The Effect of Ginger, Curcumin and Their Mixture on Blood Glucose, Lipids in Diabetic Rats

By

Al-Anood Abdul Aziz Al-Faleh
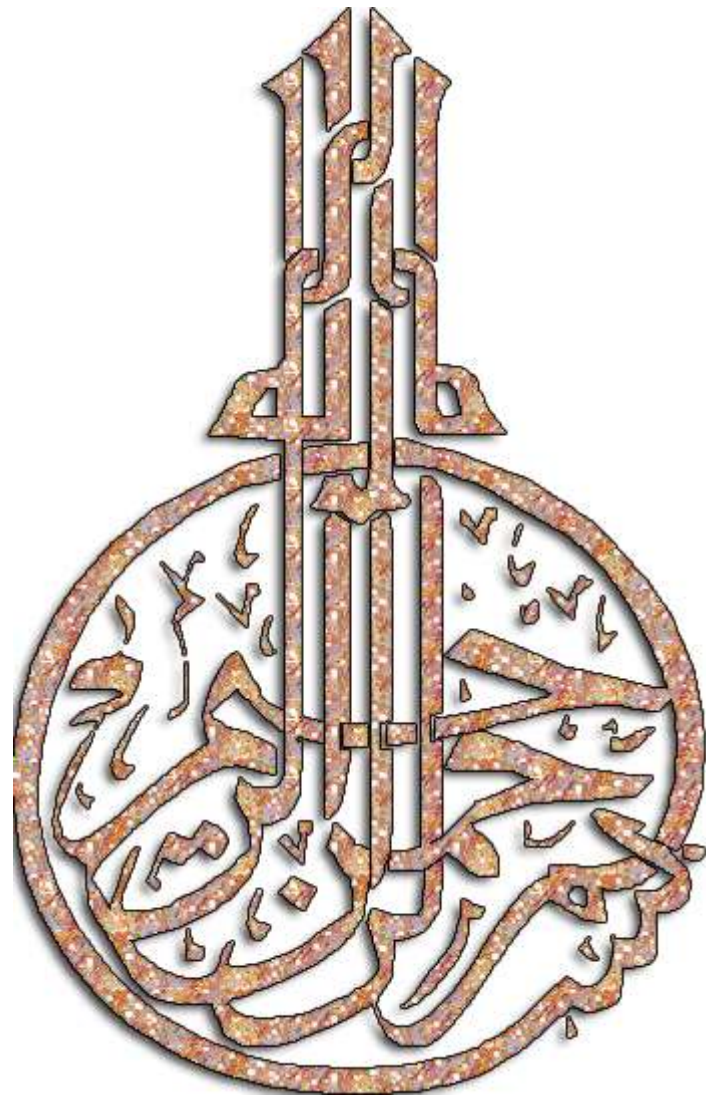
A thesis submitted for the requirements of the degree of Master of Science
[Food and Nutrition]

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Rajab 1434H – June 2013G

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This thesis has been approved and accepted in partial fulfillment of the requirements for the degree of Master of Science [Food and Nutrition]

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Dedicated to

This work was dedicated to my beloved father and mother who are support and encourage me. Also special thank and love are extended to my daughter and husband who give me love and care throughout my life, also give me kind suggestion and assistances .

ACKNOWLEDGEMENT

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Alanood Abdul Aziz Alfaleh

Abstract

Objective: The present study was conducted to evaluate the hypoglycemic, hypolipidemic and antioxidant effect of ginger, curcumin and their combination in streptozotocin (STZ)-induced diabetic rats. **Material and Methods:** Male albino rats (n=35) weighing (180-195 g) were divided into two main groups; first group: negative control (n=7) fed standard diet. and second group: diabetic rats (n=28), which divided equally to four subgroups as follows: diabetic untreated rats (positive control), diabetic rats treated with curcumin (0.5 % of diet), diabetic rats treated with ginger (3% of diet) and diabetic rats treated with (curcumin+ ginger). Diabetes was induced by a single intraperitonial injection of STZ (65 mg/kg body weight). **Results:** The results reported that the STZ-induced diabetic group exhibited very highly significant ($p<0.001$) hyperglycemia, hyperlipidemia, elevated in Malondialdehyde (MDA) accompanied with weight loss and reduced in High density lipoprotein cholesterol (HDL-C) level, Superoxide dismutase (SOD) and Catalase (CAT) enzyme activities when compared with control negative group. Treatment with curcumin, ginger or their mixture reported very highly significant ($p< 0.001$) improvement in biological evaluation, glucose, insulin, lipid profile, lipid peroxidation and antioxidant enzymes activities when compared with untreated diabetic group. Histopathological investigation of liver and pancreatic tissues of diabetic rats represented the presence of sever changes, meanwhile treatment overcome this changes, the majority of the cells tend to be normal, this improvement in the cells may explain the antidiabetic effect of the plants under study especially in their mixture. **Conclusion:** This study demonstrates that the combination of both curcumin and ginger possesses significant reduction in hyperglycemic and hyperlipidemic as well as antioxidant effect in diabetic rats. Therefore, it recommends to use both curcumin and ginger to alleviate the oxidative stress caused by diabetes. Further research is required to find out the exact mechanisms of curcumin and ginger responsible for antidiabetic and antioxidant activities.

Key words: Curcumin, ginger, diabetic, rats, hypoglycemic, hypolipidemic, antioxidant, histopathology.

تأثير الزنجبيل والكركم وخليطهما على سكر ودهون الدم للفئران المصابة بالسكر

العنود عبد العزيز الفالح

المستخلص

تهدف هذه الدراسة لتقييم التأثير الخافض للسكر و الدهون، والمضاد للأكسدة على الفئران المصابة بالسكري المُحدَث بواسطة مادة الإستربتوزوتوسين، بواسطة الكــركم و الزنجبيل و خليطهما. تم تقسيم الفئران (عددهم = 35) أوزانهم (180–195 جم) إلـــى مجموعتين رئيسيتين ، الأولى : مجموعة ضابطة سالبة (عددها 7=)، والثانية : مجموعة مصابة بالسكري (عددها 28=) ، و التي قسمت إلى 4 مجموعات فرعية: ضابطة موجبــة مصابة بالسكري (غير معالجة) ، مجموعة مصابة بالسكري تم إعطاؤها الكركم بنسبة (5، 0% من الغذاء)، مجموعة مصابة بالسكري تم إعطاؤها الزنجبيل بنسبة (3 % من الغذاء)، و مجموعة مصابة بالسكري تم إعطاؤها خليطاً من الكركم و الزنجبيل. تم إحداث مرض السكري من خلال حقن الفئران في الغشاء البريتوني بالإستربتوزوتوسين (بجرعة مقدارها 65 ملجم/كجم من وزن الجسم). أوضحت النتائج أن الفئران المصابة بالسـكري المُحــدَث بواسطة الإستربتوزوتوسين أظهرت حدوث ارتفاع ذي دلالة معنوية عالية جداً في مستوى الجلوكوز، مقاييس الدهون، الدهون فوق المؤكسدة. مع حدوث انخفاض ذي دلالة معنوية عالية جداً ($p<0.001$) في مستوى الأنسولين ،الليبوبروتينات المرتفع الكثافة، والإنزيمــات المضادة للأكسدة عند مقارنتها بالمجموعة الضابطة السالبة. بينما أدى استخدام الكــركم و الزنجبيل و خليطهما إلى حدوث تحسن ذو دلالة معنوية عالية جداً ($p<0.001$) في القياسات البيولوجية ، مستوى الجلوكوز، الأنسولين، الليبيدات، الدهون فــوق المؤكسـدة، و نشــاط الأنزيمات المضادة للأكسدة عند مقارنتها بالمجموعة المصابة بالسكري غيــر المعالجــة. أوضحت الدراسات الهستوباثولوجية لأنسجة الكبد والبنكريــاس عنــد الفئــران المصــابة بالسكري غير المعالجة ظهور تغيرات سلبية حادة على الأنسجة ، بينما أدت المعالجة إلي التغلب على هذه التغيرات؛ حيث إن أغلب الخلايا أصبحت أقرب إلى الحالة السليمة، وهذا التحسن ربما يفسر التأثير المضاد للسكري، وخاصة لخلــيط الكــركم والزنجبيــل تحــت الدراسة. الخلاصة: هذه الدراسة تبين أن خليط الكركم والزنجبيل يمتلك القدرة على خفــض مستويات الجلوكوز و الدهون، بالإضافة إلى دوره المضاد للأكسدة في الفئــران المصــابة بالسكري. و لذلك فإنه يوصى باستخدام هذا المزيج للتخفيف من الجهد التأكسدي الناجم عن السكري، كما يلزم إجراء مزيد من الأبحاث لمعرفة الآلية المسؤولة عن نشاطهما المضــاد السكري والجهد التأكسدي.

الكلمات المفتاحية : الكركم– الزنجبيل– البول السكري- الفئران– الخافض للسكري– الخافض للدهون– المضاد للأكسدة، التغييرات النسيجية.

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LIST OF ABBREVIATION

b.wt   Body weight

BWG %   Body weight gain percent

CAT   Catalase

CHD   Coronary heart disease

C ^{o} Centigrade

DFI   Daily feed intake

DM   Diabetes mellitus

FI   Feed intake

g   gram

g/rat/day   gram per rat per day

GP_X Glutathione peroxidase

GR   Glutathione reductase

GSH   Glutathione

HDL-C   High density lipoprotein cholesterol

IDF   International diabetes federation

i.p   Intraperitoneal

LDL-C   Low-density lipoprotein cholesterol

MDA   Malondialdehyde

mg / dl   milligrams per decilitre

ml   milliliter

n mol/ ml   nanomoles per milliliter

NIDDM	Noninsulin-dependent diabetes mellitus
nm	nano meter
OD	Optical density
pH	Hydrogen ion concentration
rpm	revolutions per minute
SD	Standard deviation
SOD	Superoxide dismutases
STZ	Streptozotocin
T2DM	Type tow diabetes mellitus
TBARS	Thiobarbituric acid reactive substances
TC	Total cholesterol
TG	Triglycerides
U/ ml	Unit per milliliter
HFD	High fat diet
OFR	Oxygen free radical
mg/kg	Milligram per kilogram
w/w	weight per weight
CUR	Curcumin
GIN	Ginger
TNF-α	Tumor necrosis factor-alpha
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated β-cells

INTRODUCTION

Chapter I

Introduction

Diabetes is a major threat to global public health, characterized by chronic hyperglycemia resulting from impaired insulin action/secretion or both and, it is classified into two major categories type I and type II **(Alberti and Zimmet, 2004).** The number of diabetic patients are rapidly in increasing all over the world, according to a projection of the International Diabetes Federation (IDF), 366 million people had diabetes in 2011, which will increase to 552 million by 2030 **(IDF, 2011)**. In Saudi Arabia, diabetes mellitus has become more evident in the last two decades as a result of dramatic change in life style **(Ammeri, 2004)**.

Effective control of hyperglycaemia in diabetic patients is critical for reducing the risk of micro- and macrovascular diseases **(Holman *et al*., 2008, Ray *et al*., 2009 and Ismail *et al*., 2010)**. Side effects of the presently available hyperglycaemic agents have impeded their usefulness as antidiabetic agents. This led to continuous effort to explore effective agents for control of diabetes mellitus. Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the management of this disease, amongst such plants reported to have beneficial effects in the treatment of diabetes are spices such as curcumin and ginger **(Ugwuja *et al*., 2010)**.

Curcumin powder (*diferuloylmethane*), an extract of turmeric rhizomes (*Curcuma longa*), is the most active component of spice turmeric (an essential component of curry powder) which makes 2-5% of turmeric spice (**Shishir *et al.,* 2005**). Curcumin shown to have wide spectrum of biological action, these include its anti-inflammatory, antioxidant, antidiabetic and hypocholesteremic activities (**Sigrid, 2011**).

Ginger (*Zingiberofficinale*) is one of the most widely consumed spices for the flavoring of food worldwide (**Li *et al*., 2012**), It has been that, ginger had reported several beneficial pharmacological effects such as hypoglycemic, insulinotropic and hypolipidemic activities in humans (**Andallu *et al*., 2003 and Bhandari *et al*., 2005**), and in experimental animals (**Ojewole, 2006 , Madkor *et al*., 2010 and Abdulrazaq *et al*., 2011**). The major chemical constituents of ginger rhizome are essential volatile oil and non-volatile pungent compounds, such as gingerols, shogaols, paradols and zingerone (**Masood and Tauseef, 2011**). Therefore, the present study was conducted to evaluate the effect of curcumin, ginger or their combination on blood glucose, insulin and blood lipid levels, as well as, malondialdehyde and antioxidative enzyme activities in streptozotocin-induced diabetic rats.

1.2. Objectives of the study:

This work conducted to study the following:

- The comparative effects of dietary curcumin, ginger or their mixture as hypoglycemic agents in streptozotocin (STZ)-induced rats.

