is essentially the 50% of the protein been process by the liver. Additionally, albumin plays a significant role in sustaining both transportation and osmolality of numerous hormones.
- The liver additionally creates proteins for the invulnerable framework, for example, the C - receptive protein (opsin) in which aids in the improvement of a pathogen. It additionally integrates plasma supplement proteins c1 to c9 supplement proteins which are significant for decimating attacking pathogen (Bykov et al, 2006).
- The liver likewise combines different hormones and professional hormones, for example, insulin like development factor (IGF) for development. The liver additionally delivers thrombopoietin which is significant in platelet creation as platelets are significant for homeostasis for repairing veins. The liver additionally delivers another hormone known as angiotensinogen which is a piece of a major framework known as a renin-angiotensin-aldosterone framework. This framework manages pulse by keeping up the circulatory volume (Ozougwu et al, 2017).

2.3.3 The production of bile

The liver really creates bile and secretes the bile in order to secure its away in the gallbladder. The gallbladder is below the liver. The bile pipe where the gallbladder can fundamentally release or emanate out the bile into the processing tracts to help digest fats. Right when we eat oily sustenances, they will get prepared by the stomach and will enter the little processing tracts and will energize the little assimilation tracts to release a hormone known as cholecystokinin (CCK) (Dean, 2005). The stomach related organs can't hold these oily substances essentially like that it needs the help of bile so it secretes the hormone CCK which will by then vivify the gallbladder to consent to at that point release the bile into the little stomach related organs. Thusly, it helps with emulsifying the fats spread the fats so the fats can be viably handled and devoured by the little absorption tracts.

- Bile is included two essential things it's contained bile acids or salts and moreover bile conceals. Bile acids are amphiphilic steroids which infers that if this destructive would have one side which is hydrophilic which infers it values water and the contrary side which is hydrophobic which suggests that it hates water. Exactly when we consume the lipid and when it's in little stomach related organs the bile acids will begin enveloping it where the hydrophobic regions and will fundamentally cover the lipid and the hydrophilic locale will stand up to the outside considering the way that the hydrophobic zones are unnerved of water in this manner they face inwards where is the hydrophilic region will go up against outwards and they will cover this lipid essentially and emulsify it and make what's known as a micelles. At the point when they coat this lipid this can get viably prepared and devoured by the little assimilation tracts. A couple of sorts of bile acids consolidate deoxycholic, chenic lithocholic and colic corrosive (Ozougwu et al, 2017).

- Bilirubin metabolism which is the irritability pigment is a outcome from the interruption of haemoglobin which can be an extravascular or intravascular haemolysis. Haemoglobin are the centres of red blood cell. Extravascular is the haemoglobin that will be excreted with the faeces hence the brown colour (Ozougwu *et al,* 2017). The intravascular destruction of red blood cells would result in unconjugated bilirubin which is the result of devastation of red platelets. This

unconjugated bilirubin will go through the circulation system bound to egg whites which will at that point take it to the liver. The hepatocytes of the liver now unconjugated bilirubin without the egg whites and is then changed to conjugated bilirubin in the liver (Guyton, 2006). Conjugated bilirubin is the bile shade since it is the result of our breakdown of hemoglobin so it will at that point travel through the biliary framework through the bile conduit into the digestion tracts. It will get emitted into the digestive organs the conjugated bilirubin will at that point convert to urobinogen through bacterial proteolysis. About 90% of the urobinogen will really be discharged as pee. The other 10% will be reabsorbed through the entry vein where it will at that point enter over into the liver (Dean, 2005).

2.3.4 Metabolism of carbohydrates

The liver has a critical activity in controlling starch processing in this way blood glucose levels too. After we eat glucose it will be devoured by the liver and will get changed over to a movement of sugars until it gets changed over to glycogen (Friedmann, 2008). This whole method is known as glyconeogenesis. The amalgamation of glycogen would happen if blood glucose levels are high if blood glucose levels are low glycogen it will be isolated with the objective that glucose can be released into the blood on to manufacture blood glucose levels.

2.3.5 Lipid Metabolism

Lipid assimilation the liver truly conveys a lot of lipoproteins. Lipoproteins as the name suggests is lipids and proteins anyway on a very basic level lipids which travel around the body moving lipids and proteins. The liver moreover consolidates cholesterol phospholipids. Phospholipids are our principal bit of lipoproteins and they fundamentally make up most of the telephone films (Friedmann, 2008). We have our cholesterol and it is huge for the body, if there's an exorbitant measure of cholesterol in any case the body will radiate it either in bile or feigns and be changed over into bile acids since they have similar structure and a while later it'll be produced in the assimilation tracts and be released as fertilizer (Ozougwu et al, 2017).

2.3.6 Vitamins stored in the Liver

Vitamin A is stored in greatest quantity maintains blood plasma levels of vitamin A. Prevents vitamin A deficiency for 10 months. Liver also stores vitamin D and vitamin B12. Liver

helps in the activation of vitamin D. Prevents vitamin D deficiency for 3 to 4 months. Enough vitamin B12 can be stored in liver for at least 1 year (Guyton, 2006).

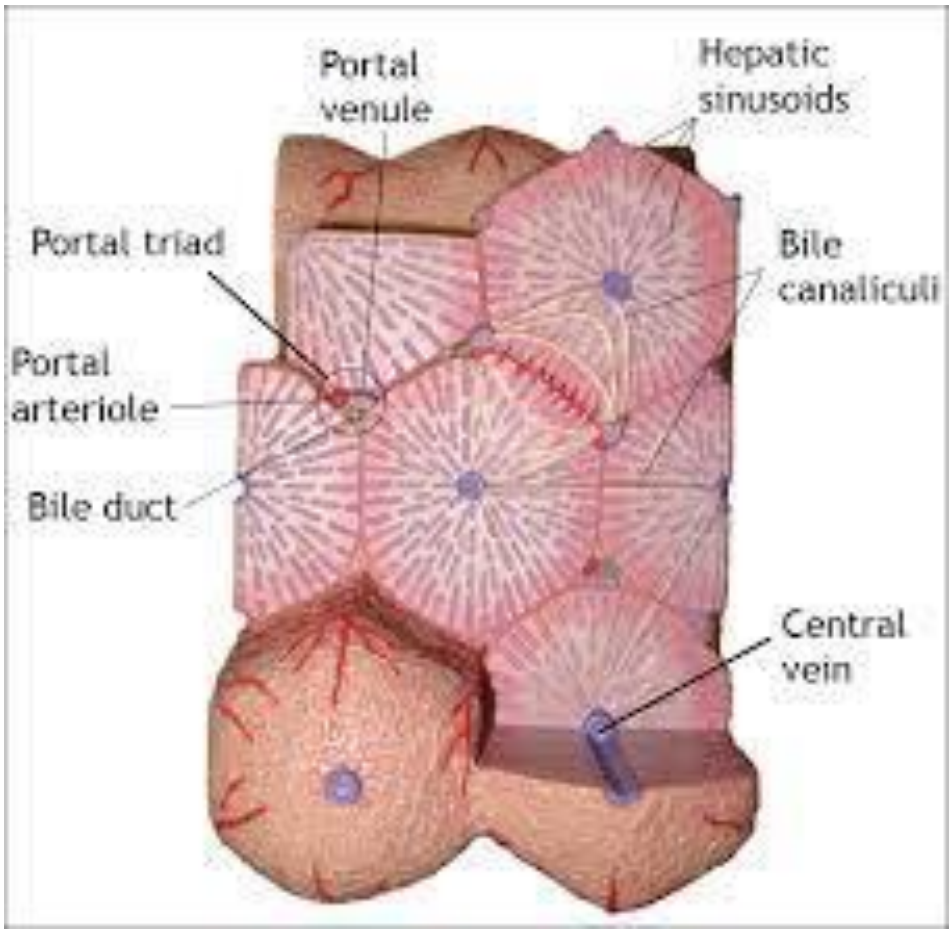


Figure 2.8. Diagram Showing Liver Lobules (Adopted from Hagen-Ansert, 2012).

2.4 Histology of the Liver

The liver lobule has a hexagonal appearance and is considered to be the basic efficient component of the liver (Figure 2.8). Structures found in the lobule include: the central vein, kupffer cells, and plates of hepatocytes, bile canaliculi and space of Disse.

2.4.1 Hepatocytes

The cells have cuboidal shape and they radiate away from the dominant vein to the portal areas (Allen, 2002). Hepatocytes are lined with sinusoids which are responsible in making sure that the central vein receives blood from the portal space (Butura, 2008). Their cellular arrangement of a tight framework to adjacent hepatocytes is aided by the presence of lateral and biliary surfaces (Figure 2.9). They are rich in mitochondria, have a round central nuclei,

well developed Golgi apparatus and eosinophilic cytoplasm (Erhardt *et al,* 2007). Hepatocytes are always transformed in to a proliferation close to threshold space which is located adjacent to the central vein.

2.4.2 Kupffer Cells

Phagocytic cells which form part of the reticuloendothelial system and they are star shaped with nuclei that is concaved and a clear to granular cytoplasm (Schumann et al, 2000). They are involved to endothelium in interplanetary of Disuse. The cells are able to degrade hemoglobin to unconjugated bilirubin and are able to multiply based on the damage of the hepatocytes and expand (Kmiec, 2001).

2.4.3 Stellate Cells

Their outline is irregular and have numerous lipid vesicles. Space of Disse is where they originate from and are able to secrete a collagenous matrix. Provide evolution factor based on the injury for regeneration of damaged liver cells. Store Vitamin A in their lipid vesicles (Friedman, 2008). They are also capable of producing an extracellular matrix when they are proliferating.

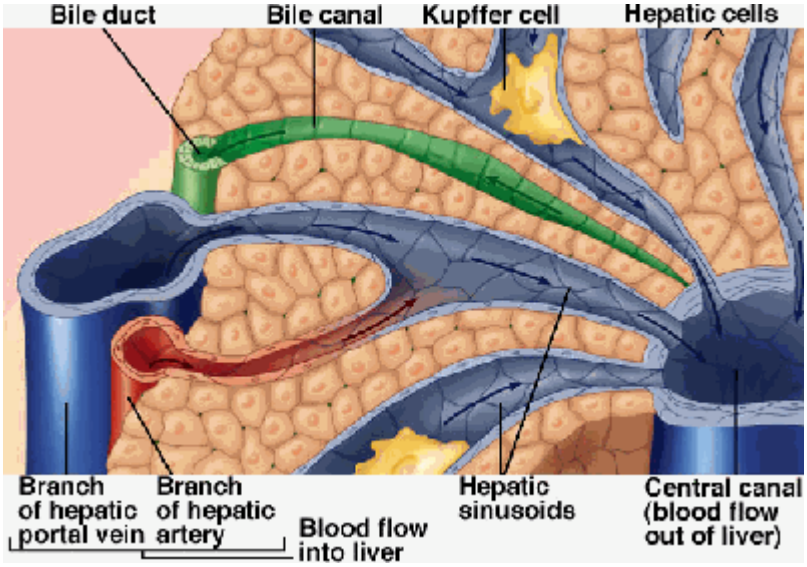


Figure 2.9: Essential Structure of Liver Lobule. Sources: Arora, 2012

3. LITERATURE REVIEW

3.0 Protocatechuic acid (PCA)

PCA is also known as the polyphenol that is found widely in plants and serve as constituents in folk medicine and still use as herbs in therapeutic plants which includes Hibiscus sabdariffa L a native of Sudan, Melissa officinalis L known as Melissa Rosmarinus officinalis L known as Rosemary, Ginkgo biloba L known as Japanese ginko, Cinnamomum aromaticum known as cynamonowa, Illicium verum commonly known as star anise (Ali et al 2005). It is common in dietary food such as brown rice, grain, bran, onions, plums, grapes, gooseberries, almonds, wine, nuts, olive oil etc. (Masella et al 2004; Al-Okbi et al 2017).

3.1 Chemical Structure of and Chemical properties of PCA

Protocatechuic acid is classified as phenolic compound due to the presence of phenols and is chemically known as 3,4-dihydroxybenzoic acid, it is found naturally and widespread among different plants species. Phenolics as secondary metabolites are obtained from phenylalanine through a process known as shikimic acid pathway. These compounds are structurally made of up aromatic ring with mono or poly hydroxyl groups. In variety of plant species, these compounds come in different derivatives such as flavonoids, iso-flavonoids, benzoic acids, tannins, lignin's, cinnamic acid and its derivatives. Other derivatives found present in plants are further classified as hydroxycinnamic acids, proanthocyanins, hydroxyl benzoic acids, ellagitannins, flavan-3-ols and anthocyanins (see figure 3.1). Like most phenolic compounds, the structure of PCA is structurally similar with caffeic acid, gallic acid, syringic acid and vanillic acid (Kakkar & Bais 2014).

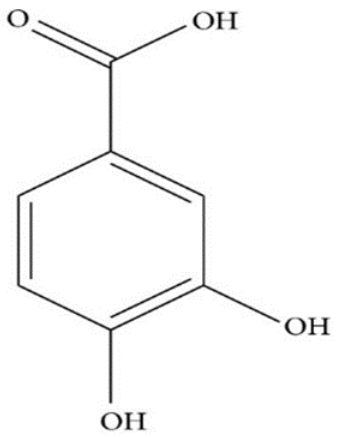


Figure 3. 1: Chemical structure of Protocatechuic acid ((Kakkar & Bais 2014).