- The effect of dietary curcumin, ginger or their mixture on lipid profile, lipid peroxidation and some antioxidant enzyme activities, as well as histological alterations of liver and pancreas tissues in diabetic rats.

REVIEW OF LITERATURE

Chapter II

Review of literature

Diabetes mellitus (DM) is a heterogeneous metabolic disorder, a high occurrence of disease in noted and the numbers of diabetic patients are gradually increasing, thus the disease constitutes a major health concern (**Narendhirakannan** *et al***., 2005 and Etuk, 2010**). According to the World Health Organization (WHO), there are approximately 347 million diabetics worldwide, the number of diabetics had been double in the last few years and WHO projects reported that, diabetes death will increase by two thirds between 2008 and 2030 (**WHO, 2012**).

It has been estimated that the global burden of type 2 diabetes mellitus (T2DM) for 2010 would be 285 million people, which is projected to increase to 438 million in 2030 (65 % increase) (**Snehalatha and Ramachnadaran, 2009**). Reasons for this rise include an increase in sedentary lifestyle, the consumption of energy-rich diet, obesity, etc… (**Yajmk, 2001**). Diabetes is the fourth or fifth leading cause of death in most high-income countries and there is substantial evidences that it is epidemic in many economically developing and newly industrialized countries (**IDF, 2011**). Due to its high prevalence and potential deleterious effect on a patient physical and psychological state, diabetes consider as a major medical concern (**Macedo** *et al***., 2005**).

2.1.Definition of diabetes:

Diabetes mellitus encompasses a heterogeneous group of disorders characterized by insulin hyposecretion and/or insensitivity, it is not a single entity but a group of conditions characterized by chronically raised plasma glucose concentration. This glucose abnormality is due to an absolute or relative lack of insulin and has many causes, the result of insufficient action of insulin is an increase in blood glucose concentration (hyperglycemia). Many other metabolic abnormalities occur, notably an increase in ketone bodies in the blood when there is a severe lack of insulin (**Paul *et al.,* 2005**).

There are two main types of DM, insulin-dependent diabetes (type 1), in most cases, this is a disease of autoimmune origin in which the insulin producing from *β*-cells in the pancreas destroyed leading to total exhaustion of insulin secretion. This type of diabetes develops rapidly, usually appearing before the age of 35, and most often between the ages of 10 and 16 year and regular insulin injections are required to survive.Non-insulin-dependent diabetes (type 2), which occurs when the body does not produce enough insulin, and the insulin that is produced becomes less effective. This type of diabetes usually appears in people over the age of 40, and tends to have a more gradual onset. In most cases, glucose levels in the blood can be controlled by diet, or tablets and diet, although sometimes insulin injection may be needed, about 90 percent of diabetics are non-insulin dependent (**Chatterjee and Shinde, 2005**).

2.1.1. Effects of DM on biological evaluation:

Studies have been shown an association between hyperglycemia and decreased body weight of diabetic animals, DM induced reduction in body weight,

and the body's inability to store or use glucose causes hunger and weight loss (**Chatrejee, 1992 and Zafar and Naqvi, 2010**).

It was reported that, streptozotocin (STZ)-induced diabetic rats showed signs of loss weight compared with rats non-injected with STZ (**Suryanarayana *et al.,* 2005 and Gupta *et al*., 2012**). Moreover, **Zafar and Naqvi (2010)** reported that STZ in a dose of 45 mg/kg induced significant reduction in the body weight of diabetic compared with non-diabetic animals. On the other hand, **Ugwuja *et al*. (2010)** showed no significant difference in the final body weight between STZ diabetic group and non- diabetic rats.

2.1.2. Effect of DM on glucose metabolism and insulin secretion:

Insulin is an endocrine hormone secreted by the *β*-cells of the islets of Langerhans in the pancreas. Its principal function is to assist the transport of glucose across the cellular membrane. When insulin is deficient or lacking, only a small amount of glucose can cross the cell membrane and used in cellular metabolism. This low rate of transport results in excess accumulation of glucose in the blood (hyperglycemia). As well as urinary excretion of glucose (glucosuria) results when the concentration of blood glucose exceeds the threefold levels for total reabsorption by the kidney (**Tharp and Woodman, 2008**).

Studies have exhibited the rats injected with STZ showed a marked raise in plasma glucose levels and decrease in insulin levels compared with control negative group (**Yaghmoor and Khoja, 2010 and Kumar *et al.,* 2012**). Moreover, **Punithavathi *et al*. (2011)** reported that injection with STZ in a dose level of 40 mg/kg induced significant increase in plasma glucose concentration and reduction in insulin level compared with normal rat.

2.1.3. Effect of DM on lipid profile parameters:

Diabetes is a complex disease where the carbohydrate and fat metabolism is impaired **(O'Keefe and Bell, 2007)**.The most typical lipoprotein pattern in diabetes, known as diabetic dyslipidemia **(Solano and Goldberg, 2006)**. Insulin affects many sites of mammalian lipid metabolism, it stimulates synthesis of fatty acids in liver adipose tissues and in the intestine, the insulin has also been reported to increase the cholesterol synthesis, and the activity of lipoprotein lipase in white adipose is also increased **(Suryawanshi *et al*., 2006).**

Diabetes affects several lipid metabolism mechanisms. Low insulin levels are associated with high levels of chylomicrons and very-low-density lipoprotein (VLDL) and lipoprotein lipase deficiency, resulting in hypertriglyceridaemia. Such hyperlipidaemia improves with tighter control of DM. Treatment of hyperlipidaemia has been shown to benefit DM patients in decreasing coronary heart diseases (CHD) risk **(Manley *et al*., 2000, Holman, 2001 and Sacks *et al.,* 2002).**

Different study on STZ-induced diabetic rats showed increase in the levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and decrease on high density lipoprotein-cholesterol (HDL-C) levels accompanied with high glucose level and low insulin level in diabetic group as compared with non-diabetic rats **(Yaghmoor and Khoja, 2010)**. Furthermore, **Kumar *et al.* (2012)** reported that, injection with STZ at a dose level of 50 mg/kg to rats showed significant increase in the plasma TG, TC, and decrease HDL-C compared with non-diabetic group. Moreover, **Ramudu *et al.* (2011a)** reported that, there were significant elevation in TC, TG and phospholipids levels in STZ-induced diabetic rat against non-diabetic rats.

2.1.4. Effect of DM on antioxidant enzyme activities and lipid peroxide:

Diabetes is characterized by hyperglycaemia together with biochemical alterations of lipid peroxidation (**Pari and Latha, 2002**), which is a free radical-related process with potentially harmful because its uncontrolled, self enhancing process causes disruption of membranes, lipids and other cell components (**Mahboob** *et al.,* **2001**). Some complications of DM are associated with increased activity of free radicals-induced lipid peroxidation and accumulation of lipid peroxidation products (**Palanduz** *et al.,* **2001**). **Arun and Nalini (2002)** reported that there was a significant increase in malondialdehyde (MDA), secondary product of lipid peroxidation, in alloxan diabetic rats compared with non diabetic rats. Moreover, **Suryanarayana** *et al.* **(2005)** found significant increase of thiobarbituric acid reactive substances (TBARS) levels in STZ-induced diabetic group compared with control group.

There are significant differences in the activities of antioxidant enzymes between diabetic and non-diabetic patients (**Jandric-Balen** *et al.,* **2003**). According to **Kowluru and Kanwar (2007)** and **Haskins** *et al.* **(2003)** the activities of antioxidant enzymes responsible for scavenging free radicals and maintaining redox homeostasis, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH) were significant decreased in STZ group as compared with non-diabetic rat group.

Hyperglycemia has been reported as a cause of increased production of oxygen free radicals, which could induce oxidative stress and become the main factor for predisposing the complications in diabetes. **Mahboob** *et al.* **(2005)** reported that there were significant elevation in MDA level and decrease in glutathione in both male and female diabetic patients in comparison to non-diabetic

controls. Moreover, **Suryanarayana *et al.* (2005)** mentioned that diabetes causes the increased level of oxidized DNA, proteins and lipids, which are also limiting factor in various diabetic complications. **Kumawat *et al.* (2009)** found significant increase in MDA, while glutathione peroxidase (GPx), reduced glutathione (GSH), glutathione reductase (GR) and superoxide dismutase (SOD) were decrease significantly in non-insulin dependent diabetes mellitus (NIDDM) patients with microvascular complications compared with healthy subjects. In addition, several studies showed that, the diabetic rats exhibited lower activities of SOD, CAT, GP_X, GR and GSH and higher level of MDA in hepatic tissues and plasma compared with normal rats **(Shanmugam *et al.*, 2011, Al-Assaf, 2012 and Kota *et al.*, 2012).**

2.1.5. Effect of DM on liver and pancreas tissue:

Hyperglycemic disorder induces in diabetes affects many organs in body such as the brain, kidney, liver, pancreas and other organs **(Aggarwal and Harikumar, 2009).** The effects of STZ on different organs have been extensively studies, it have various biological actions, including the production of acute and chronic cellular injury, carcinogenesis, treatogenesis and mutagenesis **(Zafar *et al.*, 2009)**. Studies reported that STZ stimulates H_2O_2 generation, which cause DNA fragmentation and increase oxidative stress in liver and pancreas cells **(Bolkent *et al.*, 2008 and Nirmala *et al.*, 2009)**.The incidence and severity of lesions produced by STZ in liver, pancreas and kidney, progressively increased with time from one to six weeks post injection with STZ **(Kakkar *et al.*, 1998)**.

Diabetes cause disturbs and imbalance between oxygen free radicals (OFR_S) production and cellular defense mechanisms. This imbalance can result in cell dysfunction and destruction resulting in liver and pancreas tissues, the increase level of OFR_S from glucose oxidation and protein glycosylation in DM could be due to the

increase of the production and / or decrease destruction by non-enzymatic and enzymatic antioxidant activities (CAT, SOD and GP_X) (**Kakkar *et al.,* 1998 and Waer and Helmy, 2012**). The level of antioxidant enzymes critically influence the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes (**Attia, 2009).**

Hussein and Abu-Zinadah (2010) showed that the liver of STZ diabetic rats in a dose of 65 mg/kg showed massive fatty changes, necrosis and broad infiltration of lymphocytes. **Ragavan and Krishnakumari (2006)** reported that liver sections of diabetic rats showed marked structural alterations as a result of absence of insulin, the major alterations was periportal fatty infiltration and necrosis of hepatocytes. Furthermore, **Hashemnia *et al.* (2012)** reported that the histopathological sections of untreated diabetic rats liver showed degenerative changes in the hepatocytes represented by disorganization of the hepatic cords, congestion of the central veins with mild hepatocellular necrosis and the sinusoids were infiltrated by mild nonspecific inflammatory cells, and the hepatocytes of the untreated rats showed morphological changes such as pyknosis, karyorrehexis, chromatolysis and cytoplasmic.

Waer and Helmy (2012) stated that STZ in a dose of 45 mg/kg showed severe injury illustrated in mononuclear cell infiltrate extending through hepatic tissues, kupffer cell appeared engulfing debris of the degenerated hepatocytes and hyperplasia of bile duct, as well as in pancreatic cells disorganization of the structure of the endocrine and exocrine glands in hypocellularity damaged in islets of Langerhans with necrotic pancreatic acini. Moreover, **Soetikno *et al*. (2012b)** showed that STZ in a dose of 55 mg/kg causes damage in liver tissue and showed

dilatation and congestion of central vein, also immense necrosis and fatty changes of diabetic rats.

Hyperglycemia, and its attendant effects upon cells, underlies the pathogenic lesions of DM, STZ administration to mature rats induced severe and permanent diabetes, with a decrease in insulin levels, to produce a cytotoxic model of diabetes, and it damages β-cells of the islets of Langerhans in the pancreas (**Cheville, 2009**). Diabetic pancreatic tissues showed shrinkage of islets Langerhans in size, signs of necrosis of β-cells destruction and reduction of number of islets and significant reduction in diameter after the 2nd and 4th weeks of STZ injection (**Qadori, 2011**).

It had been found that, in diabetic mice induced by STZ, the DNA in β-cells was damaged and subsequent induced inhibition of insulin biosynthesis and secretion (**Panchatcharam *et al.,* 2006 and Kanitkar *et al.,* 2008). Attia (2009)** observed histological changes in diabetic rats in acinar and endocrine β-cells of islets of pancreas. Moreover, **Gandhi and Sasikumar (2012)** and **Kulkarni *et al.,* (2012)** reported that STZ diabetic rats showed a significant reduced in mean β-cells number also the cells were smaller in size and markedly degenerated with necrosis of pancreatic islets.

Because of the importance of diabetes, its management considered a global problem and greatest expenses for health system in the whole world (**Barcelo *et al*., 2003 and Martin *et al*., 2007**). Synthetic hypoglycemic agents that are capable of reducing blood sugar level possessed most worrying side effects; most of these are gastrointestinal discomfort and nausea (**Gandhi and Sasikumar, 2012**). Therefore, finding other anti-diabetic agents especially those made from natural sources is desired (**Vishwakarma *et al*., 2010).** Plants have always been an exemplary source

of drugs, it's used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the management of this disease, amongst such plants reported to have beneficial effects in the treatment of diabetes are spices such as curcumin and ginger **(Ugwuja *et al*., 2010 and Gandhi and Sasikumar, 2012)**.

2.2. Curcumin:

Curcumin (*diferuloylmethane*) [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione], an orange-yellow crystalline powder, is the active component in turmeric rhizomes (*Curcuma Long Linn*, *Zingiberaceae*), at a content of 3 to 5%, it's a lipophilic polyphenol that is nearly insoluble in water **(Anand *et al*., 2007)**. Curcumin widely used in traditional Indian medicine to cure biliary disorders, anorexia, cough, diabetic wounds and hepatic disorders **(Aggarwal *et al.,* 2003 and Shishir *et al.,* 2005).**

Curcumin containing more than one phenol group, each of which consists of a ring of six carbon atoms with a hydroxyl (OH) group attached, it contains two phenol groups and each phenol is resonant. A chain of each carbon atoms pin the two phenols together. On each phenol, the point of attachment is opposite the hydroxyl group. An OCH3 group unites with each phenol on a carbon adjacent to the hydroxyl attachment site. In the chain, the third and fifth carbon each unites with an oxygen atom. Each carbon oxygen group may form either a keto, or an enol **(Jan McBarron, 2012)**. Curcumin is a potent antioxidant, whose antioxidant capacity is 100-fold stronger than that of vitamin E/C **(Sreejayan, 1994).** Curcumin containing 95% pure curcuminoids (curcumin 80 %, desmethoxycurcumin 10% and bisdesmethoxycurcumin 5%) **(Weisberg *et al.,* 2008)**.

Traditionally, it has been used as spice and colorant in Indian curries, as well as a component of Chinese medicines, and has been approved as a safe food in the US (**Shimatsu *et al.,* 2012**). In recent years, a number of studies have investigated the various biological effects of curcumin, attributed to polyphenol's potential to modulate multiple signaling molecules. Animal studies have suggested that curcumin may be active against a wide range of human diseases, including diabetes, obesity, neurologic, psychiatric disorders and cancer. Also, chronic illnesses affecting the eyes, lungs, liver, kidneys, gastrointestinal and cardiovascular systems, Curcumin plays a beneficial role in terms of being an antioxidant, anti-tumurogenic, and anti-inflammatory agent (**Bengmark, 2006 and Gupta *et al.,* 2012**). Human clinical trials also indicated that, curcumin has no toxicity when administered at doses of 10 g/day (**Aggarwal *et al.,* 2003**) and at a dose level 8 g/day (**Chainani, 2003**).

2.2.1. Effect of curcumin on biological evaluation:

Loss of body weight and increase in the percentage of lean mass are extremely beneficial toward decreased insulin resistance and improved cardiovascular health (**Weisberg *et al.,* 2008**). **Suryanarayana *et al*. (2005)** studied the effects of curcumin on STZ-induced diabetic rats, and found increased in food intake accompanied by decreased in the body weight of diabetic rats group when compared with non diabetic rats , while diabetic rats treated with curcumin showed significant improvement in body weight as compared with diabetic untreated rats.

Moreover, study in high-fat-fed mice demonstrated that curcumin supplementation (at a dose level of 500 mg/kg diet) can increase the basal metabolic rate, thereby contributing to increase energy expenditure and weight loss (**Ejaz *et al.,* 2009**).

Moreover, other studies concluded that treatment with curcumin prevent body weight loss in STZ-induced diabetic rat compared with diabetic rat untreated group (**Hie *et al.,* 2009 and Soetino *et al.,* 2012a).** In addition, **Hussein and Abd El-Maksoud (2013)** reported decreased in body weight and increased in food intake in diabetic rats treated with curcumin when compared with normal rats, the same study also reported improvement in body weight and food intake in diabetic rats treated with curcumin compared with diabetic untreated rats. On the other hand, another studies showed no significant difference of final body weight between diabetic rats, diabetic rats treated with curcumin and non diabetic rats (**Kowluru and Kanwar, 2007 and Ugwuja *et al.,* 2010**). These changes in results could be attributed to several factors such as dose used, administration route and duration of experiment.

2.2.2. Hypoglycemic and hypolipidemic effect of curcumin:

Curcumin has been shown to improve the symptoms associated with diabetes. The efficacy of curcumin has been widely observed in reducing various diabetic secondary complications such as diabetic nephropathy/renal lesions (**Sharma *et al.,* 2006),** retinopathy (**Kowluru and Kanwar, 2007**) and wound healing (**Panchatcharam *et al.,* 2006**). It has potential as a hypoglycemic agent in many animal studies (**Arun and Nalini, 2002, Hussain, 2002, Pari and Murugan, 2005, Meghana *et al.,* 2007 and Gupta *et al.,* 2012**).

In a STZ-induced diabetic mouse model, curcumin (at 60 mg/kg body weight) was shown to act as an anti-diabetic agent , which in turn to maintain the normal structure of the kidney (**Sawatpanich *et al*., 2010**). The effect of curcumin on the progression of insulin resistance and type 2 diabetes mellitus (T2DM) was investigated in another study, insulin resistance and T2DM were induced in male

Sprague Dawley rats by high-fat diet feeding for 60 and for 75 days, curcumin was administered in the last 15 days of high-fat diet feeding after induction of T2DM. The results showed that curcumin revealed an anti-hyperglycemic effect and improved insulin sensitivity **(Arun and Nalini, 2002 and Gupta *et al*., 2012).**

Despite the positive results of some studies, some studies have shown conflicting results. **Kowluru and Kanwar (2007)** revealed similar results of blood glucose in diabetic rats and diabetic rats treated with dietary curcumin, and reported that curcumin did not prevent STZ-induced hyperglycemia. The underlying mechanisms by which curcumin can lower blood glucose is not fully defined.

The effect of curcumin administration on total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels was investigated. Interestingly, curcumin was found to be more effective at low doses than at high doses in reducing TC and LDL-C levels in patients with acute coronary heart diseases **(Alwi *et al*., 2008)**.

Babu and Srinivasan (1997) studied the hypolipidemic action of 0.5% of dietary curcumin for 8 weeks in STZ-induced diabetic rats, the results showed that the blood TC, TG and LDL-C was significantly lowered, on the other hand HDL was significantly increased by dietary curcumin as compared with untreated diabetic group. In addition, the effect of curcumin on T2DM and insulin resistance showed that, the lipid profile parameters were improved through the hypolipidemic and hypoglycemic effects of curcumin compared with diabetic untreated group **(El-Moselhy *et al*., 2011)**.

The hypolipidemic effect of curcumin has also been found to be effective in treating diabetic rats **(Hussein and Abd El-Maaksod, 2013)**. In another study, dietary curcumin effectively reduced the elevated serum and hepatic TG

concentration in high- fat-fed rats as compared with high fat-fed rats without curcumin **(Manjunatha and Srinivasan, 2007).**

2.2.3. Antioxidant effect of curcumin:

Oxidative stress plays a major role in the pathogenesis of both types of DM. Free radicals formed disproportionately in diabetes by glucose oxidation, non enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins **(Maritim and Sanders, 2003)**. Moreover, **Hussein *et al*. (2012)** mentioned that hyperglycemia is a metabolic disorder that results in excessive production of free radicals, which lead to severe oxidative damage of cell components like lipids, proteins and DNA. The antioxidant activities of curcumin was reported, it acts as a scavenger of oxygen free radicals **(Subramanian *et al*., 1994 and Rubya *et al*., 1995**).

Curcumin activity as an antioxidant and free-radicals scavenger has been demonstrated from several studies. *In vitro*, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H_2O_2 and nitrite radical generation, by activated macrophages, which play an important role in inflammation **(Joe and Lokesh, 1994)**. Curcumin prevents the oxidation of hemoglobin and inhibits lipid peroxidation **(Gupta *et al*., 2012)**. This activity can arise either from the phenolic hydroxyl group or the methylene group of the β-diketone (heptadiene-dione) moiety **(Ligeret *et al*., 2004, Suzuki *et al*., 2005, Chen *et al*., 2006 and Anand *et al*., 2008).**

Furthermore, **Arun and Nalini (2002)** reported that curcumin elevated significantly the GSH and GPx activities in diabetic animals compared to the untreated diabetic rats. The effect of curcumin on the malondialdehyde and glutathione content in red blood cells were assessed results showed that, curcumin

therapy was correlated with a significant reduction in the erythrocyte malondialdehyde content and a significant increase in glutathione levels in diabetic patients **(Durgaprasad, 2005)**. **Kowluru and Kanwar (2007)** reported that curcumin decrease lipid peroxidation, increase intracellular antioxidant, GSH and regulate antioxidant enzymes in diabetes rats. In addition, **Pallas *et al*. (2008)** suggested that curcumin treatment appear to have countered the hyperglycemia-induced oxidative stress, because there was a reversal of change with respect to lipid peroxidation, reduced glutathione and activities of antioxidant enzymes in significant manner.

Hussin and Abo-Zinadah (2010) studied the antioxidant effect of curcumin in STZ-diabetic rats and the authors found that, increased MDA content in the liver and kidney in STZ-diabetic rats compared to non-diabetic rats and diabetic rats treated with curcumin, also the activities of SOD and CAT activities were significantly reduced in the STZ groups, while there were significantly elevated in the enzyme activities, their values tend to be near the normal values in the group treated with curcumin compared to the non-treated group.

2.2.4. Effect of curcumin on liver and pancreas tissues:

Antioxidants can exert beneficial effects on both liver and pancreatic β-cell function in diabetes rats **(Calabrese, 2003)**. Curcumin in diabetic rats can suppress blood glucose levels, increase the antioxidant status of pancreatic β-cells, hepatic cells and enhance the activation of peroxisome proliferator-activated receptor gamma (PPAR-γ) **(Nishiyama *et al*., 2005).**

Previous study has pointed to the protective effect of curcumin on acute liver injury by inhibiting oxidative stress **(Reyes-Gordillo *et al.,* 2007)**. The ability of curcumin to affect the occurrence of oxidative stress in the liver of rats with diabetes

was also examined. Curcumin was found to prevent liver lipid peroxidation in rats with STZ-induced diabetes and showed graduate restoration of hepatocytes and blood sinusoids, also most of the hepatocytes were showed normal and regular pattern **(Soetikno *et al*., 2012b and Waer and Helmy, 2012).** In addition, **Murguan and Pari (2006)** found that curcumin improve the pathological changes and reduced congestion of portal triad and inflammation when compared with STZ diabetic rats. Moreover, **Hussein and Abu-Zinadah (2010)** reported that the histological architecture of liver sections of the STZ rats treated with curcumin showed normal patterns, with a mild degree of fatty change, necrosis and lymphocyte infiltration, almost comparable to those of control group.

Studies have been conducted to determine whether curcumin had a direct stimulatory effect on the pancreatic β-cells which could be can contribute to the hypoglycemic activity of this compound. In a study by **Best *et al.* (2007)** stated that, curcumin induced electrical activity in rats pancreatic β-cells by activating the volume-regulated anion channel, as a result of increased channel open probability, this effect was accompanied by potential depolarization of the cell membrane, the generation of electrical activity, and enhanced insulin release.

Curcumin also decreased β-cell volume, presumably reflecting loss of Cl($-$), and hence water, as a result of anion channel activation. These findings were consistent with the suggestion that Cl(-) fluxes play an important role in regulating β-cell function, the stimulation of β-cell function by curcumin might be contributed to the hypoglycemic actions of this compound **(Best *et al.*, 2007).** Additionally, curcumin was found to induce heme oxygenase-1 expression, which has been reported to have cytoprotective effects in mouse pancreatic β-cells **(Pugazhenthi *et al.*, 2007).**

Pancreatic islet cells death is the cause of deficient insulin production in diabetes mellitus, Generation of oxidative stress is implicated in STZ injection, especially in *β*-cells **(Aggarwal and Harikumar, 2009)**. Approaches to prevent cells death have prophylactic significance in the management of hyperglycemia, the role of curcumin in STZ-induced islet damage was examined in *vitro* by **Meghana *et al*. (2007)** and the results showed that, curcumin retarded islet ROS (Reactive oxygen species) generation and inhibited apoptosis, indicating that curcumin protects islets against STZ-induced oxidative stress by scavenging free radicals. In addition, **Waer and Helmy (2012)** reported that STZ- induced diabetic rats treated with curcumin showed gradual restoration of pancreatic endocrine cells, degeneration in some of pancreatic acini, and after 3 weeks the cells showed healthy structure. Curcumin has been shown to protect islets against streptozotocin (STZ)-induced oxidative stress by scavenging free radicals, also showed that islet viability and secreted insulin in curcumin-pretreated islets were significantly higher than in islets in group injected with STZ alone **(Meghana *et al.,* 2007).** Moreover, **Kanitkar *et al.* (2008)** demonstrated that curcumin can protect islets from cytokine-induced cell death by scavenging ROS (Reactive oxygen species) and prevent the progression of diabetes induced by STZ.