3.2 Antiulcer Activity of PCA

Ulcer is mostly cause in organs or tissue that are in contact with stomach enzymes or acid. There are different types of ulcers, the most common ones are peptic ulcer (gastric ulcer) and duodenal ulcer. Antiulcer agents or drugs are also called antacid which are expended for the management of diverse type of ulcers, gastrointestinal related diseases (Gadekar et al 2010; Sharifi-Rad et al 2018). In folk medicine, different kinds of plants species also understood to treat a lot of different classifications of ulcer cases of which most are found to contain phenolic compound. A study induced gastric ulcer on rats through oral administration of aspirin or ethanol and through pyloric ligation to study the effect of Protocatechuic acid ethyl ester, the result has shown a significant effect of Protocatechuic acid ethyl ester at a dose range of 30 mg/kg and 60 mg/kg i.p. after 30 minutes of administration with less ulcer index in rat fed with PCA ethyl ester compare with control animals (those administered with aspirin or ethanol without Protocatechuic acid ethyl). The study suggested that antiulcer activity of Protocatechuic acid ethyl may be due to cytoprotective accomplishment as a result of the drug.

3.2.1 Cardiac and Hepatoprotective Activity of PCA

Cardiovascular disease are ailments are only related to the heart and other blood vessels around it, and mostly related to atherosclerosis. There are different types of cardiovascular diseases, some include coronary artery disease (such as myocardial infraction commonly known as heart attack and angina), peripheral artery diseases, arrhythmia, and stroke (Grundy 2004; Libby 2006). There are wide range of plant reported to possess cardiac activity. Kumar et al 2010 extracted bioactive components from Azadirachta indica A. (Neem flowers), Ocimum sanctum, Ocimum tenuiflorum, Cissus quadrangularis and flowers of Alangium salvifolium and the result has shown Ocimum tenuiflorum to exhibit more cardiac stimulant cardiotonic effect than other plants. A study extracted PCA from the petals of Hibiscus sabdariffa and inject it into rat models. The result has shown PCA to possess cardioprotective and antihypertensive effect on stages of 2-kidney. Zhou et al 2012 studied Salvia miltiorrhiza which has remained expended as herbal drug for the remedy of heart ischemic diseases. The study extracted bioactive components from the plant with PCA among the phenolic components. They use rat model induced with isoproterenol and the result has shown a positive cardiac activity in acute myocardial infraction rats.

Liu et al 2012 studied in vivo protective effect of PCA on rat induced with hepatoxicity using tert-butyl-hydroperoxide which is a free radical that cause both hepatotoxicity and inflammation. PCA is extracted from Hibiscus sabdariffa L which has been reported to function as free radical searching compound against oxidative pressure cause by free radicals. The outcome has demonstrated that the utilization of extricated PCA (50-100mg/kg) fundamentally diminished serum levels of hepatic proteins markers LDH (lactate dehydrogenase), aspartate, aminotransferase, alanine and decreased the subsequent oxidative worry in the live by GSH (glutathione) and MDA (malondialdehyde) evaluation. The rat liver further undergoes histopathological test and the result has shown the effectiveness of PCA to reduce hepatocyte swelling and liver lesions.

3.2.2 Hyperlipidemic Activity of PCA

Hyperlipemia is term used to describe high amount of lipid or fats in the blood. Hyperlipidaemic can be in form of high cholesterol level (also known as hypercholesterolemia) or high triglycerides. High amount of cholesterol in the blood leads to blockage in the arteries due to increase deposits of fats. Several studies have reported hyperlipidaemia to increase rate of coronary illness (Jain et al 2007). Different restorative plants have been investigated for capability of hostile to hyperlipidaemic activity, mostly which are hypolipidemic in nature (i.e. drugs that reduces fats) (Bahamani et al 2015). Some of the medicinal plants reported to possess hypolipidemic activity include Amaranthus spinosus, Glycrrhiza glabra, Withania somnifera, Moringa oleifera, hibiscus cannabinus etc. (Dhaliya et al 2013).

3.2.3 Anti-atherosclerotic Activity of PC

Atherosclerosis is classified as a chronic inflammatory disease which form as a result of narrowing of the artery due to plague. Formation of Atherosclerosis can lead to stroke, coronary artery diseases, kidney diseases, peripheral artery diseases, diabetes, obesity, high blood pressure. (Sedighi et al, 2017) studied plant-based compound such as flavonoid, antioxidants and phenols. that can be employ to prevent atherosclerosis. The study reported that the use of ascorbic acid (vitamin C), tocopherol (vitamin E) derived from plant have potential as anti- atherosclerotic drugs. Other medicinal plant associated with anti-atherosclerotic include Valeriana officinalis, Medicago sativa Linn, Pulicaria gnaphalodes, Quercus, Sesamum indicum Linn, Gundelia tournefortii, Allium ampeloprasum etc. A study by (Borate et al, 2011) has found PCA to possess anti-atherosclerotic activity by inhibiting

adhesion of monocyte to tumour nerolis in induced mouse aortic endothelial cells. The ability of PCA to function as anti-atherosclerotic is due to its anti-inflammatory properties. Salvia miltiorrhiza is a Chinese customary herb that has been utilized since folk medicine as a remedy for vascular diseases.

3.2.4 Antibacterial Activity of PCA

Scientist have been extracting bioactive components of variety of plant species for use as antibacterial agents, some of these plants extract has shown susceptibility against wide range of bacterial species, such as ginger, garlic, lemon etc. (Klančnik et al 2010; Smullen et al 2007). A research by Chao &Yin 2009 on Hibiscus sabdariffa L (Roselle calyx) extract has shown to contain protocatechuic which help maintain pH value, moisture, protein and fat content during storage at 4°C for 15 days and contamination from bacteria such as campylobacter and aerobes and was also establish to decline oxidation of lipids in beef meat. This research supports the idea of using PCA extracted from plants for use as local preservative for short period of time.

3.3 Diabetes

Diabetes is among the major health issue globally. It is a disorder related to high glucose level and absence of insulin. There are two kinds of diabetes, type 1 and type 2.Chronic diabetes (also known as chronic hyperglycemia) and it long time effect can lead to many organs failure and dysfunction, especially, the kidneys, blood vessels, eyes, heart etc. the major treatment of diabetes involve reducing concentration of glucose or intake of food rich in glucose (hyperglycemic diet), consistent exercise and usage of insulin drugs such as sulphonylureas, meglitinides, thiazolidinediones, biguanides, α-glucosidase inhibitors in addition to insulin and D-phenylalanine derivatives are some method use to regulate and treat diabetes. These drugs mechanism of actions varies from one another, some of the drugs help reduce hepatic gluconeogenesis, delay absorption and digestion of glucose, stimulate sensitivity of insulin receptor, aid in stimulation of insulin secretion. Different studies have reported the side effects of these drugs, some of the listed side-effects include weight gain, severe and critical hypoglycemia and gastrointestinal complications. To avoid these complications, scientist have reconsidered the use of extract derive from medicinal plants for their antidiabetic activities (Stein et al 2013).

3.3.1 Antidiabetic Activity of PCA

Scazzocchio et al 2011 utilized 1 and 2% PCA to study d-galactose treated mice for time period of 8 weeks and the result has shown decrease in fructose, Reactive Oxygen species (ROS) levels, carboxymethyl lysine, methylglyoxal, protein carbonyl, sorbitol and pentosidine. Other study utilized both PCA and cyanidin-3-O-β-glucoside and the result has shown both compounds to exert insulin-like characteristics by using PPAPγ activation. These result and other studies have demonstrated PCA as a potential antidiabetic agent. The same study utilized 2% and 4% PCA which is supplied to diabetic mice for period of 12 weeks has shown to prevent glycation-associated to diabetic disorder (Scazzocchio et al 2011).

Harini & Pugalendi 2010 studied the outcome of PCA on Streptozotocin stimulated diabetic rat and compare the result with Glibenclamide (a synthetic drug known as glyburide used for treatment of type 2 Diabetes Miletus). Wester Albino rats were induced with Streptozotocin (40mg/kg BW, i.p.) and PCA was orally managed at dissimilar dosage concentration (200, 100, 50 mg/kg) per day for the period of 45 days. The result has shown decrease in plasma hemoglobin (HB) and insulin and increase in glycosylated hemoglobin (HBA 1C) and plasma glucose. Other enzymes involve in gluconeogenic were found to increase and some glycolytic enzymes were found to decrease. The study was compared with rats treated with Glibenclamide in saline for the same period of time as PCA administered rats and the result demonstrated that PCA at 100mg/kg can be at the same effectiveness level with Glibenclamide as antihyperglycemic (antidiabetic) agents.

Adisakwattana et al 2005 studied the antihyperglycemic outcome and mechanism of P-methoxycinnamic acid (p-MCA) in diabetic induced rats using Streptozotocin. P-MCA is extracted from derivative of cinnamic acid and different concentrations ranging from 10-100mg/kg PO were administered to both diabetic rats and normal rats (those that were not induced with Streptozotocin) for period of 4 weeks. The results have demonstrated P-MCA to reduced concentration of plasma glucose in both initiated diabetic and ordinary rodents. Different tests have given a few varieties in initiated rodents and ordinary rodents. There were no watched changes in the exercises in compounds include in controlling glucose chemicals, glucose 6 phosphate and hepatic glycogen in ordinary rodents treated with P-MCA. The investigation recommended that antihyperglycemic action of p-MCA might be expected to watch reduction of gluconeogenesis, increment in glycolysis and emission of insulin.

Celik et al 2009 studied both antidiabetic (hypoglycemic) and liver-protective effect of Caffeic acid phenethyl ester (CAPE) on Wistar rats induced with streptozotocin (STZ). To induced diabetes on the models, STZ was injected in a single dose (45mg/kg b.w. i.p.) for two days. Different concentration of CAPE was injected into models 2 days after STZ injection at 10, 20 and 30μM/kg (i.p.) doses. The results were compared with control (rats not induced with STZ). In STZ induced rats, models, CAPE has demonstrated to increase level of glycogen which was lowered by diabetes, a significant decrease triglyceride, cholesterol, alanine aminotransferase, levels of fasting blood glucose. The liver histopathological evaluation carried out has shown elevation of connective tissue in portal region, the effect of CAPE to reduce hepatocytic anisonucleosis and necrosis.

Gandhi et al 2011 studied antidiabetic and antioxidant activities of Solanum torvum Swartz (STS) fruit which was reported to contain different phenolic compound analyzed by High performance liquid chromatography (HPLC). The extracted were administered on Male albino Wistar rats (190–200 g) induced with streptozotocin. Methanol is used to extract the phenolic compounds present in STS and 2 different doses of the extract (400 and 200mg/kg per day) were used to administered to rats induced with STZ for 30 days. The study analyzed antioxidants, marker enzymes involve in carbohydrate metabolism, insulin, levels of glucose, glycated hemoglobin, hemoglobin, hepatic function, total protein and liver glycogen. The HPLC result has shown the presence of different phenolic compounds such as caffeic acid, catechin, rutin and garlic acid. The hypoglycemic result has shown that the higher the concentration (i.e. at 400mg/kg) the lower blood glucose level. The studied has shown that Solanum torvum Swartz (STS) possess antidiabetic activity.

Scazzocchio et al 2011 study the effect of Cynidin-3-O-β-Glucoside (C3G) and PCA exert on insulin like effect by upregulating human oriental adipocyte's peristome proliferative-activated-receptor-γ (PPAR-γ). The research main objective is to test PCA and C3G in place of many sensitizing drugs which are reported to have adverse side effects. Human adipocytes were treated with 100μmmol/L of PCA and 50μmmol/L of C3G. The result has shown increase of uptake of glucose on adipocyte cells as a result of PCA and C3G treatment. Thus, this experiment has shown both PCA and C3G possess antidiabetic activity.

3.4 Streptozotocin

Streptozotocin is a compound isolated as a result of broth fermentation of a bacteria called Streptomyces achromogenes. These drugs are reported to have broad application on tumour cells (malignant melanoma, mycosis fungoides, lymphomas, lung cancer, multiple myeloma and glioma (see figure 3.2). Some of the chemical properties of STZ includes relative stability at 37^0C and pH of 7.4. beta cell-toxic glucose analogue and hydrophilic (Bolzan & Blanchi 2002).