Clear evidence of pancreatic islets growth as a respond as a curcumin treatment in diabetic mice showed that, curcumin might promote hormone or growth factors for pancreatic islets in diabetic **(Sharma *et al.,* 2006 and Weber *et al.,* 2006).**

2.3. Ginger:

Ginger (*Zingiber officinale*, Roscoe Zingiberaceae) is one of the most widely spices worldwide. It originated in South-East Asia and then became widespread in many ecological zones. It has been cultivated since long as a spice and condiment to add flavor to Indian food **(Park and Pizzuto, 2002)**. Besides its extensive use as a spice, the rhizome of ginger has also been used in traditional herbal medicine. The health-promoting perspective of ginger is often attributed to its rich phytochemistry **(Shukla and Singh, 2007)**.

The constituents of ginger are numerous and vary depending on the place of origin and form of rhizomes; fresh or dry. It contains appreciable amounts of vitamins and minerals, as well as some enzymes, for example, a potent proteolytic enzyme called zingibain . **Jolad *et al*. (2004)** identified more than 60 compounds in fresh ginger grouped into two broader categories; volatiles and non-volatiles. Volatiles compounds including sesquiterpene and monoterpenoid hydrocarbons providing the distinct aroma and taste of ginger and non-volatile compounds include gingerols, shogaols, paradols and zingerone. Moreover, **Gong *et al*. (2004)** and **Zhan *et al*. (2008)** analyzed both fresh and dried ginger using GC-MS, the number of components that were reported were 140 and 136, respectively; its main volatile components are α-zingiberene (22.29%), *β*-sesquiphellandrene (8.58%), *α*-farnesene (3.93%), *β*-bisabolene (3.87%), *α*- curcumine (2.63%), with [6]-gingerol (9.38%) and [6]-shogaol (7.59%) being the promising pungent compounds, as well as zingerones (9.24%) are present in a significant amount as they are produced during thermal degradation of gingerols or shogaols.

Ginger has enormous health-promoting potential effects as an evident when using it for treating a number of ailments including degenerative disorders (arthritis

and rheumatism), digestive health (indigestion and constipation), cardiovascular disorders (atherosclerosis and hypertension), diabetes mellitus and cancer. Also it has anti-inflammatory properties, and these properties are beneficial in controlling the process of aging, as well as it is recommended for sore throat and vomiting. Moreover, it has antimicrobial potential, which can help in treating infectious diseases and helminthiasis (**Jiang *et al*., 2006, Shukla and Singh, 2007, White, 2007, Ali *et al*., 2008 and Nicoll and Henein, 2009**).

2.3.1. Effect of ginger on biological evaluation:

Diabetes is a chronic metabolic disorder associated with low physical activity and high energy intake (**Eckel *et al*., 2005**). A study on high fat diet (HFD) fed rats reported the protective effect of ginger in the development of various parameters of metabolic syndrome, a condition predisposing to a high risk of type 2 diabetes as compared with HFD group. After treatment with an ethanolic extract of ginger at doses in decrease level of 100, 200 or 400 mg/kg for 6 weeks, the results showed that there were marked raises in body weight and insulin level, while decreases in serum glucose, total cholesterol, LDL-cholesterol, triglycerides, free fatty acid and phospholipids induced by high-fat diet, compared to untreated group (**Nammi *et al*., 2009**).

Two different studies about the effect of ginger on diabetic rats reported that, there was an increase in the food intake in diabetic treated and untreated groups, while the body weight of diabetic and diabetic animals treated with ginger decreased significantly when compared to control negative animals. The results showed that treatment with ginger induced significant improvement in diabetic treated rats as compared with untreated group (**Ansari *et al*., 2008, Islam and Choi, 2008 and Saraswat *et al*., 2010**).

2.3.2. Hypoglycemic and hypolipidemic effect of ginger:

Recent evidence revealed the potential effect of ginger for the treatment of diabetes mellitus. Data from *vitro*, *vivo*, and clinical trials has been demonstrated the antihyperglycaemic effect of ginger. The mechanisms underlying these actions were associated with insulin release and action, improve carbohydrate and lipid metabolism (**Li *et al*., 2012**). It was reported that oral administration of ginger significantly decrease fasting blood glucose level after injection with STZ-induced type 1 diabetic rat, ginger producing a 24% to 53% reduction in blood glucose at doses ranging from 100 to 800 mg/kg b.wt (**Ojewole, 2006**). Another study showed that a single dose of ginger aqueous extract prevented 5-hydroxytryptamine-(5-HT) induced acute hyperglycemia (**Akhani *et al*., 2004**). Moreover, **Saraswat *et al*. (2010)** reported the effect of dietary ginger (3%) in the diet for 8 weeks in STZ-diabetic rats induced decreasing in blood glucose levels while insulin non significantly affected by ginger. Limited clinical studies have been conducted that the potential beneficial effects of ginger in patients. After consuming 3 g of dry ginger powder in divided dose for 30 days, resulting in significant reduction in blood glucose, TG, TC, LDL, and VLDL cholesterol in diabetic patients (**Andallu *et al*., 2003**).

Long-term treatment with ginger not only affected blood glucose levels, but also decreased serum triglyceride and total cholesterol, increased insulin, and effectively prevented body weight, liver, and kidney weight loss in type 1 diabetic animals (**Akhani *et al*., 2004, Al-Amin *et al*., 2006 and Abdulrazaq *et al*., 2011**). In a nicotinamide and low dose of STZ-induced type 2 diabetic rats, oral administration of ginger powder (200 mg/kg) alleviated signs of metabolic syndrome. In treated diabetic group there was a significant decrease of blood

glucose, total lipid, and an increase in total antioxidant enzyme activities as compared with untreated rats **(Madkor *et al*., 2010)**.   Furthermore, the major pungent component in ginger, [6]-gingerol (100 mg/kg b.wt), which significantly decrease fasting blood glucose and improved glucose tolerance in db/db type 2 diabetic mice and lowered plasma triglyceride, total cholesterol, free fatty acid and low density lipoprotein **(Singh *et al*., 2009).**

2.3.3. Antioxidant effect of ginger:

Ginger, as an antioxidant, improves diabetes induced oxidative stress and its complications through prevention of lipid peroxidation and protein oxidation **(Afshari *et al*., 2007)**. The rich phytochemistry of ginger includes components that scavenge free radicals produced in food chains or biological systems **(Ramaa *et al*., 2006)**. Increased production of free radicals and reduction in cellular homeostasis results in oxidative stress that can lead to DNA damage **(Hussein *et al*., 2005).** Ginger is one of the richest sources of antioxidants that paved the way for its utilization to scavenge free radicals and allied health discrepancies, the active ingredients of ginger include gingerols, which exhibit antioxidant activity, and inhibit xanthine oxidase enzyme, that involved in the generation of reactive species **(Chang *et al*., 1994 and Dugasani *et al*., 2010).**

Kota *et al*. (2008) studied the effect of ginger at different doses and their findings the authors indicated that, there was a significant reduction in MDA in liver and kidney tissues, which attributed to enhancement of the antioxidant status of the host tissue or inhibition of oxidative products. **Ahmed *et al*. (2008)** revealed that dietary feeding of ginger (1% w/w) significantly attenuated lindane-induced lipid peroxidation, accompanied by the modulation of oxygen free radical (OFR) scavenging enzymes such as reduced glutathione (GSH), GSH-dependent enzymes

glutathione peroxidase (GSH-px), glutathione reductase (GSH-R) and glutathione-S-transferase (GSH-ST). Moreover, **El-Sharaky *et al*. (2009)** reported that ginger significantly lowered lipid peroxidation and raised the levels of antioxidant enzyme activities.

Furthermore, **Afshari *et al*. (2007)** studied the effect of dietary ginger (5% of their consumed food daily) on STZ-induced diabetic rats, and reported that the MDA level in diabetic rats treated with ginger were significantly lower than in untreated group, while plasma antioxidant activities in ginger treated rats were higher than in the diabetic and control groups. The effect of ginger on oxidative stress in diabetic rats were studied, twenty-four male Wistar rats were divided into three groups, control group, non-treated diabetic group, and diabetic group treated with ginger powder as 5% of their daily food, after 6 weeks the results revealed that, diabetes caused significant increase of lipid peroxidation and protein oxidation and decrease of catalase activity, while consumption of ginger induced attenuated in lipid peroxidation and increased catalase activity in diabetic treated rats as compared with untreated diabetic rats **(Ansari *et al*., 2008).**

2.3.4. Effect of ginger on liver and pancreas tissues:

Several studies reported the protective effects of ginger on different organs tissues. **Nwaopara *et al*. (2008)** and **Shati and Elsaid (2009)** demonstrated that ginger showed significant amelioration on oxidative stress induced by alcohol abuse and changes in liver, kidney and brain of rats tissue. **Ramudu *et al*. (2011b)** showed that ginger protected the liver tissue from STZ-induced oxidative damage. In addition, treatment with 1 % of dietary ginger for 4 weeks to rats improved antioxidant status, which may have protective role in hepatic tissue **(Mallikarjuna *et al*., 2008).** Moreover, treatment by ginger showed marked regeneration and

improvement in the hepatic tissues of albino rats and significant decrease in serum levels of liver enzymes compared with untreated diabetic group (**Abd-El Aty and Morgan, 2011**).

Bhandari *et al*. (**2005**) reported that ginger could protect the liver and pancreas tissues from lipid peroxidation on STZ diabetic rats. **Aggarwal (2010)** reported that gingerol one of the active components of ginger appeared to inhibit and intervene cyto-degeneration of pancreatic *β*-cells and hepatocytes and helped in scavenging the free radicals. Furthermore, **Chakraborty *et al*. (2012)** revealed that ginger has been shown to modulate insulin release in pancreatic *β*-cells and reported that gingerol whish is an active component of ginger it showed a protective effects of pancreatic *β*-cells.

MATERIAL AND METHODS

Chapter III

Material and Methods

3.1. Material:

3.1.1. Chemicals and Kits:

Streptozotocin, Zansor (STZ;product number S1312), was obtained from Sigma-Aldrich (St. Louis, MO) Chemical Co. Citric acid, trisodium citrate dehydrate, glucose solution (5%) purchased from Pharmaceutical Solutions Industry Ltd, Jeddah. Enzymatic glucose kits, enzymatic colorimetric kits for total cholesterol, triglyceride and high density lipoprotein cholesterol were obtained from Human Gesellschaft for Biochemical, Germany. ALPCO immunoassay insulin ELISA kits, Cayman's kits for assays of thiobarbituric acid reactive substances (TBARS), catalase (CAT) and superoxide dismutase (SOD) were purchased from Cayman Chemical Company, Ann Arbor, MI, USA.

3.1.2. Ginger and Curcumin:

Fresh ginger rhizomes were purchased from the local market. Curcumin powder (95% curcuminoids) was purchased from GNC, Pittsburgh, PA.

3.1.3. Experimental animals:

Male Wister albino rats (n=35 rats) weighing about (180-195 g) were obtained from the animal experimental unit of King Fahd Center for Medical

Research, King Abdulaziz University. All animals were allowed to one week to acclimatize in animal housing conditions before being used for the study. The rats were housed in standard laboratory conditions at a temperature of (22 ± 3 °C), relative humidity (50-55%) and a 12 h light/dark cycle (2 rats / cage). All animals fed standard nutritionally balanced diet according to **(Reeves, 1997)** and drinking water *ad libitum*.

3.1.4. Diet formula:

Standard nutritionally balanced diet according to AIN-93 **(Reeves, 1997)** was obtained from King Fahd Center for Medical Research, the diet consists of the following ingredients; crude protein 20.0%, crude fat 4.0 %, crude fiber 5.0 %, vitamin mix 1.0%, mineral mix 3.50%, choline chloride 0.25%, the remained formula up to 100% cornstarch, and its energy equals 2850 kcal/kg. The diet manufactured by Grain Silos & Flour Mills Organization, KSA**.**

3.2. Methods:

3.2.1. Preparation of ginger powder:

Fresh ginger rhizomes 5 kg were washed and cut into small thin pieces, then lyophilized for 54 hrs, it yield 13.6% (w/w) of the fresh, the obtained powder were stored at -20ºC until further use according to **(Rai *et al*., 2010)**. Lyophilization was conducted by using Freeze-Dryer Lyophilizer Millorock Bench-Top Freeze Dryer, Germany.

3.2.2. Induction of diabetes mellitus:

Diabetes was induced by a single intraperitonial (i.p.) injection of freshly prepared STZ (at a dose level of 65 mg/kg of body weight dissolved in 0.02 ml of 0.05 M citrate buffer pH 4.5) according to **(Nafiu *et al*., 2011)**. The citrate buffer was prepared by adding 47 ml of 0.05 M citric acid to 53 ml of 0.05 M trisodium

citrate dehydrate, pH of citrate buffer adjusted exactly at 4.5 by the use of pH meter according to **(Dawson *et al*., 1986).** After i.p. injection, the animals allowed to drink 5 % glucose solution overnight to overcome the death from hypoglycemia shock. Seventy-two hrs later, the blood samples obtained from orbital plexus vein of each injected rat by a fine capillary glass tube and the blood glucose concentration was determined to confirm induction of diabetes, the non-diabetic rats excluded from the study, and diabetes established with non- fasting blood glucose levels of ≥ 300 mg/dl.

3.2.3. Experimental design:

After the adaption period, animals divided into two main groups, as follows:

First group: Negative control (negative non-diabetic) group, rats (n=7) received a single i.p. injection with 0.2 ml of 0.05 M citrate buffer pH: 4.5, and fed standard diet.

Second group: Diabetic rats (n=28), which divided equally to four subgroups as follows:

- **Subgroup (1): Diabetic untreated rats** (positive control group), animals fed standard diet.

- **Subgroup (2):Diabetic rats treated with curcumin**; animals fed on diet containing curcumin powder (0.5 g / 100 g diet) according to **(Manjunatha and Srinivasan, 2007).**

- **Subgroup (3): Diabetic rats treated with ginger**; animals fed on diet containing ginger powder (3g/100 g diet) according to **(Saraswat *et al*., 2010)**.

- **Subgroup (4):Diabetic rats treated with (curcumin+ ginger)**; animals fed on diet containing curcumin and ginger powder (0.5% and 3%, respectively).

3.2.4. Biological evaluation:

During the experimental period food intake (FI) was recorded every second day per each group, and the animals were weighted twice weekly in all groups. The biological values of different diets were assessed by the determination of body weight gain% (BWG %) which was calculated at the end of the experimental period,using the following equation:

$$\textbf{Body weight gain\% (BWG \%) =} \frac{\text{Final b.wt} - \text{Initial b.wt}}{\text{Initial b.wt}} \text{X } 100$$

And feed efficiency ratio (FER) according to the method of **Chapman *et al*. (1959)**, was calculated using the following equation:

Feed efficiency ratio (FER) =Gain in body weight (g)/ feed consumed (g).

3.2.5.Blood collection and serum separation:

At the end of the experimental period (8 weeks), rats were fasted overnight before scarification. Blood samples withdrawn by heparinized capillary tube from the retro orbital plexu of each rat under anesthesia with diethyl ether, then centrifuged at 3000 rpm for 15 min to separate serum,which stored at -20° C until biochemical analysis. Immediately after blood sampling, animals sacrificed and the liver and pancreas of each animal dissected out, and then fixed in 10% formalin for histopathological studies.

3.2.6. Biochemical analysis:

3.2.6.1. Determination of glucose:

Serum glucose measured by enzymatic GOD / POD kits according to **(Trinder, 1969).** The glucose is determined after enzymatic oxidation in the presence of glucose oxidation. The formed hydrogen peroxide reacts under catalysis of peroxidase with pheole and 4-aminophenazone to a red-violet quinoneimine dye as indicator.

3.2.6.2. Determination of insulin:

Insulin estimated using enzyme linked immunosorbent assay ELISA methodas described by **Clark and Hales(1994)**. Monoclonal antibodies specific for insulin are immoblized to the 96-well microplate as the solid phase. Standards, controls, and samples are added to the appropriate wells with a horseradish peroxidase enzyme labeled monoclonal antibody (conjugate), resulting in insulin molecules being sandwiched between the solid phase and the conjugate. The optical density (OD) is measured by microplate reader at 450 nm. The intensity of the color generated is directly proportional to the amount of insulin in the sample.

3.2.6.3. Determination of lipid profile parameters:

3.2.6.3.1. Determination of total cholesterol (TC):

Total cholesterol assessed by using enzymatic colorimetric kit as described by **(Roeschlau et al., 1974)**. It determined after enzymatic hydrolysis and oxidation, the indicator quinoneimine formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase.

3.2.6.3.2. Determination of triglyceride (TG):

Enzymatic colorimetric GPO-PAP kitused for measured triglycerides as described by (**Fossati and Prenape, 1982**). The triglyceride was determined after enzymatic hydrolysis with lipases. Indicator is quinoneimine form hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase.

3.2.6.3.3. Determination of high-density lipoprotein cholesterol (HDL-C):

An enzymatic colorimetric kit used for the determination of HDL-C as described by (**Lopes-Virella *et al*., 1977**). The assay combines tow specific steps; in 1st step chylomicrons, VLDL and LDL cholesterol are specifically eliminated and destroyed by enzymatic reaction. In the 2nd step remaining cholesterol from the HDL fraction is determined by well established specific enzymatic reaction in the presence of specific surfactants for HDL.

Low-density lipoprotein-cholesterol (LDL-C)and very low-density lipoprotein-cholesterol (VLDL-C)were calculated according to the equation of (**Marchall, 1992**) as follow:

LDL-C = TC -(HDL-C +V LDL-C), VLDL-C estimated as triglyceride/5

3.2.6.4.Assay of antioxidant enzymes activities:

3.2.6.4.1. Estimation of malondialdehyde (MDA):

Malondialdehyde (MDA) as a measure of lipid peroxidation, which used as an indicator of oxidative stress. Cayman's Thiobarbituric Acid Reactive Substances TBARS assay kit used to assay MDA according to (**Yoshioka *et al*., 1979**) .The MDA-TBA adduct formed by the reaction of MDA and TBA under high temperature and acidic conditions measured calorimetrically at 530-540 nm. If lipoproteine fractions are first acid precipitated from the sample, interfering soluble

TBARS are minimized, lipids with greater unsaturation will yield higher TBARS values.

3.2.6.4.2. Estimation of superoxide dismutase (SOD) activity:

Superoxide dismutase (SOD) is metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism.Cayman's superoxide dismutase assay kitwas used for measured SOD activity by the method of **(Wheeler *et al*., 1990).** SOD activity was measures by the inhibition of nitrobluetetrazolium by superoxide anion ($O_2^{\cdot}$) produced by potassium superoxide dissolved in dimethyl sulfoxide the absorbance was read at 550 nm. One unit of enzymatic activity defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

3.2.6.4.3. Estimation of catalase (CAT) activity:

Catalase (CAT) is involved in the detoxification of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS). Cayman's catalase assay kit used for determination of enzyme activity as described by **Sinha (1972)**. The method based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured calorimetrically with 4-amino-3hydrazino-5-mercapto-1,2,4-triazole (purpled) as chromogen. Purpled specifically forms a bicyclicheterocycle with aldehydes, which is upon oxidation changes form colorless to a purple color.

3.2.7.Histopathological examination:

Specimens from the liver and pancreas were placed in 10% neutral buffered formalin. The fixed tissues were then trimmed, washed with ice saline and

dehydrated in ascending grades of isopropyl alcohol and cleared in xylene. The wax impregnated tissues were embedded in paraffin blocks using the same grade wax,the paraffin blocks were cut with rotary microtome at 3-5µ thickness. The sections were floated on a tissue floatation bath at 40°C and taken on glass slides. The sections were then melted in an incubator at 60°C and after 5 min. the sections were allowed to cool and stained with Hematoxylen and Eosin according to **(Bancroft and Cook, 1998)**, and examined microscopically.

3.2.8. Statistical analysis:

Results were expressed as a (mean ±SD). Data were analyzed statistically by analysis of variance, for statistical significance using L.S.D. test, one way ANOVA, post hoc multiple comparisons according to **Snedecor and Cochron (1989)**. An DELL computer with a software system SPSS version 20 was used for these calculations.

RESULTS

Chapter IV

Results

4.1. Biological evaluations.

Table (4.1) and Figures (4.1, 4.2, 4.3 and 4.4) showed the effect of curcumin, ginger or their mixture on final body weight, BWG %, DFI and FER in STZ-diabetic male rats. The results reported that diabetic group showed very highly significant differences ($p < 0.001$) in final body weight, BWG%, DFI and FER as compared with control (-ve) group. Diabetic groups treated with curcumin, ginger or their mixture demonstrated very highly significant differences in all biological parameters ($p < 0.001$) when compared with control negative group. The results also showed that final body weight, BWG%, DFI and FER in curcumin, ginger or their mixture groups recorded very highly significant differences ($p < 0.001$) with respect to untreated diabetic group, There were non-significant differences in final body weight, BWG%, DFI and FER between the three treated groups.

Table 4.1. Initial and final body weight (g), body weight gain %, feed intake (g/rat/ day) and feed efficiency ratio (FER) in control and diabetic rats treated with curcumin, ginger or their mixture after 8 weeks of treatment.

Experimental groups	Initial body weight (g)	Final body weight (g)	BWG %	DFI (g/rat/day)	FER
Control (- ve)	188.96 ± 4.04	354.59 ± 8.97	87.65 ± 4.96	19.41± 1.71	0.152 ± 0.012
Diabetic (+ ve)	189.16 ± 2.84	250.94 ± 10.85 [a#]	32.66 ± 2.68 [a#]	27.11±1.69 [a#]	0.041 ± 0.003 [a#]
Diabetic + Curcumin	186.26 ± 2.61	307.75± 7.02 [a#b#]	65.23 ± 3.78 [a#b#]	22.79 ± 1.01 [a# b#]	0.095 ± 0.009 [a#b#]
Diabetic + Ginger	188.16 ± 3.16	302.00 ± 11.02 [a#b#]	60.50 ± 3.73 [a# b#]	23.60 ± 0.83 [a#b#]	0.086 ± 0.008 [a#b#]
Diabetic + (Curcumin & Ginger)	188.67 ± 3.47	310.89 ± 8.57 [a#b#]	64.78 ± 2.67 [a#b#]	23.39 ± 0.97 [a#b#]	0.093 ± 0.007 [a#b#]

BWG%: Body Weight Gain , **DFI:** Daily Food Intake , **FER**: Feed Efficiency Ratio.

Each value represents the mean of 7 rats ± SD.

[a]: Significant difference between control and diabetic groups.

[b]: Significant difference between diabetic and diabetic treated groups.

[c] : Significant difference between diabetic treated with curcumin and diabetic treated with ginger .

[d] : Significant difference between diabetic treated with curcumin or ginger and diabetic treated (curcumin + ginger) .

([*]$p < 0.05$, [+] $p < 0.01$ and [#] $p < 0.001$)

Figure 4.1. Final body weight in control and diabetic rats (DM) treated with curcumin (CUR), ginger (GIN) or their mixture (CUR & GIN).