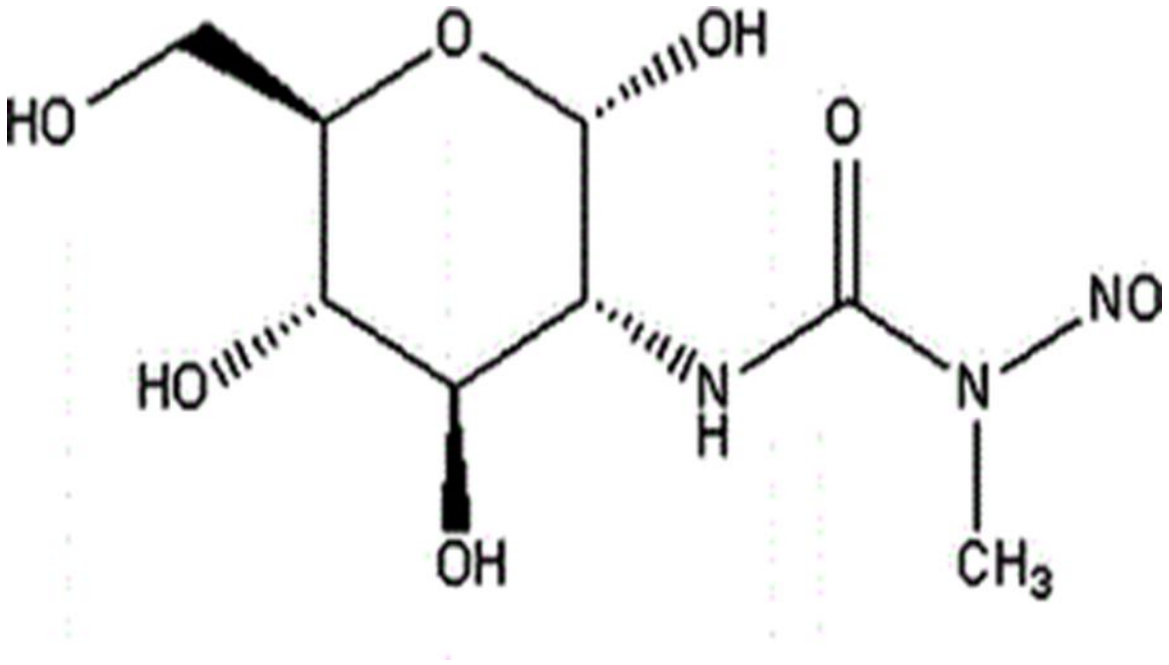


Figure 3. 2 Streptozotocin (Lenzen 2008).

3.4.1 Inducing diabetic in rats using Streptozotocin

Injection of STZ in rats lead to swelling of pancreas and degeneration in Langerhans islet beta cell and thus induces diabetes mellitus. Akbarzadeh et al 2007 used STZ on adult rat weighing between 250-300g for 75 to 90 days. The rats were intravenously injected with 60mg/kg STZ. Controls are used (without STZ injection) and kept separately. The result has shown STZ to decrease pancreatic islet beta cells nicotinamide-adenine dinucleotide (NAD), C-peptide, volume of insulin level, body weight. Serum concentration was found to increase and degeneration of Langerhans islet beta cells after swelling of pancreas were observed.

4. MATERIALS AND METHOD

4.0 Chemicals

The chemical use in this project includes Streptozotocin, protocatechuic acid, stains (hematoxylin, eosin, Masson, periodic acidic stiff).

4.1 Animal Study

The experiments were carried out on healthy Wister rat animals weighing (200 -250 g). The animals were kept in cages with free access to food and water and kept under room temperature.

The four weeks diabetic Wister rat animals were randomly divided into 4 distinct groups for treatments procedures. These groups include:

- Group I: Normal (n =6)
- Group II: Rat induced with Streptozotocin (60mg/kg) i.e. diabetic rat (DM, n = 6).
- Group III: Diabetic Rat administered with PCA (dose: 20 mg/kg b.w) for 3weeks (DM+PCA20 n = 6)
- Group IV: Rat administered with PCA (dose: 20 mg/kg b.w) for three weeks (PCA20 n = 6).

4.2 Diabetes Induction

After fasting for 4 hours, and the selected rats' samples were rendered diabetic after injecting the rats of Group II and III with STZ-Na-citrate solution. Among the 12 rats (6+6) for group II and III, one of the rats was anesthetized by placing it on isoflurane Jar. The rat was removed from the jar after it breathing has slow down and injected with STZ solution with a dosage of 60mg/kg (Jelodar et al, 2007). After diabetic induction, all the rats were awoken and transferred back into the cage. To avoid the occurrence of sudden hypoglycemia, the rats were supplied with 10% sucrose solution overnight. A diabetic test is carried out to confirm the rats that were successfully diabetic as a result of STZ injection. However, for the rat to be considered diabetic their sugar level must be above 250mg, see figure 4.1 below.

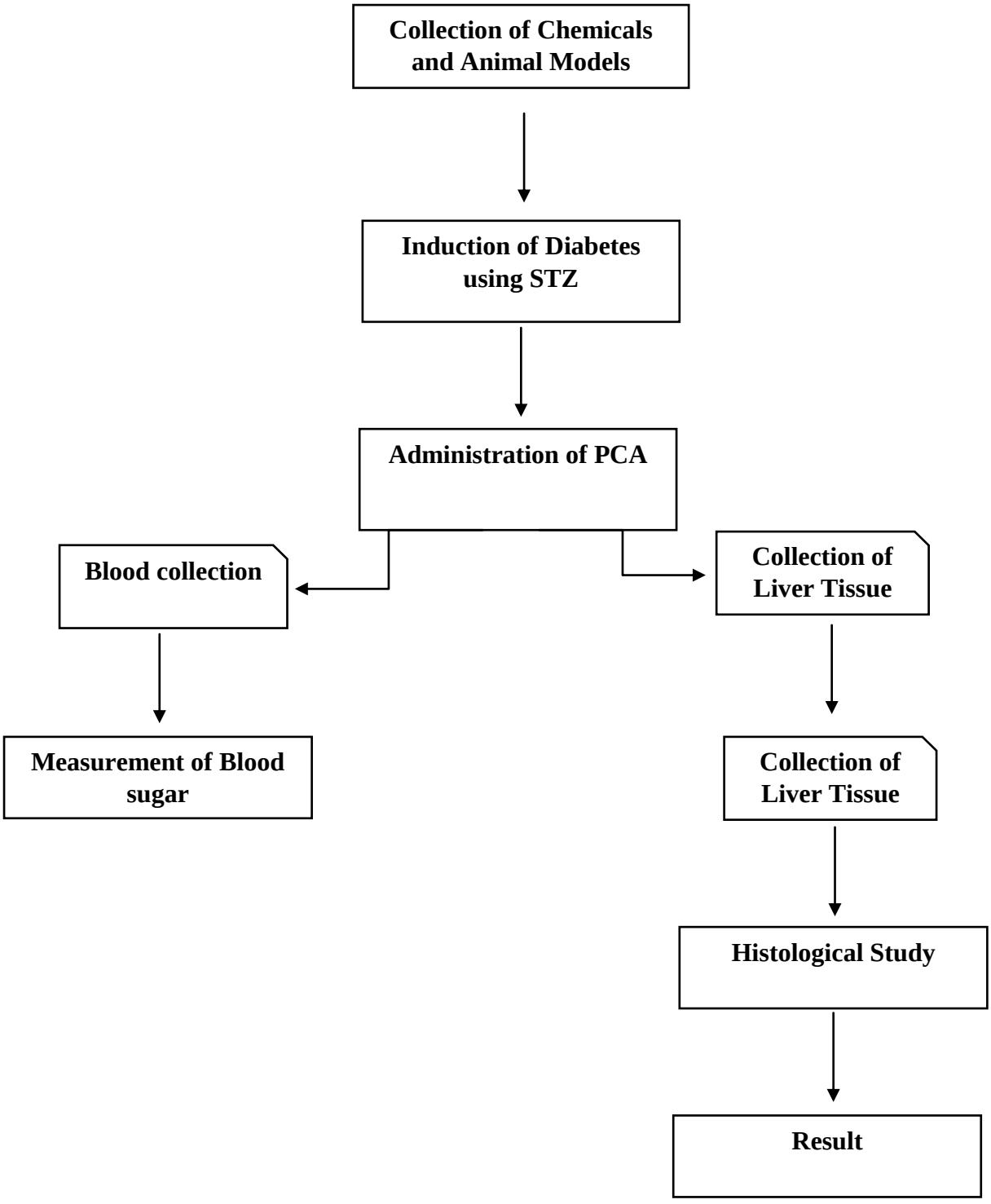


Figure 4. 1**:** Flowchart of Methodology

4.3 Data Collection

Prior to scarification, all the rats were kept in metabolic cage for 12 hours and fasted overnight before blood collection. The preparation and anesthetizing procedure for the rats were performed using cocktail of ketamine: xylamine (90:00 mg/kg b.w.). Whole blood was extracted through heart punching, collected in tubes and under centrifugation to obtain plasma and serum. During euthanasia, liquid nitrogen was used to dissolved the collected liver tissues from storage. In later analyses, the extracted tissues and samples of urine and blood are kept/maintained at a temperature of -800C.

4.4 Histology

Histological assessment was done utilizing little bits of tissues with sizes of 2mm which were gathered from anatomical areas of the rodent's liver and investigation was done to decide territorial contrasts between the tissues. For safeguarding, the tissues were put away in 10% proper saline answer for 48 hours. The fixed tissues were additionally washed in running faucet water for 6-10 hours followed by drying out utilizing liquor in a climbing grade, clearing, inserting in paraffin wax at a softening purpose of 58-600 ^{0}C. squares were set up by cutting segment of 5-6 µm thick and mounting of the segment on to albumenized slides. Drying of the segments were done before recoloring with the accompanying routine histological stains so as to break down various compartment of the liver tissue.

4.4.1 Periodic Acid Schiff (PAS) Staining Protocol (Bio-Optica-04-130808-Milan-Italy)

PAS is a method that is employ for easy identification or tracing of glycogen in tissues, for instance, cardiovascular, liver, skeletal muscle utilizing formalin-fixed, paraffin implanted tissue segments as well as frozen sections. The solutions utilized in this procedure include distilled water and periodic acid. The details of the stains is put 10 drops of reagent A on the section and left to act for 10 minutes then the section was rinsed using distilled water. After this put 10 drops of reagent B are poured on the section and left to act for 10 minutes ,and then the section was rinsed using distilled water. Next put 10 drops of reagent C are poured on the section and left to act for 10 minutes then the section was rinsed using distilled water ,and the slides were drained without rinsing and 10 drops of reagent D is poured on the section and left to act for two minutes. After that the section was rinsed using distilled water. Next put 10 drops of reagent E is poured on the section and left to act for three minutes, then

the section was rinsed using running tap water. next the section was dehydrated through ascending alcohol and cleared in xylene before mounting.

4.4.2 Masson Trichrome Stain (Bio-Optica-04-010802-Milan-Italy)

MTS also known as connective tissue stain is a staining method used for the histological visualization of collagenous connective tissue fibres in tissue sections such as liver, collagen fibres and muscles. The sample type required for the staining procedure include rehydrated and Deparaffinized tissue sections. The details described stain is start with put six drops of reagent A (Wiegert's iron hematoxylin A solution) and six drops of reagent B (Wiegert's iron hematoxylin B solution) are poured on the section and left to act for 10 minutes then the slides were drained without rinsing and 10 drops of reagent C (Picric acid alcohol solution) is poured on the section and left to act for four minutes and then the section was rinsed quickly (three-four seconds) using distilled water and 10 drops of reagent D (Ponceau acid fuchsin according to Mallory) is poured on the section and left to act for 4 minutes after this the section was rinsed using distilled water and 10 drops of reagent E (Phosphomolybdic acid solution) is poured on the section and left to act for 10 minutes then the slides were drained without rinsing and 10 drops of reagent F (Masson aniline blue) is poured on the section and left to act for five minutes after that the section was rinsed using distilled water and dehydrated rapidly through ascending alcohol left for one minute at the last absolute alcohol, cleared in xylene before mounting.

4.4.3 Hematoxylin and Eosin Staining (Hematoxylin: HX69657153, Eosine: HX69574839, Merk Germany)

Haematoxylin and Eosin also known as H&E stain is the most popular staining method used in histology for viewing detail structure of cells (such as nuclear, cytoplasmic, and extracellular matrix features) and tissues. The details of the stain is start with tissues were kept in an oven at 60 ° C for 90 minutes. After drying, the tissues were immediately taken to hot xylol and left for 1 minute then the tissues were kept in xylol at room temperature for 1 minute. After the completion of xylol processes, tissues were further kept in 100% (v/v) alcohol for 1 minute and then tissues were immediately transferred to 80% (v/v) alcohol and left for 1 minute. Immediately after alcohol treatment, tissues were kept in distilled water for 1 minute and then the tissues were transfer to Hematoxylin and kept in for 1 minute and the tissues were kept in acid alcohol for 30 seconds after that tissues were transferred to 80% (v/v) alcohol and then tissues were kept in Eosin for one minute and tissues were then kept in

100% (v/v) alcohol then the Tissues were transferred into xylol and kept at room temperature finally tissues were closed with coverslip using entellan.

4.5 Data Analysis

Statistical package for social sciences (IBM SPSS version 21) was utilized for entry and analysis of data. The data analysis is based on Kruskal Wallis test (which is anis an extension of the Mann-Whitney U test) and Mann-Whitney U test is used when there are differences in independent groups.

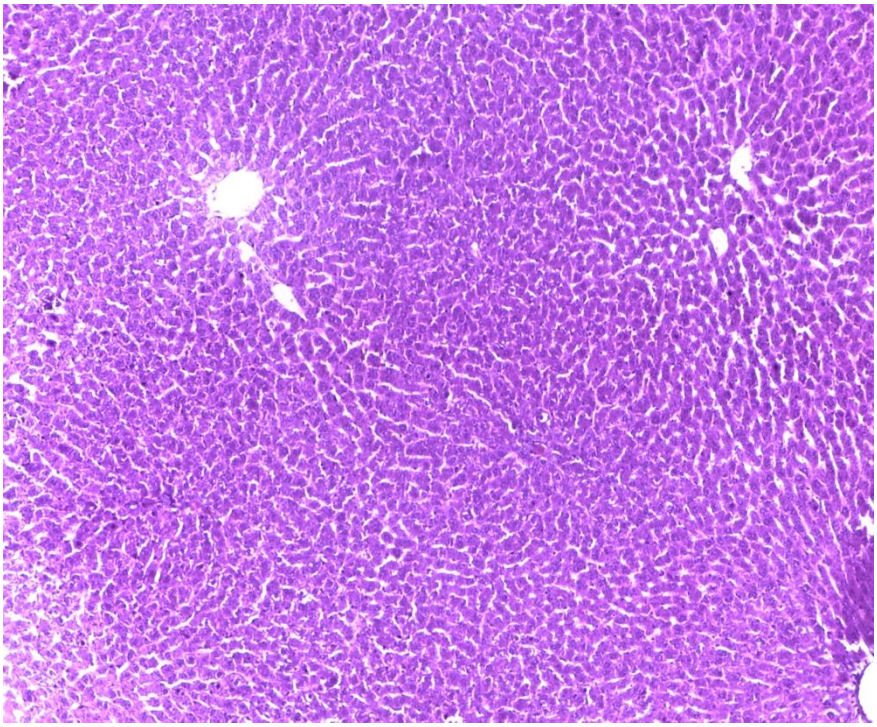
5. RESULTS

5.1 Histological Results

Figure 5.1 shows the Portal area, hepatocytes and sinusoids in the liver tissue of the control group and it can be seen in normal structure. Figure 5.2 shows the HE x40. Group I (control group) and it can be seen that Portal area, hepatocytes and sinusoids in the liver tissue of Group I (control group) appear to be normal. Figure 5.3 shows PAS x40 of Group I (control group) with no observed Kupffer cell increase and PAS (+) staining is normal in the hepatocyte's zones Figure 5. 4 shows the Masson Trichrome x40 of Group I (control group) and it can be seen that the glycogen level is normal in the hepatocytes around Vena Sentralis. of the control group. PAS x40. It can be seen there is no increase in connective tissue of the portal area in the control group. Figure 5. 5: HE x10 of Group IV (administered with only PCA) and It can be seen that the liver appears normal. Figure 5. 6 shows PAS x40 of Group IV (administered with only PCA) and it can be seen that glycogen staining is similar to the control group in hepatocytes.

Figure 5.7 shows the Masson Trichrome x40 of Group IV (administered with only PCA) and it can be seen that there is no increase in connective tissue in the PCA group. Figure 5. 8 shows the Vena Sentralis (vs) HE x10.Group II (rat induced with STZ) and it can be seen that hepatocytes around the portal area (Pa) in the liver tissue of the diabetic group are stained openly. Figure 5. 9 shows the HE x40 of Group II (rat induced with STZ) and congestion is observed in the liver tissue sinusoids of the diabetic group. Figure 5. 10 shows the PAS x40 of Group II (rat induced with STZ) and Kupffer cells (shown in the arrow) can be seen in the liver tissue of the diabetic group. Figure 5. 11 shows the PAS x40 of Group II (rat induced with STZ) and it can be seen that hepatocytes around the portal area of the diabetic group are stained openly and glycogen decreased. Figure 5. 12 shows the PAS x40 of Group II (rat induced with STZ) and it can be seen that hepatocytes around the v. centralis in Group II (rat induced with STZ) are normal PAS staining (as shown using arrows). Figure 5. 13 shows the Masson Trichrome x40 of Group II (rat induced with STZ) and there is no increase in connective tissue of Group II (rat induced with STZ). Figure 5. 14 shows the PAS x10 of Group III (diabetic group treated with PCA) and it can be seen that the liver structure of Group III (diabetic group treated with PCA) is similar to Group I (Normal/Control). Figure 5. 15 shows the Trichrome staining x40 of Group III (diabetic group treated with PCA) and it

can be seen there is no increase in connective tissue in Group III (diabetic group treated with PCA). Figure 5. 16 shows the PAS x40 of Group III (diabetic group treated with PCA) and it can be seen that hepatocytes around the portal area in Group III (diabetic group treated with PCA) are normal PAS staining.



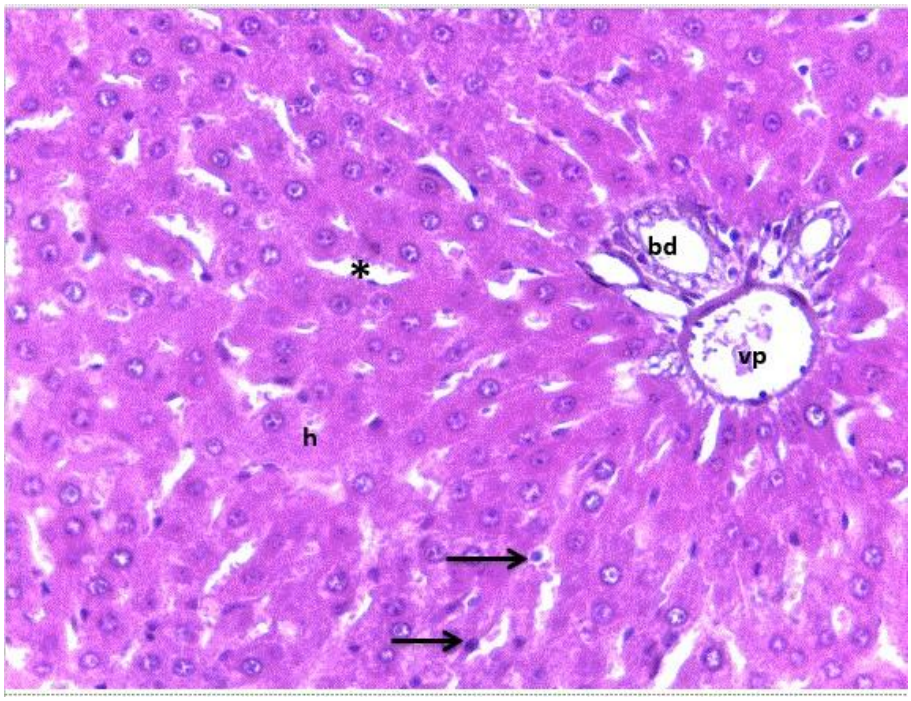


Figure 5. 1: Liver tissue. HE x10 Group I (control group)

Figure 5. 2: HE x40. Group I (control group).

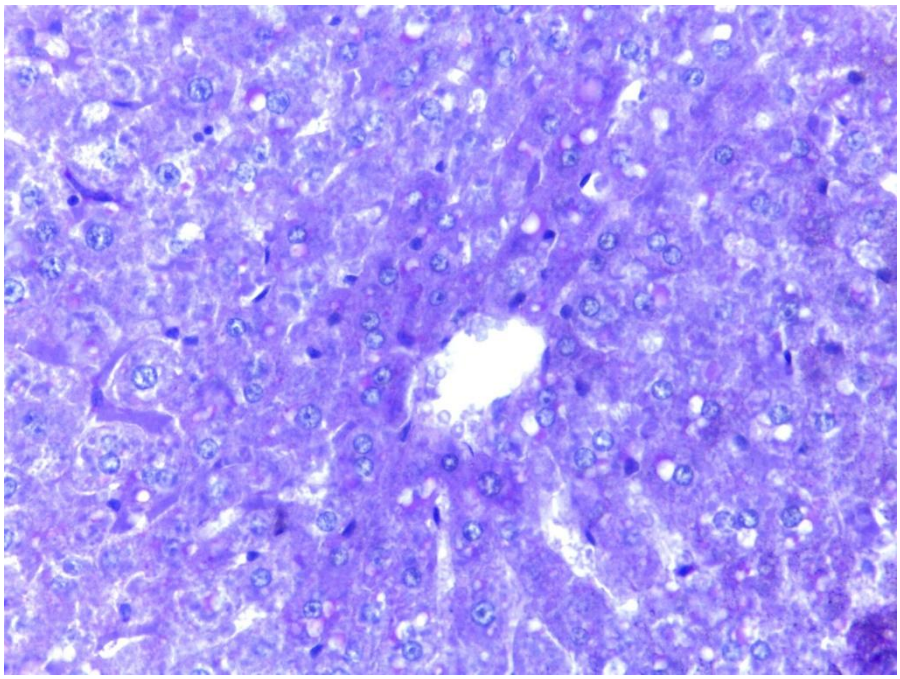


Figure 5.3: PAS x40. Group I (control group)

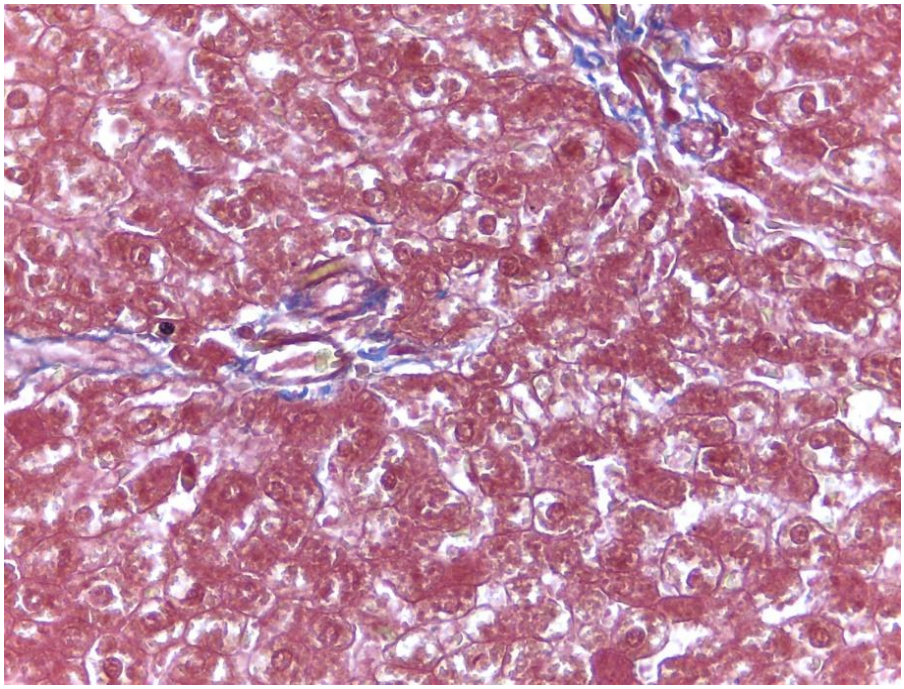


Figure 5. 4**:** Masson Trichrome x40. Group I (control group)

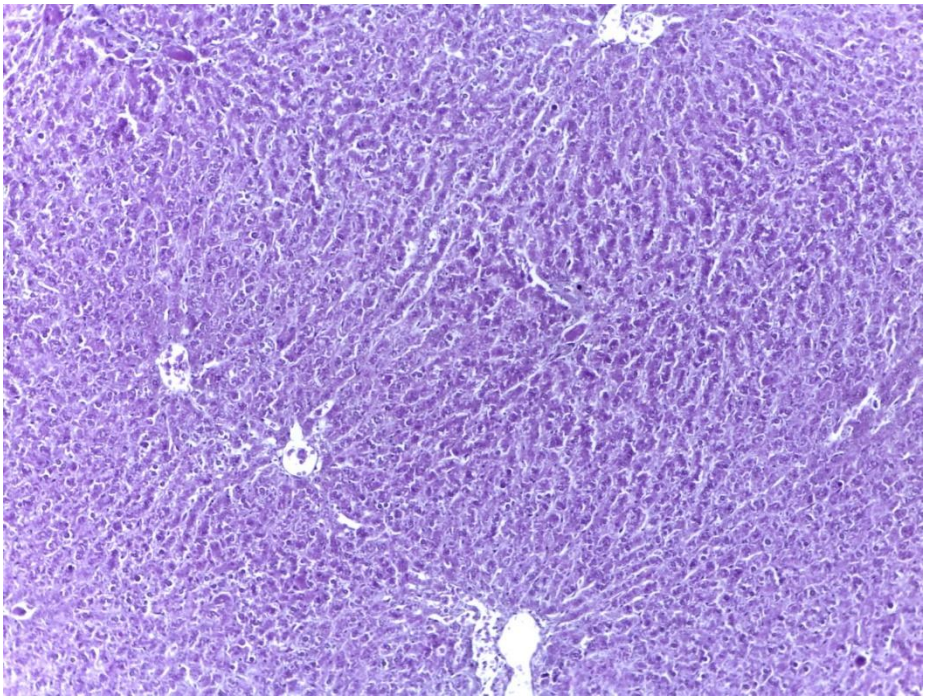


Figure 5. 5**:** HE x10 of Group IV (administered with only PCA).