Figure 4.2. Body weight gain in control and diabetic rats (DM) treated with curcumin (CUR), ginger (GIN) or their mixture (CUR & GIN).
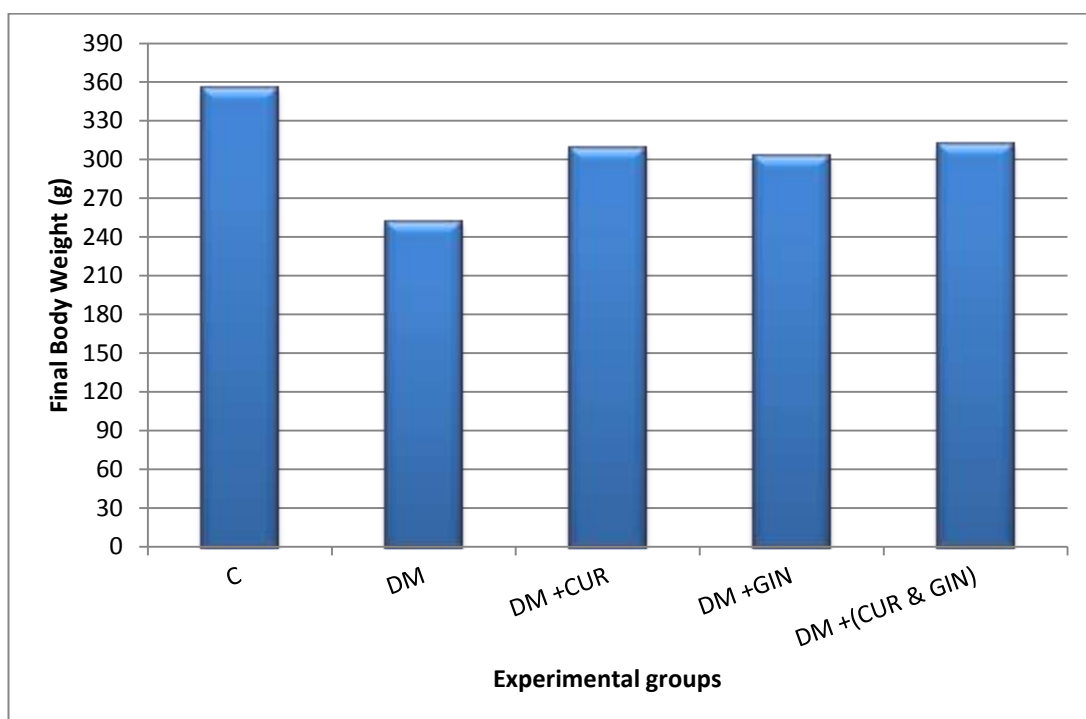
Figure 4.3. Daily food intake in control and diabetic rats (DM) treated with curcumin (CUR), ginger (GIN) or their mixture (CUR & GIN).
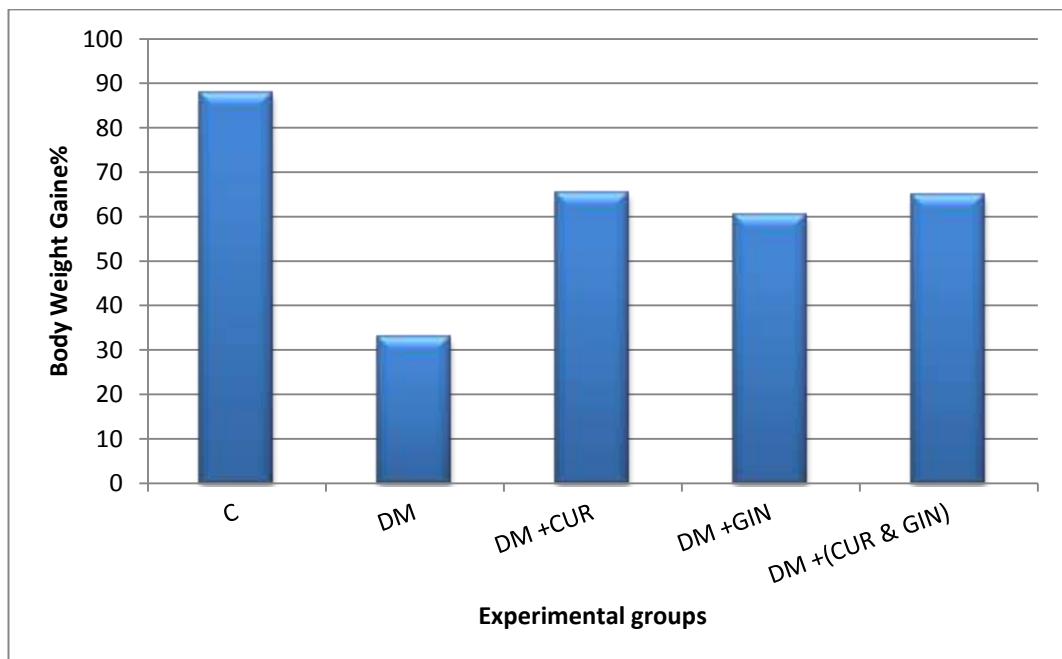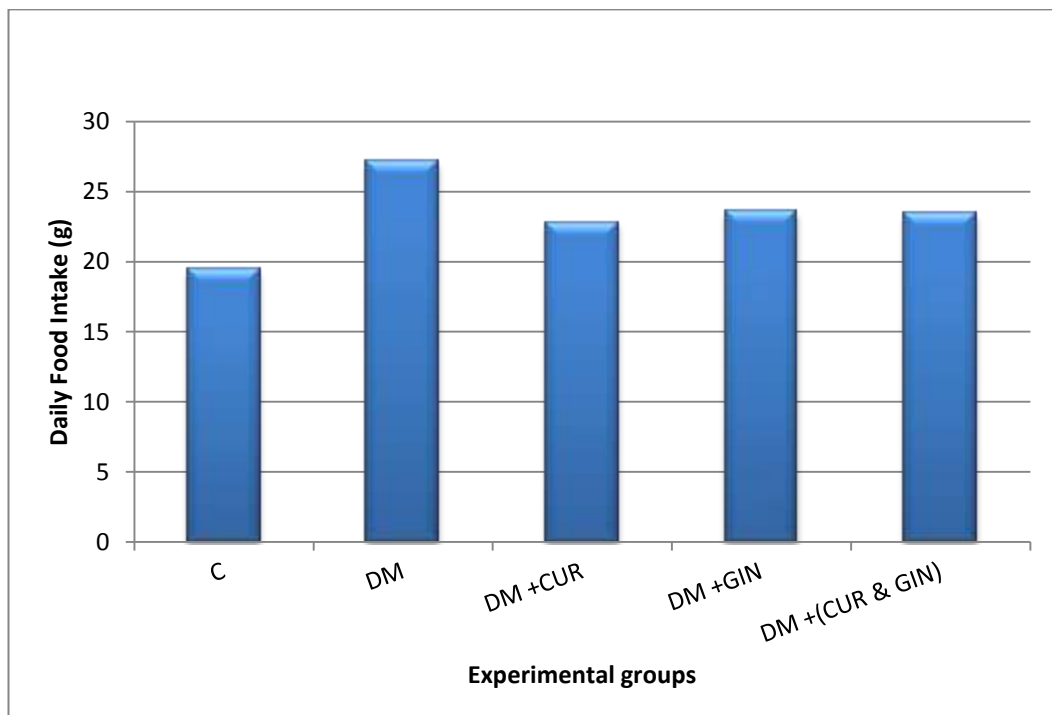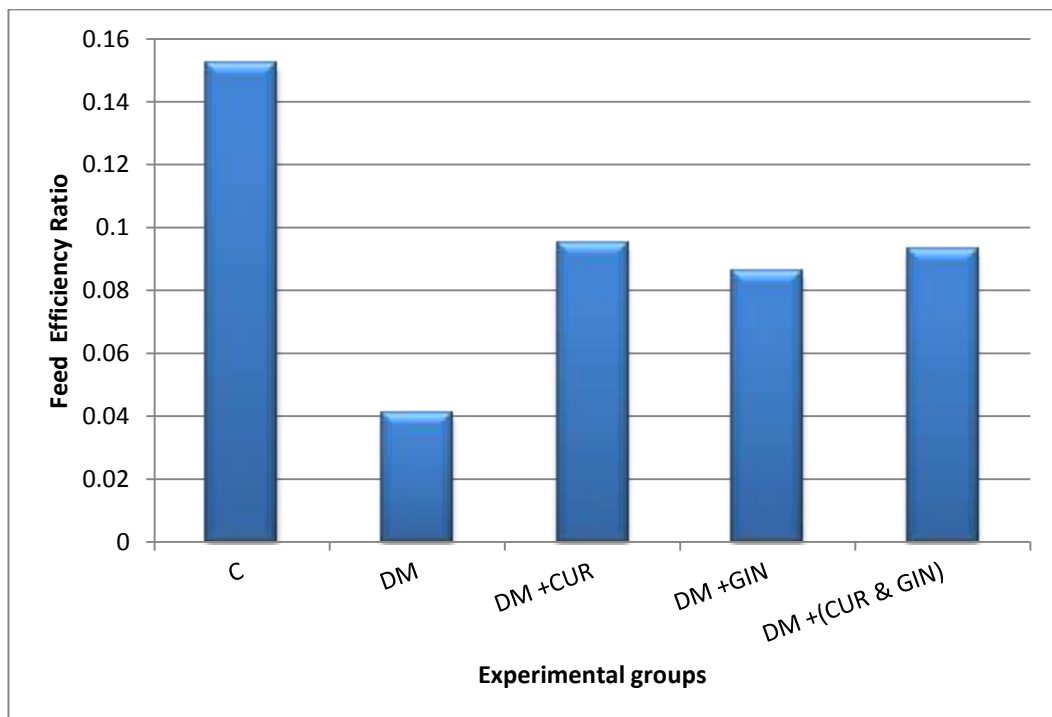
Figure 4.4. Feed efficiency ratio in control and diabetic rats (DM) treated with curcumin (CUR), ginger (GIN) or their mixture (CUR & GIN).

4.2. Serum glucose concentration and insulin level.

Table (4.2) and Figures (4.5&4.6) showed the effect of curcumin, ginger or their mixture on serum glucose concentration and insulin level in STZ-diabetic male rats. In diabetic rats there were a very highly significant ($p < 0.001$) elevation in glucose concentration accompanied with a very highly significant ($p < 0.001$) reduction in insulin level as compared with control (-ve) group, with percentage (273.93% and -58.46%, respectively) as percent change from control group. Diabetic groups treated with curcumin, ginger or their mixture showed improvement in glucose concentration and insulin secretion level, but there values showed significant differences ($p < 0.001$) as comparing with control (-ve) group.

Administration of curcumin, ginger or their mixture to diabetic rats showed remarkably ameliorated the elevation in glucose concentration and the reduction in insulin level, there were very highly significant ($p < 0.001$) improvement in glucose concentration and insulin level as compared with diabetic untreated group. The results also demonstrated that serum glucose concentration and insulin level in diabetic group treated with curcumin recorded significant differences ($p < 0.05$) when compared with ginger treated group. Treatment with curcumin and ginger mixture showed non-significant differences when compared with curcumin treated group, while demonstrated highly significant differences ($p < 0.01$) with respect to ginger treated group in both glucose concentration and insulin levels.

Table 4.2. Serum glucose (mg/dl) and insulin (µU/ml) level in control , diabetic rats treated with curcumin, ginger or their mixture after 8 weeks of treatment.

Experimental groups	Glucose (mg/dl)	Insulin (µU/ml)
Control (- ve)	99.73±7.13	68.13±3.70
Diabetic (+ ve)	372.92±18.03 [a #]	28.30±2.11 [a #]
Diabetic + Curcumin	193.27±3.79 [a # b #]	47.61±3.16 [a # b]
Diabetic + Ginger	204.54±8.69 [a # b # c * d +]	43.56±2.55 [a # b # c * d +]
Diabetic + (Curcumin & Ginger)	186.96±7.27 [a # b #]	50.16±2.16 [a # b #]

Each value represents the mean of 7 rats ± SD.

[a] : Significant difference between control and diabetic groups.

[b] : Significant difference between diabetic and diabetic treated groups.

[c] : Significant difference between diabetic treated with curcumin and diabetic treated with ginger .

[d] : Significant difference between diabetic treated with curcumin or ginger and diabetic treated (curcumin + ginger) .
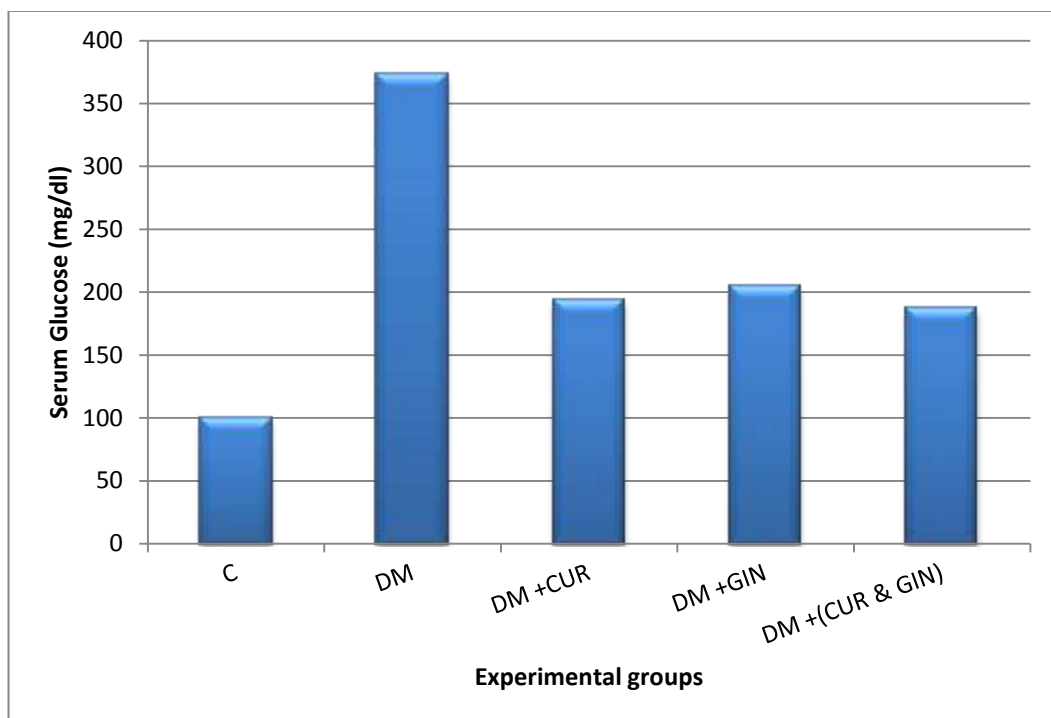
($^{*}p < 0.05$, $^{+}p < 0.01$ and $^{\#}p < 0.001$)