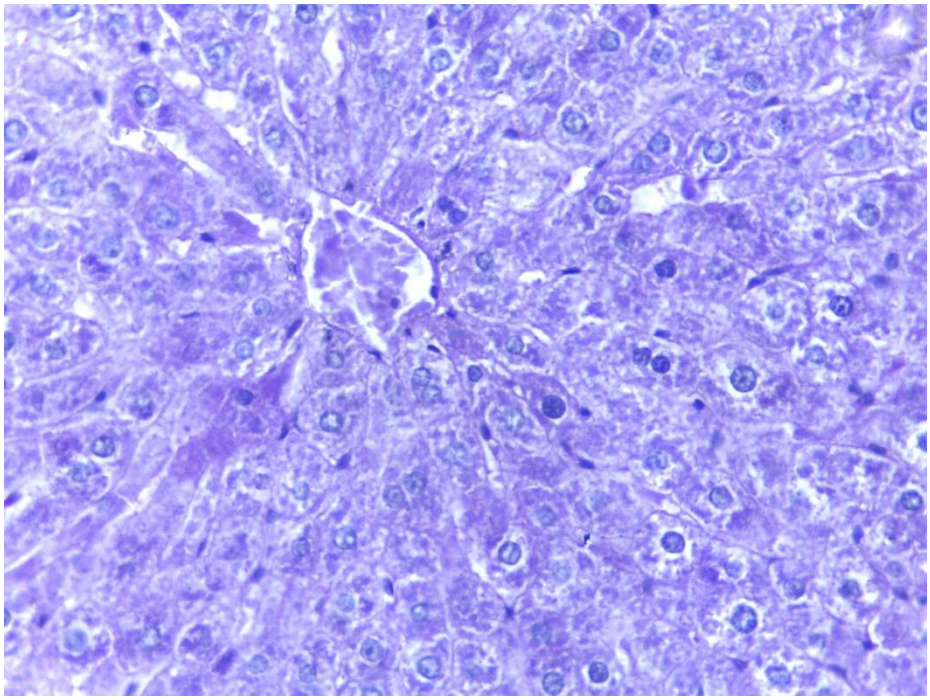


Figure 5. 6**:** PAS x40. Group IV (administered with only PCA).

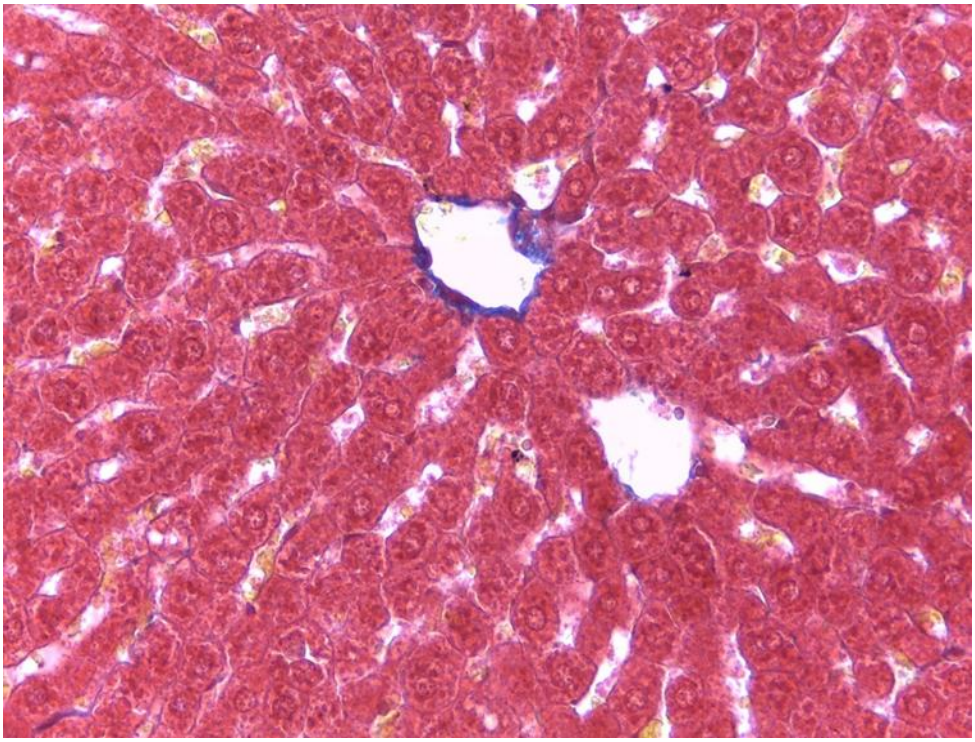


Figure 5. 7**:** Masson Trichrome x40. Group IV (administered with only PCA).

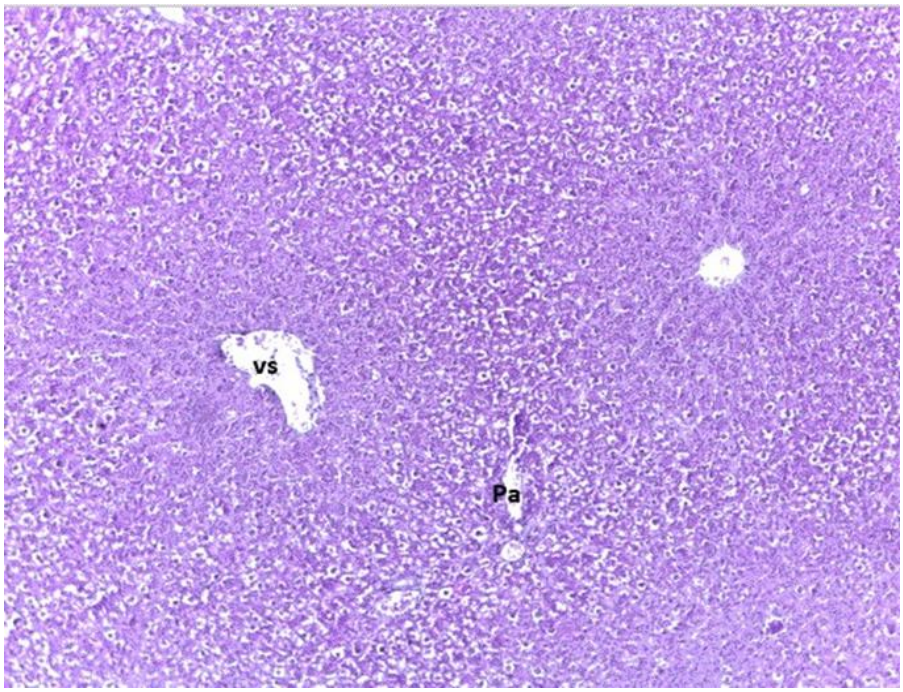


Figure 5. 8: Vena Sentralis (vs). HE x10.Group II (rat induced with STZ).

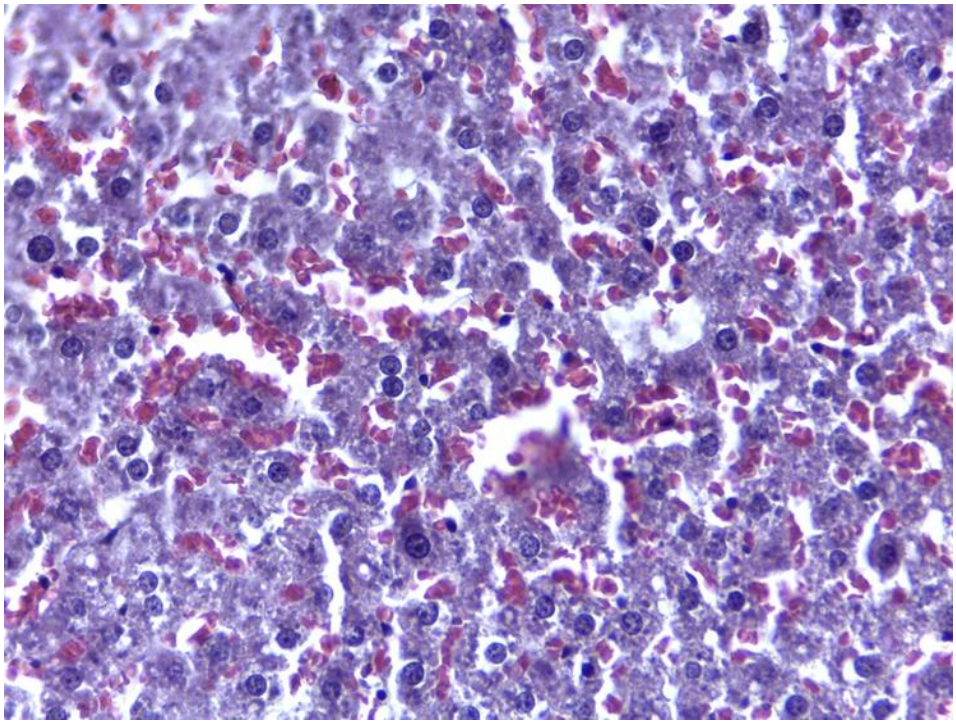


Figure 5. 9**:** HE x40. Group II (rat induced with STZ).

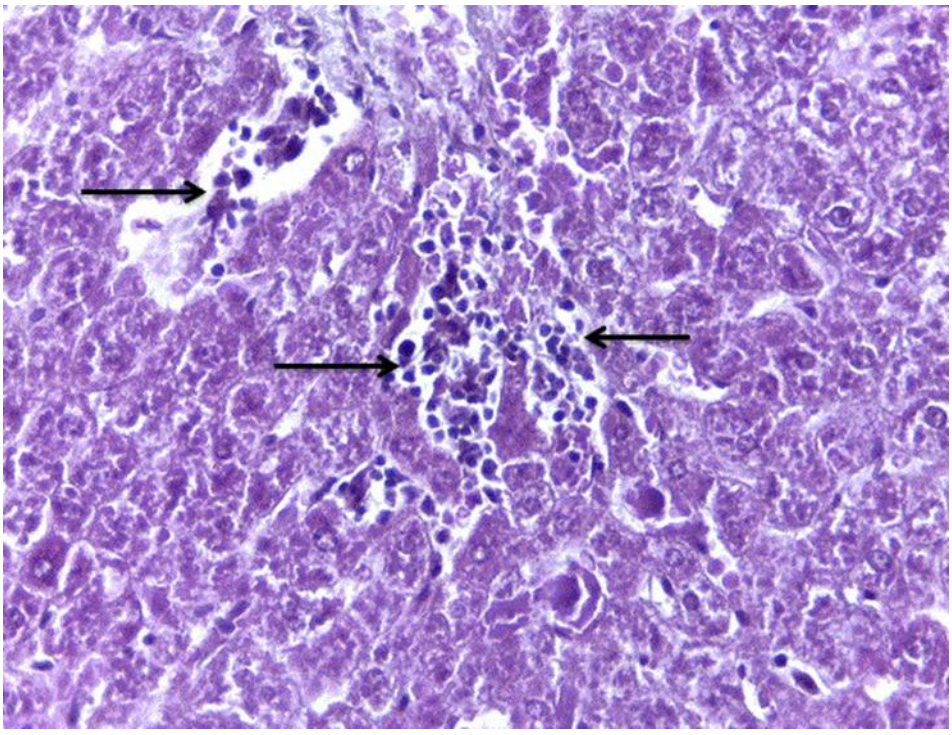


Figure 5. 10**:** PAS x40. Group II (rat induced with STZ).

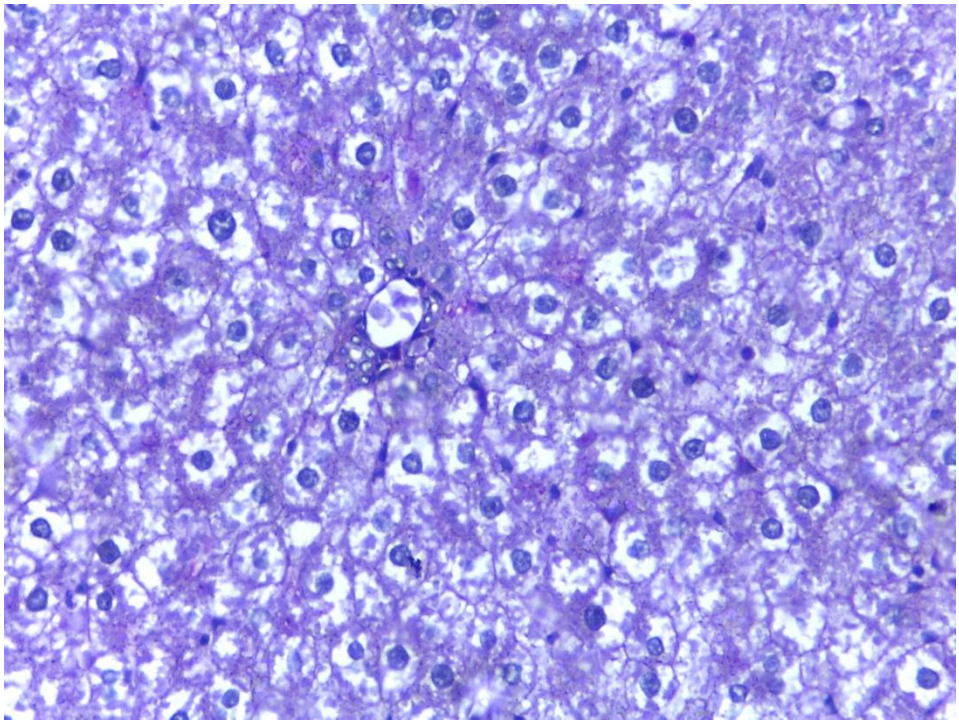


Figure 5. 11**:** PAS x40. Group II (rat induced with STZ)

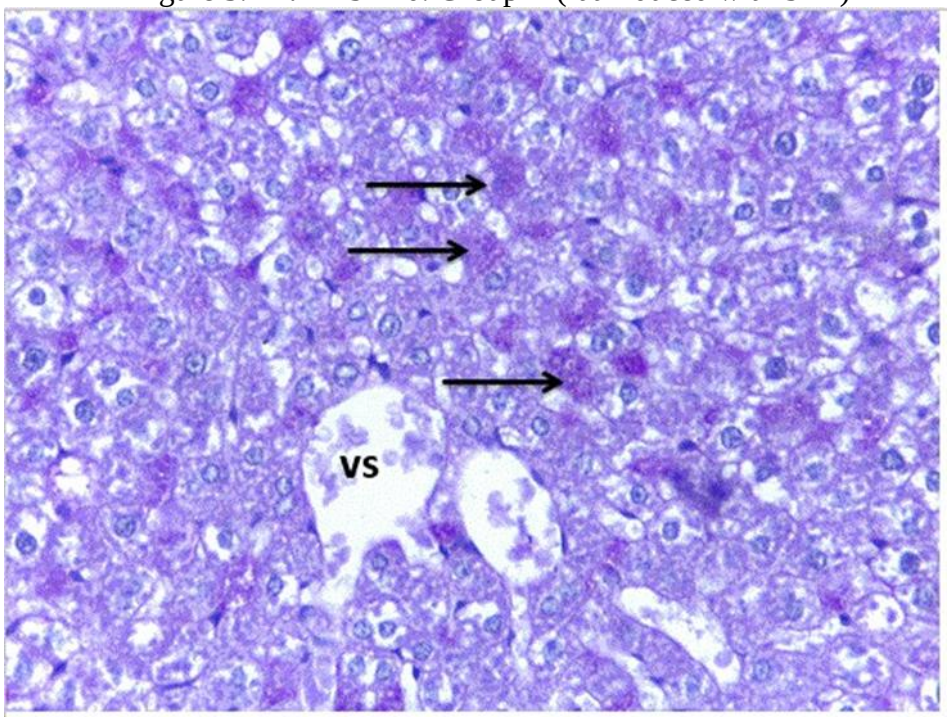


Figure 5. 12**:** PAS x40. Group II (rat induced with STZ)

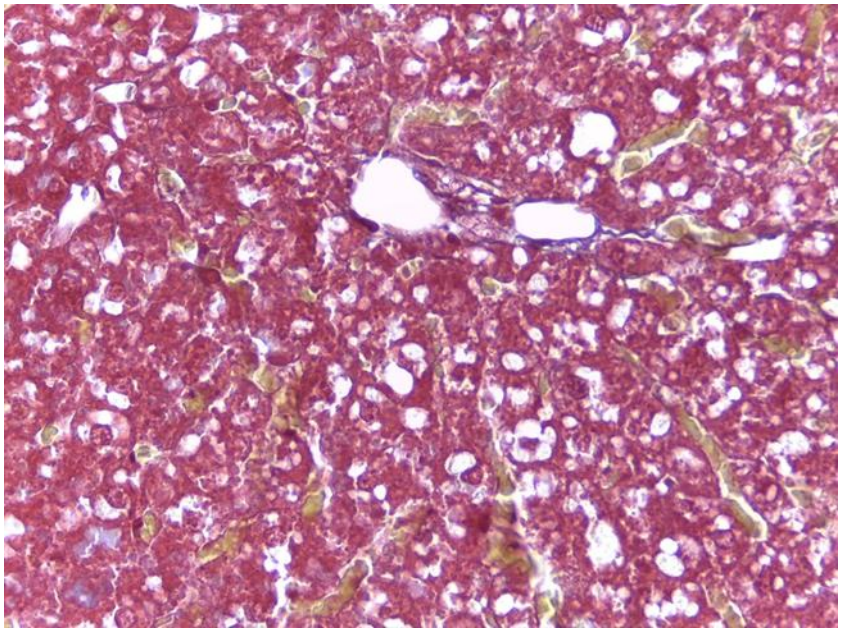


Figure 5. 13**:** Masson Trichrome x40 of Group II (rat induced with STZ)

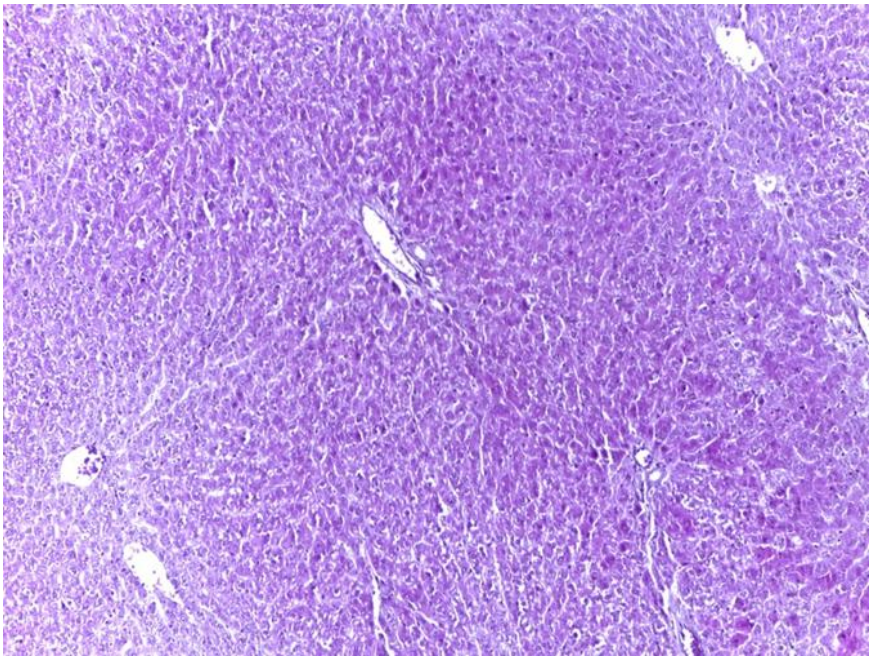


Figure 5. 14**:** PAS x10 of Group III (diabetic group treated with PCA)

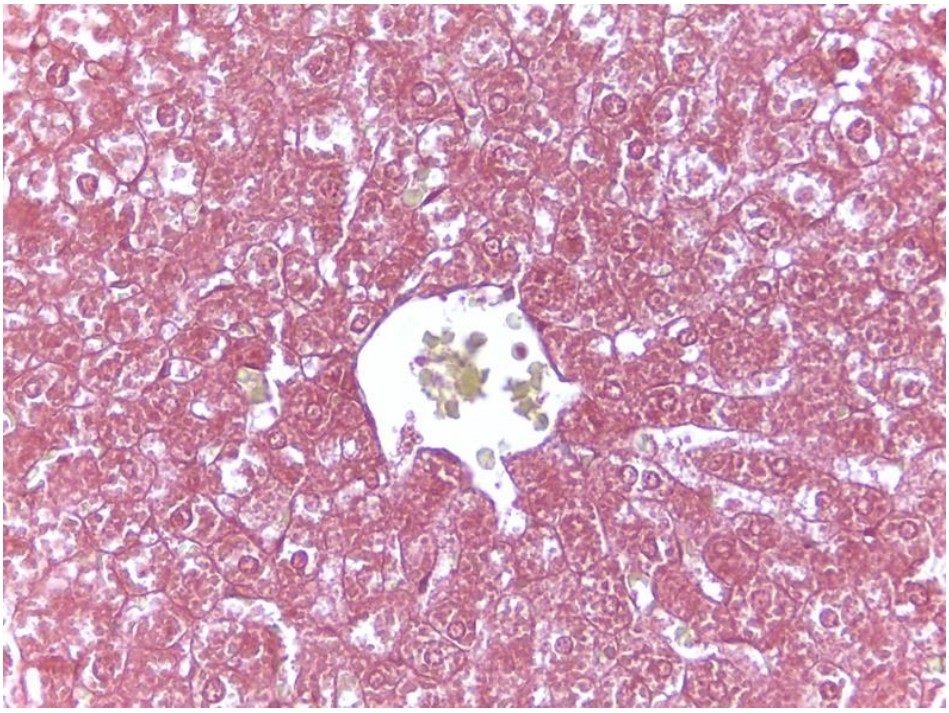


Figure 5. 15**:** Trichrome staining x40. Group III (diabetic group treated with PCA).

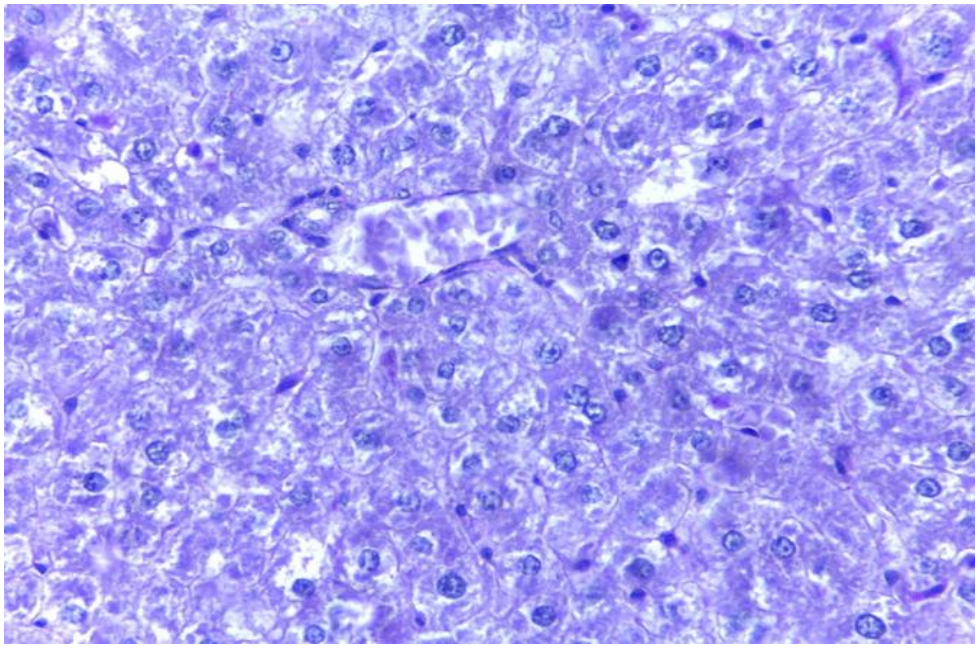


Figure 5. 16**:** PAS x40. Group III (diabetic group treated with PCA)

5.2 Statistical analysis

Statistical result values are given as means ± S.D. for six rats in each group. Data were analyzed based on Kruskal Wallis and Mann-Whitney using SPSS version 10 (IBM SPSS version 21). The limit of statistical significance was set at $P < .05$.

Overall description of the statistical test analysis is given in table 5.1 which shows biochemical parameters (Aspartate, Alanine and Alkaline Phosphatase) and mean ranks of each group. Table 5.2 and 5.3 shown the Kruskal Wallis test for all the groups. All groups are compared with each other to see the differences in mean ranks of Liver enzymes (AST, ALT and ALP). Table 5.4 and 5.5 shows the comparison of Mann Whitney test for group I and group II (Control Vs DM) based on differences in mean ranks of Liver enzymes (AST, ALT and ALP). Table 5.6 and 5.7 shows the comparison of Mann Whitney test of group I and group IV (Control Vs PCA) based on the differences in mean ranks of Liver enzymes (AST, ALT and ALP). Table 5.8 and 5.9 shows the comparison of Mann Whitney test of group I and group III (Control Vs DM+PCA) based on the differences in mean ranks of Liver enzymes (AST, ALT and ALP). Table 5.10 and 5.11 shows the comparison of Mann Whitney test of group I and group III (DM Vs PCA) based on the differences in mean ranks of Liver enzymes (AST, ALT and ALP). Table 5.12 and 5.13 shows the comparison of Mann Whitney test of group I and group III (DM Vs DM+PCA) based on the differences in mean ranks of Liver enzymes (AST, ALT and ALP). Table 5.14 and 5.15 shows the comparison of Mann Whitney test of group III and group IV (DM+PCA Vs PCA) based on the differences in mean ranks of Liver enzymes (AST, ALT and ALP). Table 5.16 and 5.17 shows the case comparison and reports of percentage, standard deviation, mean, maximum and minimum of all groups.

Table 5. 1**:** Biochemical parameters of all Groups

Experimental Groups	Liver enzyme (AST)	Liver enzyme (ALT)	Liver enzyme (ALP)
Control (n:6)	89.0±17.5	40.6 ±9.41	220.0 ±33.9
DM (n:6)	414.3±421.5*	386. 1±416.8*	539.6±109.8*
PCA (n:6)	106.6±16.6 #	58.8±8.65*#	187.0±45.5#
DM+PCA (n:6)	94.1±11.3#	52.6±8.45#	296.6±62.0 # ρ

Values are mean ± SD of six rats from each group

*values when compared to control statistically significant $p<0.05$.

values when compared to DM statistically significant $p<0.05$.