Figure 4.5. Serum glucose (mg/dl) level in control , diabetic rats (DM) treated with curcumin (CUR), ginger (GIN) or their mixture (CUR & GIN).
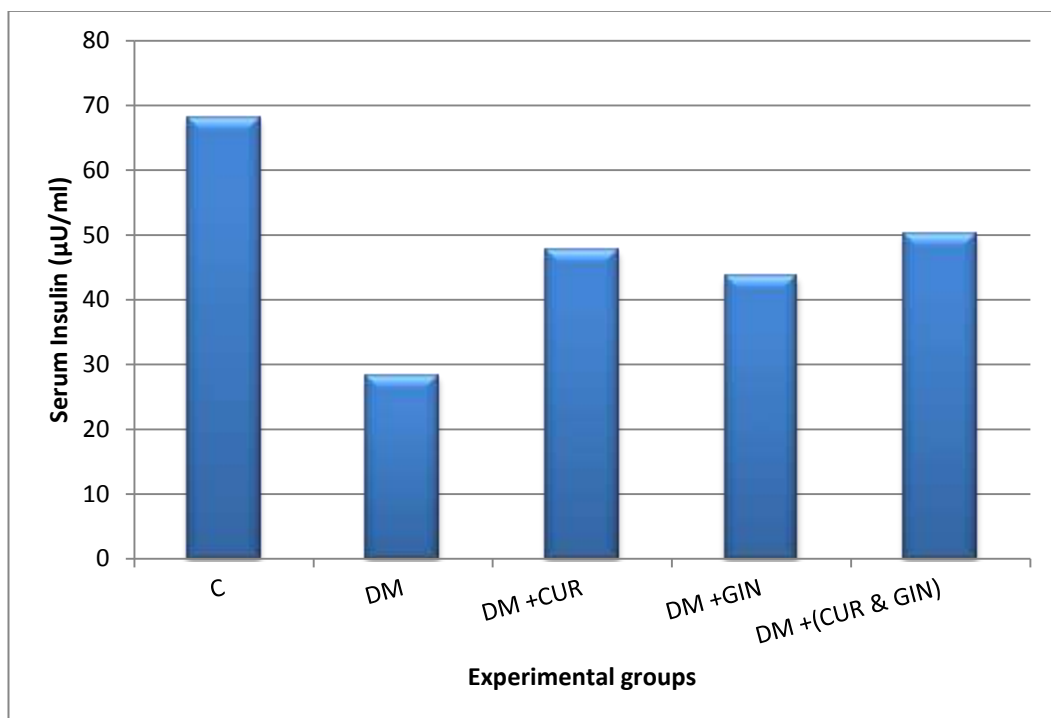
Figure 4.6. Serum insulin (µU/ml) level in control , diabetic rats (DM) treated with curcumin (CUR), ginger (GIN) or their mixture (CUR & GIN).

4.3. Serum lipid profile parameters .

Table (4.3) and Figure (4.7) showed the effect of curcumin, ginger or their mixture on serum lipid profile levels in STZ-diabetic male rats. In diabetic group there were very highly significant (p<0.001) elevation in TC, TG, LDL-C VLDL-C, accompanied with a very highly significant ($p < 0.001$) reduction in HDL-C level as compared with control (-ve)   group, with percentage (92.74%, 79.36%, 313.11%, 79.36% and -41.49% , respectively) as percent change from control group.

Diabetic groups treated with curcumin, ginger or their mixture showed improvement in lipid profile levels, but curcumin group and ginger group showed very highly significant differences ($p < 0.001$) in lipid profile levels comparing with control group, while the curcumin and ginger mixture group recorded that TC and HDL-C values showed highly significant differences   ($p < 0.01$) and TG, LDL-C and VLDL-C recorded very highly significant differences ($p < 0.001$)   comparing with control group (-ve).

 Administration of curcumin, ginger or their mixture to diabetic rats showed remarkably amelioration in the elevation in TC, TG, LDL-C, VLDL-C and the reduction in HDL-C levels, there were very highly significant   ($p < 0.001$) improvement for lipid profile   levels as compared with diabetic untreated group. Also, the data demonstrated that serum TC, TG, VLDL-C levels recorded highly significant differences ($p < 0.01$), but LDL-C level showed a very highly significant difference ($p < 0.001$) and HDL-C level recorded a significant difference ($p < 0.05$) in diabetic group treated with curcumin when compared with ginger treated group.

Treatment with curcumin and ginger mixture showed non-significant difference when compared with curcumin treated group, while demonstrated very highly significant differences ($p < 0.001$) with respect to ginger treated group in lipid profile levels.

Table 4.3. Serum lipid profile levels (mg/dl) in control and diabetic rats treated with curcumin, ginger or their mixture after 8 weeks of treatment.

Experimental groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control (- ve)	90.16 ± 5.95	80.33± 5.19	45.49 ± 2.68	28.60 ± 1.50	16.07 ± 1.04
Diabetic (+ ve)	173.77 ± 4.23 [a#]	144.08 ±7.52 [a#]	26.80 ± 1.89 [a#]	118.15 ± 3.25 [a#]	28.82 ± 1.50 [a#]
Diabetic+ Curcumin	108.80 ± 8.25 [a#b#]	100.49 ± 7.25 [a#b#]	38.23 ± 2.88 [a#b#]	50.47 ± 1.10 [a#b#d#]	20.10 ± 1.45 [a#b#]
Diabetic + Ginger	118.75± 6.43 [a#b#c+d#]	112.16 ±7.38 [a# b#c+d#]	35.33 ± 2.59 [a# b#c*d#]	60.99 ± 3.89 [a#b#c#d#]	22.43 ± 1.48 [a#b#c+d#]
Diabetic + (Curcumin &Ginger)	101.80 ±7.26 [a+ b#]	93.22 ± 5.87 [a#b#]	40.91 ±2.38 [a+b#]	42.25 ± 2.59 [a#b#]	18.64 ± 1.17 [a#b#]

TC: Total Cholesterol, **TG:** Triglycerides, **HDL-C:** High Density Lipoprotein Cholesterol, **LDL-C:** Low Density Lipoprotein Cholesterol and **VLDL-C:** Very Low Density Lipoprotein Cholesterol.

Each value represents the mean of 7 rats ± SD.

[a] : Significant difference between control and diabetic groups.

[b] : Significant difference between diabetic and diabetic treated groups.

[c] : Significant difference between diabetic treated with curcumin and diabetic treated with ginger .

[d] : Significant difference between diabetic treated with curcumin or ginger and diabetic treated (curcumin + ginger) .

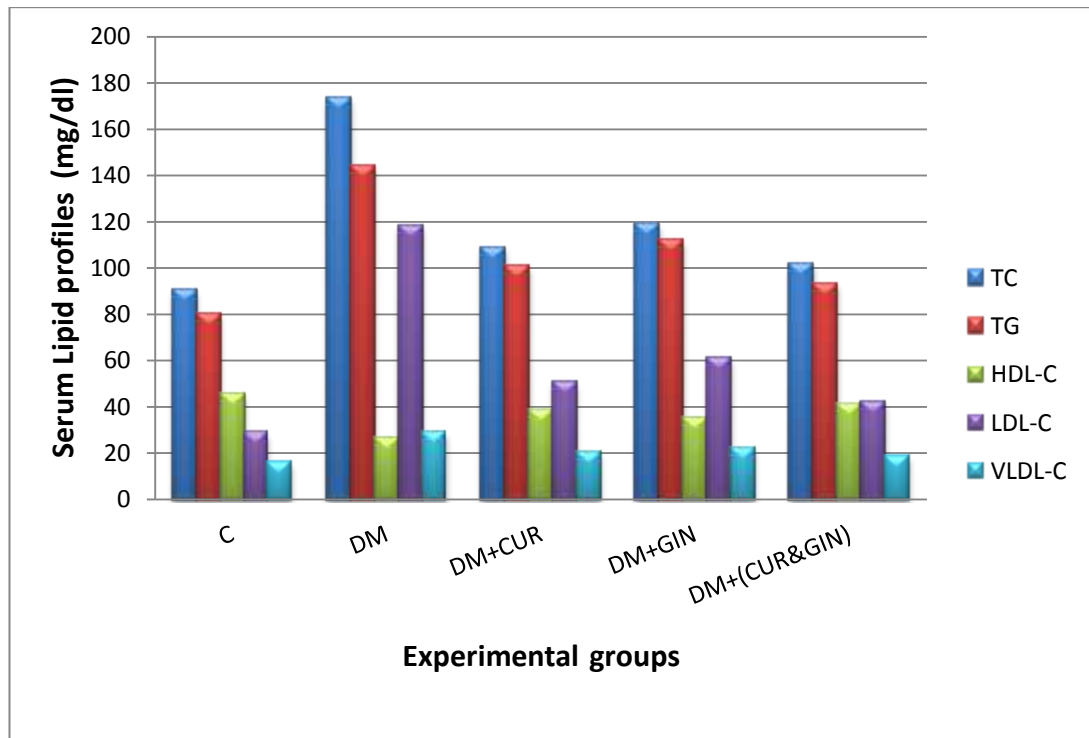($^*p < 0.05$, $^+ p < 0.01$ and $^\# p < 0.001$)

Figure 4.7. Serum lipid profile levels (mg/dl) in control and diabetic rats (DM) treated with curcumin (CUR), ginger (GIN) or their mixture (CUR & GIN).

4.4. Serum malondialdehyde and some antioxidant enzymes activity.

Table (4.4) and Figures (4.8) showed the effect of curcumin, ginger or their mixture on serum MDA level and some antioxidant enzymes activity SOD, CAT in STZ-diabetic male rats. In diabetic group there were very highly significant ($p < 0.001$) elevation in MDA levels accompanied with very highly significant ($p < 0.001$) reduction in SOD and CAT enzymes activity as compared with control (-ve) group, with percentage (289.42%, -58.87% and -60.34, respectively) as percent change from control group. Diabetic groups treated with curcumin, ginger or their mixture showed improvement in MDA level , SOD and CAT enzymes activity, but there values showed significant differences ($p < 0.001$) with respect to control negative group. Administration of curcumin, ginger or their mixture to diabetic rats showed remarkably amelioration the elevation of MDA level and the reduction in SOD, CAT enzymes activity. There were very highly significant ($p < 0.001$) improvement for MDA level and SOD, CAT enzyme activities as compare with diabetic untreated group. The data also demonstrated that serum MDA level and SOD, CAT enzymes activity in diabetic group treated with curcumin recorded significant differences ($p < 0.05$) when compared with ginger treated group.

Treatment with curcumin and ginger mixture showed non-significant difference when compared with curcumin treated group, while demonstrated highly significant improvement ($p < 0.01$) as compared with ginger treated group in MDA level, SOD and CAT enzyme activities.

Table 4.4. Serum Malondialdehyd (MDA) (nmol/ml) Antioxidant enzymes activities in control and diabetic rats treated with curcumin, ginger or their mixture after 8 weeks of treatment.

Experimental groups	MDA (nmol/ml)	SOD (U/ml)	CAT (nmol/ml)
Control (- ve)	10.21±0.88	0.231±0.021	32.53±1.87
Diabetic (+ ve)	39.76± 1.49 [a #]	0.095±0.008 [a #]	12.90±1.10 [a #]
Diabetic + Curcumin	20.80±1.76 [a # b #]	0.194±0.016 [a # b #]	23.63±2.01 [a # b #]
Diabetic + Ginger	22.67±1.64 [a # b # c* d+]	0.179±0.005 [a # b # c* d+]	21.70±1.51 [a # b # c* d+]
Diabetic + (Curcumin & Ginger)	19.85±1.64 [a # b #]	0.201±0.010 [a # b #]	24.83±1.10 [a # b #]

MDA: Malondialdehyde, **SOD**: Superoxide dismutases, **CAT**: Catalase .

Each value represents the mean of 7 rats ± SD.

[a] : Significant difference between control and diabetic groups.

[b] : Significant difference between diabetic and diabetic treated groups.

[c] : Significant difference between diabetic treated with curcumin and diabetic treated with ginger .

[d] : Significant difference between diabetic treated with curcumin or ginger and diabetic treated (curcumin + ginger) .

($^{*}p < 0.05$, $^{+}p < 0.01$ and $^{#}p < 0.001$)

Figure 4.8. Serum Malondialdehyd (MDA) (nmol/ml) in control and diabetic rats (DM) treated with curcumin (CUR), ginger (GIN) or their mixture (CUR &GIN).