P vales when compared to PCA statistically significant $p<0.05$.

Figure 5.17 shows Bar Chart of Mean Rank and Groups for Aspartate. Group II (administered with streptozotocin) has shown the highest mean rank of 19.92 for aspartate compare to other groups 6.58, 10.25 and 13.25 for group I, III and IV respectively. Figure 5.18 shows Bar Chart of Mean Rank and Groups for Alanine. Group II (administered with streptozotocin) has shown the highest mean rank of 20.25 for alanine compare to other groups 5.17, 10.17 and 14.42 for group I, III and IV respectively. Figure 5.19: Bar Chart of Mean Rank and Groups for Alkaline. Group II (administered with streptozotocin) has shown the highest mean rank of 21.50 for alkaline compare to other groups 9.17, 14.17 and 5.17 for group I, III and IV respectively.

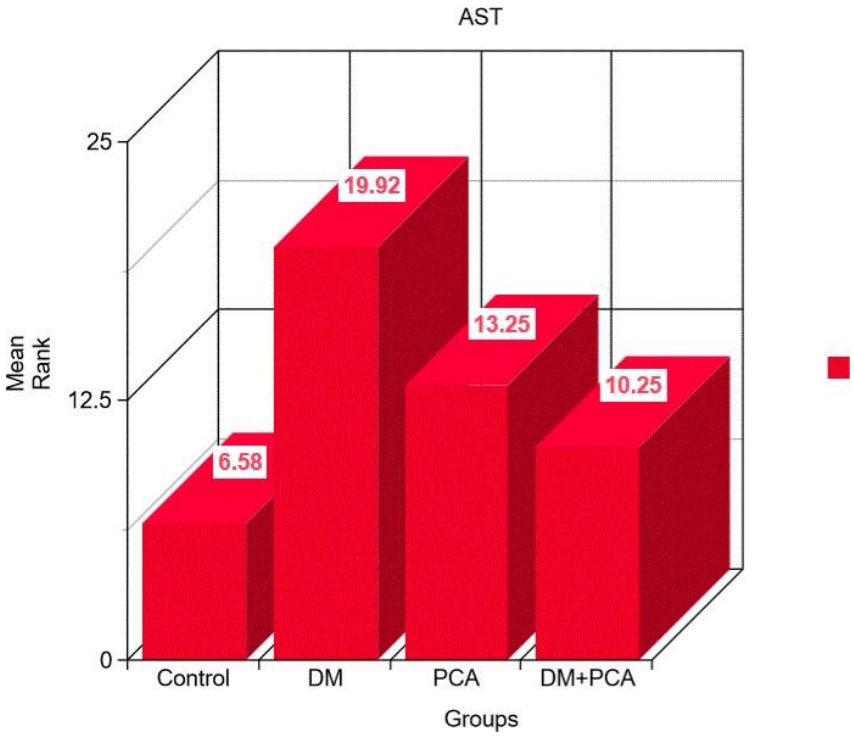


Figure 5.17**:** Bar Chart of Mean Rank and Groups for Aspartate

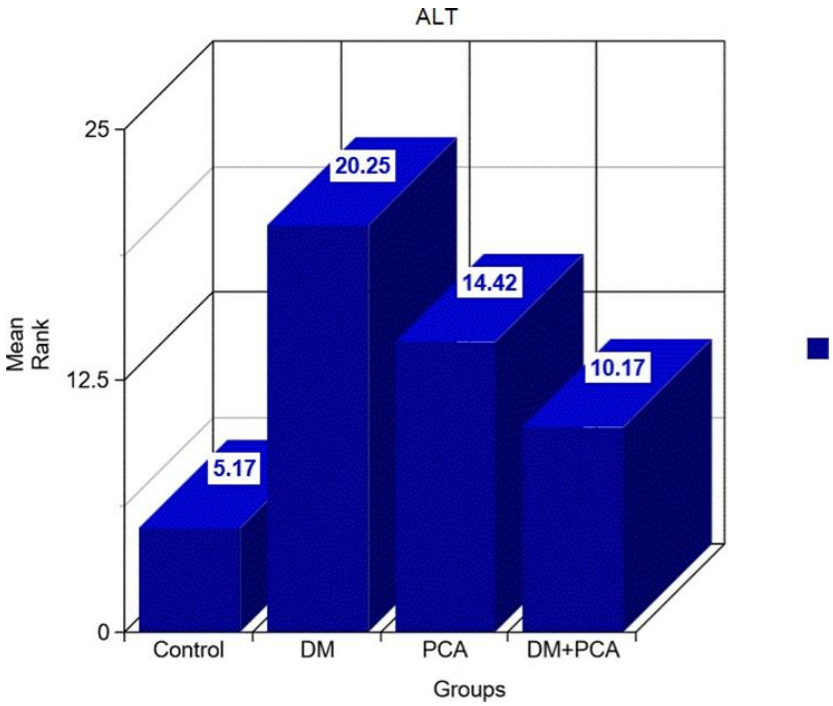


Figure 5.18**:** Bar Chart of Mean Rank and Groups for Alanine.

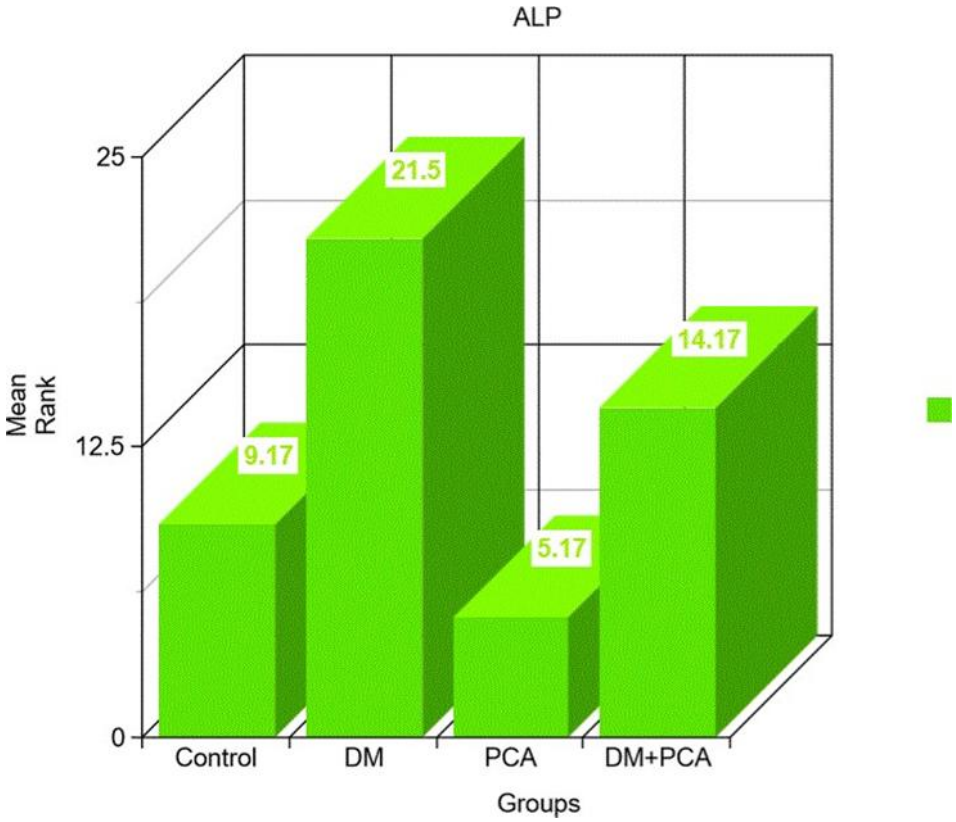


Figure 5.19**:** Bar Chart of Mean Rank and Groups for Alkaline

Table 5. 2**:** Kruskal-Wallis Test for all the Groups

Ranks			
	GROUP	N	Mean Rank
Liver enzyme (AST)	I (control)	6	6.58
	II (DM)	6	19.92
	IV (PCA)	6	13.25
	III(DM+PCA)	6	10.25
	Total	24	
Liver enzyme (ALT)	I (control)	6	5.17
	II (DM)	6	20.25
	IV (PCA)	6	14.42
	III(DM+PCA)	6	10.17
	Total	24	
Liver enzyme (ALP)	I (control)	6	9.17
	II (DM)	6	21.50
	IV (PCA)	6	5.17
	III (DM+PCA)	6	14.17
	TOTAL	24	

Table 5. 3**:** Test statistics for Kruskal Wallis Test

Test Statistics[a, b]			
	Liver enzyme (AST)	Liver enzyme (ALT)	Liver enzyme (ALP)
Chi-square	11.492	14.774	17.840
Df	3	3	3
Asymp. Sig.	.009	.002	.000
a. Kruskal Wallis Test b. Grouping Variable: GROUP			

Table 5. 4**:** Mann-Whitney Test for all the group I and II

Ranks				
	GROUP	N	Mean Rank	Sum of Ranks
Liver enzyme (AST)	I (control)	6	3.67	22.00
	II (DM)	6	9.33	56.00
	Total	12		
Liver enzyme (ALT)	I (control)	6	3.58	21.50
	II (DM)	6	9.42	56.50
	Total	12		
Liver enzyme (ALP)	I (control)	6	3.50	21.00
	II (DM)	6	9.50	57.00
	Total	12		

Table 5. 5: Test Statistics for Mann-Whitney of Group I and II

Test Statistics[b]			
	Liver enzyme (AST)	Liver enzyme (ALT)	Liver enzyme (ALP)
Mann-Whitney U	1.000	.500	.000
Wilcoxon W	22.000	21.500	21.000
Z	-2.722	-2.807	-2.882
Asymp. Sig. (2-tailed)	.006	.005	.004
Exact Sig. [2*(1-tailed Sig.)]	.004[a]	.002[a]	.002[a]
a. Not corrected for ties.			
b. Grouping Variable: GROUP			

Table 5. 6**:** Mann-Whitney Test for Group I and IV

Ranks				
	GROUP	N	Mean Rank	Sum of Ranks
Liver enzyme (AST)	I (control)	6	4.50	27.00
	IV (PCA)	6	8.50	51.00
	Total	12		
Liver enzyme (ALT)	I (control)	6	3.83	23.00
	IV (PCA)	6	9.17	55.00
	Total	12		
Liver enzyme (ALP)	I (control)	6	8.17	49.00
	IV (PCA)	6	4.83	29.00
	Total	12		

Table 5. 7**:** Test statistics for Mann-Whitney Test of Group I and IV

Test Statistics[b]			
	liver enzyme (AST)	liver enzyme (ALT)	liver enzyme (ALP)
Mann-Whitney U	6.000	2.000	8.000
Wilcoxon W	27.000	23.000	29.000
Z	-1.925	-2.562	-1.601
Asymp. Sig. (2-tailed)	.054	.010	.109
Exact Sig. [2*(1-tailed Sig.)]	.065[a]	.009[a]	.132[a]
a. Not corrected for ties. b. Grouping Variable: GROUP			

Table 5. 8**:** Mann-Whitney Test for Group I and III

Ranks				
	GROUP	N	Mean Rank	Sum of Ranks
Liver enzyme (AST)	I (control)	6	5.42	32.50
	III (DM+PCA)	6	7.58	45.50
	Total	12		
Liver	I (control)	6	4.75	28.50
Enzyme (ALT)	III (DM+PCA)	6	8.25	49.50
	Total	12		
Liver Enzyme	I (Control)	6	4.50	27.00
	III (DM + PCA)	6	8.50	51.00
	Total	12		

Table 5. 9**:** Test statistics for Mann-Whitney Test of Group I and III

Test Statistics[b]			
	Liver enzyme (AST)	Liver enzyme (ALT)	Liver enzyme (ALP)
Mann-Whitney U	11.500	7.500	6.000
Wilcoxon W	32.500	28.500	27.000
Z	-1.043	-1.684	-1.922
Asymp. Sig. (2-tailed)	.297	.092	.055
Exact Sig. [2*(1-tailed Sig.)]	.310[a]	.093[a]	.065[a]
a. Not corrected for ties. b. Grouping Variable: GROUP			

Table 5. 10**:** Mann-Whitney Test for Group II and IV

Ranks				
	GROUP	N	Mean Rank	Sum of Ranks
Liver enzyme (AST)	II (DM)	6	8.75	52.50
	IV (PCA)	6	4.25	25.50
	Total	12		
Liver enzyme (ALT)	II (DM)	6	8.67	52.00
	IV (PCA)	6	4.33	26.00
	Total	12		
Liver enzyme (ALP)	II (DM)	6	9.50	57.00
	IV (PCA)	6	3.50	21.00
	Total	12		

Table 5. 11**:** Test statistics for Mann-Whitney Test of Group II and IV

Test Statistics[b]			
	liver enzyme	liver enzyme	liver enzyme
Mann-Whitney U	4.500	5.000	.000
Wilcoxon W	25.500	26.000	21.000
Z	-2.169	-2.082	-2.882
Asymp. Sig. (2-tailed)	.030	.037	.0044
Exact Sig. [2*(1-tailed Sig.)]	.026[a]	.014[a]	.002[a]
a. Not corrected for ties. Grouping Variable: GROUP			

Table 4. 12**:** Mann-Whitney Test for Group II and III

Ranks				
	GROUP	N	Mean Rank	Sum of Ranks
Liver enzyme (AST)	II (DM)	6	8.83	53.00
	III (DM+PCA)	6	4.17	25.00
	Total	12		
Liver enzyme (ALT)	II (DM)	6	9.17	55.00
	III (DM+PCA)	6	3.83	23.00
	Total	12		
Liver enzyme (ALP)	II (DM)	6	9.50	57.00
	III (DM+PCA)	6	3.50	21.00
	Total	12		

Table 5. 13**:** Test statistics for Mann-Whitney Test of Group II and III

Test Statistics[b]			
	liver enzyme	liver enzyme	liver enzyme
Mann-Whitney U	4.000	2.000	.000
Wilcoxon W	25.000	23.000	21.000
Z	-2.242	-2.562	-2.882
Asymp. Sig. (2-tailed)	.025	.010	.004
Exact Sig. [2*(1-tailed Sig.)]	.026[a]	.009[a]	.002[a]
a. Not corrected for ties. b. Grouping Variable: GROUP			

Table 5. 14**:** Mann-Whitney Test for Group III and IV

Ranks				
	GROUP	N	Mean Rank	Sum of Ranks
Liver enzyme (AST)	IV (PCA)	6	7.50	45.00
	III DM+PCA	6	5.50	33.00
	Total	12		
Liver enzyme (ALT)	IV (PCA)	6	7.92	47.50
	III DM+PCA	6	5.08	30.50
	Total	12		
Liver enzyme (ALP)	IV (PCA)	6	3.83	23.00
	III DM+PCA	6	9.17	55.00
	Total	12		

Table 5. 15**:** Test statistics for Mann-Whitney Test of Group III and IV

Test Statistics[b]			
	Liver enzyme (AST)	Liver enzyme (ALT)	Liver enzyme (ALP)
Mann-Whitney U	12.000	9.500	2.000
Wilcoxon W	33.000	30.500	23.000
Z	-.962	-1.363	-2.562
Asymp. Sig. (2-tailed)	.336	.173	.010
Exact Sig. [2*(1-tailed Sig.)]	.394[a]	.180[a]	.009[a]
a. Not corrected for ties. b. Grouping Variable: GROUP			

Table 5. 16**:** Case Processing Summary

Case Processing Summary						
	Cases					
	Included		Excluded		Total	
	N	Percent	N	Percent	N	Percent
Liver enzyme * GROUP	24	100.0%	0	.0%	24	100.0%
Liver enzyme * GROUP	24	100.0%	0	.0%	24	100.0%
Liver enzyme * GROUP	24	100.0%	0	.0%	24	100.0%

Table 5. 17**:** Report of all the groups

Report				
GROUP		liver enzyme (AST)	liver enzyme (ALT)	liver enzyme (ALP)
I (Control)	Mean	89.0000	40.6667	220.0000
	Std. Deviation	17.51571	9.41630	33.92344
	Median	85.0000	38.0000	209.5000
	Minimum	73.00	29.00	181.00
	Maximum	123.00	53.00	270.00
II (DM)	Mean	414.3333	386.1667	539.6667
	Std. Deviation	421.51947	416.80663	109.83199
	Median	293.5000	169.5000	542.0000
	Minimum	94.00	53.00	418.00
	Maximum	1244.00	1035.00	724.00
IV (PCA)	Mean	106.6667	58.8333	187.0000
	Std. Deviation	16.66933	8.65833	45.50165
	Median	105.5000	57.5000	170.5000
	Minimum	85.00	46.00	138.00
	Maximum	125.00	70.00	244.00
III (DM+PCA)	Mean	94.1667	52.6667	296.6667
	Std. Deviation	11.33872	8.45380	62.06019
	Median	98.0000	50.5000	311.0000
	Minimum	75.00	43.00	195.00
	Maximum	106.00	68.00	367.00
Total	Mean	176.0417	134.5833	310.8333
	Std. Deviation	242.02164	244.69962	155.00117
	Median	99.5000	54.0000	254.0000
	Minimum	73.00	29.00	138.00
	Maximum	1244.00	1035.00	724.00

6. DISCUSSION AND CONCLUSION

6.1 Discussion

Due to the high abundance of high calorie diets and unhealthy living habits of people around the globe, the prevalence of hyperglycemia is increasing along with related complications such as cerebrovascular and cardiovascular diseases, morbidity and mortality (Zeng et al 2018). However, there is high need for exploring and analyzing plant-derived materials with low side effects to prevent the occurrence of the diseases and protect against the risk of diabetes and its complications.

The fundamental objective of this study is to evaluate the anti-diabetic and hepato-protective activity of PCA in STZ-induced diabetic rats. It was observed that hepatocyte cords of the liver tissue were regular in the Group I (control group), there was no observed Kupffer cell increase, PAS (+) staining was normal in the hepatocytes zones, and there was no connective tissue increase (as shown in figure 5.1, 5.2, 5.3 and 5.4). In the Group IV (administered with only PCA), the liver structure and glycogen distribution were similar to the Group I (control group) as shown in figure 5.5, 5.6 and 5.7. In Group II (rat induced with STZ) i.e. the diabetic group, while open stained cytoplasm was observed in many hepatocytes around the portal area, v. Hepatocytes around Sentralis were stained normally (as shown in figure 5.8), congestion in sinusoids (as shown in figure 5.9), and Kupffer cell increase (as shown in figure 5.10). It was observed that PAS staining performed to define glycogen decreased in hepatocytes around the portal area (as shown in figure 5.11) and normal in the hepatocytes nearby the vital vein (as shown in figure 5.12). Furthermore, it was observed that there was no collagen increase in portal area and other regions, and vacuolization in hepatocytes (as shown in figure 5.13). In Group III (diabetic group treated with PCA), hepatocytes have very low vacuolization, there was no Kupffer cell increase (as shown in figure 5.14), hepatocytes around the portal area have normal PAS (+) staining (as shown in figure 5.15) and no connective tissue fibers increased as in the control and diabetes group (as shown in figure 5.16).

Histological study carried out in this study has shown that the administration of Protocatechuic acid (PCA) in Streptozotocin (STZ) induced in diabetic rats' tissue has demonstrated positive histologic effects. However, similar results were reported by Scazzocchio et al 2011 who studied the effect of PCA on diabetic rats. Moreover, closed

results were reported by Harini & Pugalendi 2010 studied the effect of PCA on Streptozotocin induced diabetic rat. The study has shown decrease in plasma haemoglobin (HB) and insulin, increase in glycosylated haemoglobin (HBA 1C), plasma glucose, decrease in enzymes involve in gluconeogenesis and increase in enzymes involve in glycolysis.

Another study that demonstrated the antidiabetic effect of PCA were reported by Adisakwattana et al 2005. The results have shown PCA to reduced concentration of plasma glucose in both induced diabetic and normal rats. Moreover, some studies utilized different chemical compounds which demonstrated antidiabetic properties on rats' models. Some of these studies include the analysis of Caffeic acid phenethyl ester (CAPE) administered on Wistar rats induced with streptozotocin (STZ) by Celik et al 2009 and the use of Solanum torvum Swartz (STS) fruit extracts on Wistar rats induced with streptozotocin by Gandhi et al 2011.

The overall description of the statistical test analysis is given in table 5.1 which shows biochemical parameters (Aspartate, Alanine and Alkaline Phosphatase) and mean ranks of each group. Table 5.2 and 5.3 shown the Kruskal Wallis test for all the groups. To see the difference in mean ranks of all the groups, supplementary tables are provided to compare groups against each other. Moreover, table 5.4 and 5.5 shows the comparison of Mann Whitney test for mean ranks of group I and group II (Control Vs DM). Table 5.6 and 5.7 shows the comparison of Mann Whitney test for mean ranks of group I and group IV (Control Vs PCA). Table 5.8 and 5.9 shows the comparison of Mann Whitney test for mean ranks of group I and group III (Control Vs DM+PCA). Table 5.10 and 5.11 shows the comparison of Mann Whitney test for mean ranks of group II and group IV (DM Vs PCA). Table 5.12 and 5.13 shows the comparison of Mann Whitney test for mean ranks of group II and group III (DM Vs DM+PCA). Table 5.14 and 5.15 shows the comparison of Mann Whitney test for mean ranks of group III and group IV (DM+PCA Vs CA). However, table 5.16 and 5.17 shows the case comparison and reports of percentage, standard deviation, mean, maximum and minimum of all groups.

The Kruskal Wallis test was preformed to see whether there is a difference between the biochemical parametric of the groups. The mean rank for Aspartate (AST) is shown in Figure 5.17, Alanine (ALT) is shown in Figure 5.18, Alkaline phosphatase (ALP) is shown in Figure 5.19. More so, table 5.17 has shown that Group II (DM) group have the highest mean rank of 19.92 for AST,20.25 for ALT and 21.50 for ALP. However, Group IV (administered with

only PCA) has shown a mean rank of 13.25 for AST,14.42 for ALT and 5.17 for ALP. Contemporary to Group II, Group II (DM+PCA) has shown a mean rank of 10.25 for AST, 10.17 for ALT and 14.17 for ALP. Finally, the Group I (control group) has shown mean rank of 6.58 for AST ,5.17 for ALT and 9.17 for ALP

6.2 Conclusions

Diabetes and its subtypes are treated using broad range of modern drugs extracted or isolated form plants sources with diverse medicinal properties and bioactive compounds. There are wide range of bioactive metabolites that forms building blocks of therapeutic drugs synthesis, nutraceuticals and pharmaceuticals. Since ancient time where folk medicine is practiced, plant have been the major source of drugs against diseases worldwide due to low side effect. Bioactive compounds and metabolites derived from plants have been isolated and have been used as anticancer, antioxidant, antidiabetic, antihypertensive, anticoagulants and other cardiovascular disorder. The major treatment of diabetes involves reducing concentration of glucose or intake of food rich in glucose (hyperglycemic diet), consistent exercise and usage of insulin drugs such as sulphonylureas. Different studies have reported the side effects of these drugs, some of the listed side-effects include weight gain, severe and critical hypoglycemia and gastrointestinal complications.

PCA has shown broad application such as antidiabetic activity, antioxidant activity, cardiac and hepatoprotective activity, neurological and nephron-protective activity, anti-inflammatory and analgesic activity, antiviral activity, antibacterial activity and anticancer activity. In this study, the analysis of the antidiabetic activity of PCA is carried out based on histological studies and statistical analysis using Wistar rat models which are categorized into 4 groups with 8 number of samples in each group namely; Group I which are Normal rats, Group II are Rats induced with Streptozotocin (60mg/kg), Group III are diabetic Rat administered with PCA (dose: 20 mg/kg b.w) for 4 weeks and Group IV which contained Rat administered with PCA (dose: 20 mg/kg b.w) for 4 weeks.

The histological studies have shown that the hepatocytes of group III (diabetic group treated with PCA), have very low vacuolization, with no Kupffer cell increase, hepatocytes around the portal area appear to be normal PAS (+) staining with no connective tissue fibres increased as shown in the control and diabetes group. The statistical analysis has shown that

Group II (DM) group have the highest mean rank of 19.92 for AST, 20.25 for ALT and 21.50 for ALP. This result can be attributed or explain due to the fact that the group is not administered with PCA compare with Group IV (administered with only PCA) which has shown lower parametric mean rank values of 13.25 for AST,14.42 for ALT and 5.17 for ALP as well as Group II, Group II (DM+PCA) which has shown a mean rank values of 10.25 for AST, 10.17 for ALT and 14.17 for ALP and the Group I (control group) which has shown mean rank values of 6.58 for AST,5.17 for ALT and 9.17 for ALP. However, within the limits of this experimental study, the administration of Protocatechuic acid in Streptozotocin induced in diabetic rats' tissue has demonstrated positive histologic effects supported by statistical test analysis.

6.3 Study Recommendation

Recommendations in the future are to use this extract with other concentrations and compare it with therapeutic groups. In this study we used one concentration of PCA extract ,and if it can be used as a treatment on different categories and to know the effectiveness of this extract and be an effective treatment in the future for some chronic and acute diseases.

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Yakın Doğu Üniversitesi, Tıp Fakültesi'nden, sorumlu araştırmacı Prof Dr. Aysel Kükner tarafından hazırlanan "Deneysel Diyabet Oluşturulmuş Sıçanlarda Protocatechuic Asitin Karaciğer dokusu Üzerine Etkisinin Işık Mikroskobik ve Biyokimyasal Olarak İzlenmesi " isimli tez çalışması, 2018/20-35 karar numaralı "Deneysel Diyabet Oluşturulmuş Sıçanlarda Chorchorus Olitoriusun testis dokusu üzerindeki etkilerinin ışık mikroskobik olarak incelemesi." isimli çalışmadan elde edilen karaciğer dokusunda yapılmıştır. İsimli çalışmadan elde edilen karaciğer dokusunda, 27/9/2019 tarih 2019/09 sayı etik kurul onayı ile yapılmıştır.

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