Figure 4.9. Serum SOD (U/ml) in control and diabetic rats (DM) treated with curcumin (CUR), ginger (GIN) or their mixture (CUR &GIN).
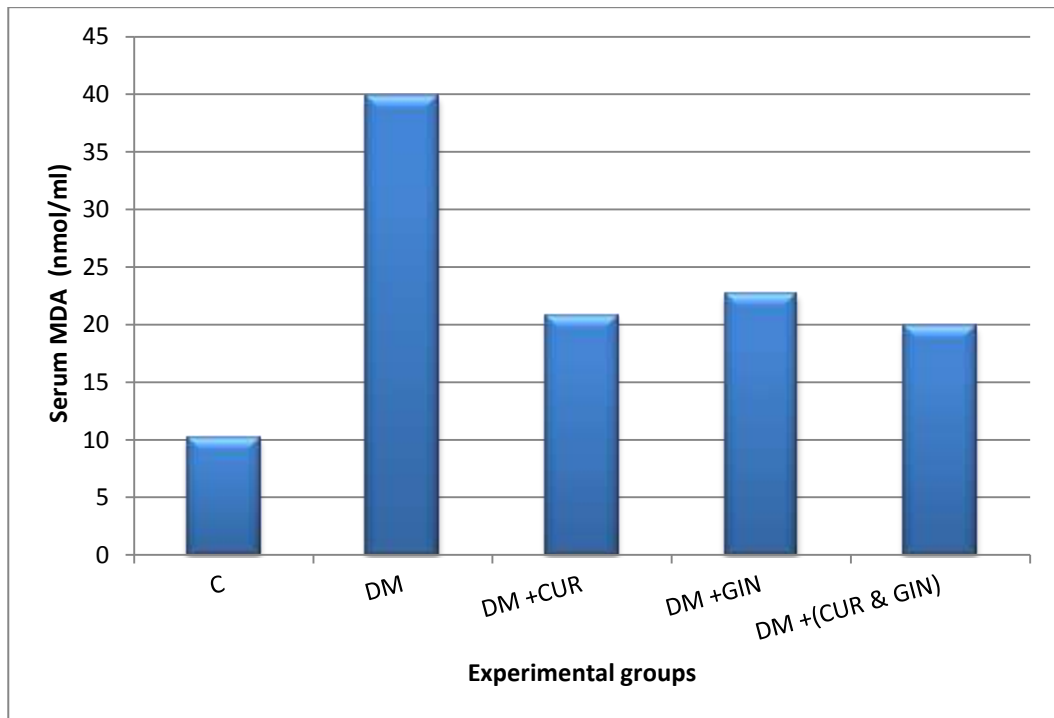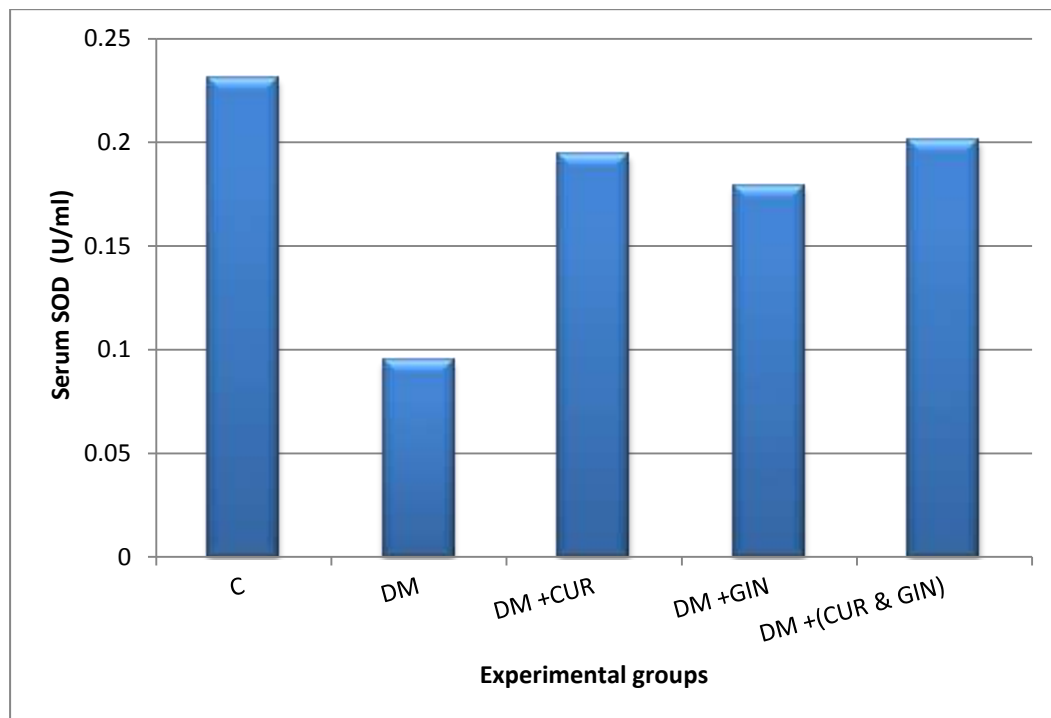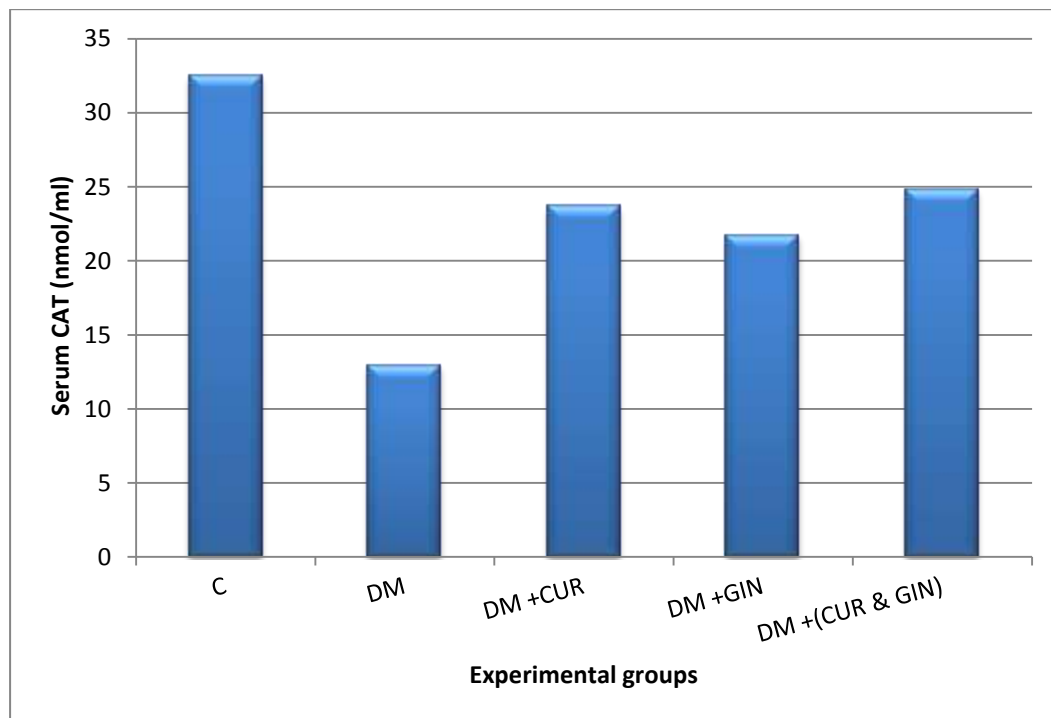
**Figure 4.10. Serum CAT (nmol/ml) in control and diabetic rats (DM) treated
with curcumin (CUR), ginger (GIN) or their mixture (CUR &GIN).**

4.5 . Histopathological investigation:

4.5.1. Liver:

Microscopically, liver from control rat group showed the normal histological structure of hepatic lobule and portal vein without alterations (Fig. 4.11), the figure showed that hepatocytes were arranged in the form of branching cords, separated by blood sinusoids and radiated from the central vein, the hepatocytes appeared polyhedral in shape and containing basophilic granules and central rounded vesicular nuclei. Liver tissues in diabetic rats showing activation of kupffer cells, sinusoidal leucocytosis, and apoptosis of hepatocytes (Fig. 4.12) , marked dilatation and congestion of central vein with necrosis of sporadic hepatocytes (Fig. 4.13), as well as congestion of central vein and focal hepatic necrosis replaced by mononuclear infiltration (Fig. 4.14).

Liver tissues in diabetic rat treated with curcumin showed apparent normal histological structure (Fig. 4.15), except slight kupffer cells activation (Fig. 4.16). While, liver tissues in diabetic rats treated with ginger showed kupffer cells activation (Fig. 4.17 and 4.18), slight congestion of hepatic sinusoids with binucleation of hepatocytes (Fig. 4.19 and 4.20). Meanwhile, in diabetic rats treated with both curcumin and ginger examined liver sections showed kupffer cells activation (Fig. 4.21), and other sections of diabetic treated rats revealed no histopathological alteration (Fig. 4.22 and 4.23).
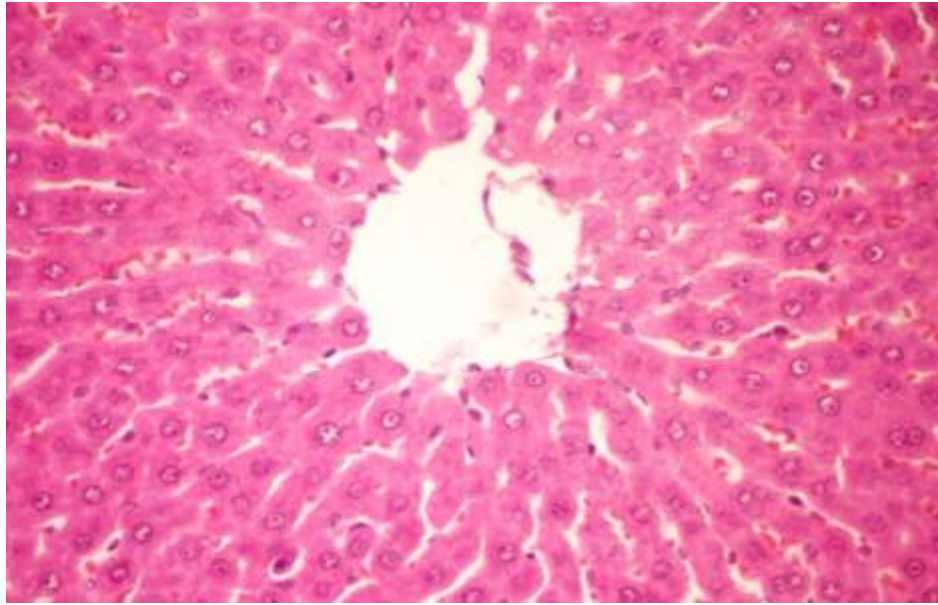
**Figure 4.11. Liver of control negative (-ve) showing no
histopathological alteration.**
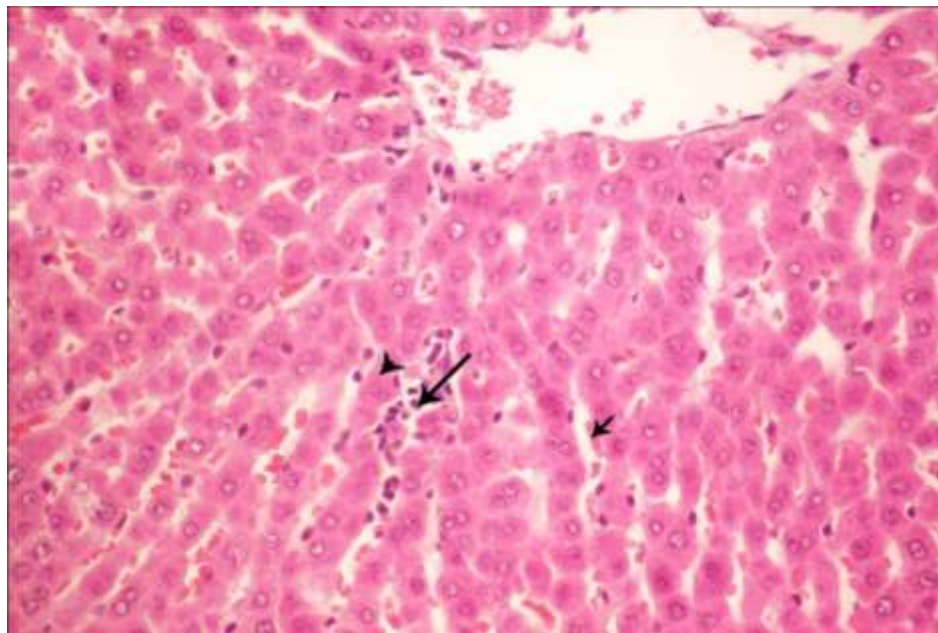
(H&E stain x 400)

**Figure 4.12. Liver of diabetic rats showing activation of kupffer
cells (small arrow), sinusoidal leucocytosis (large arrow), and
apoptosis of hepatocytes (arrow head).**

(H&E stain x 400)
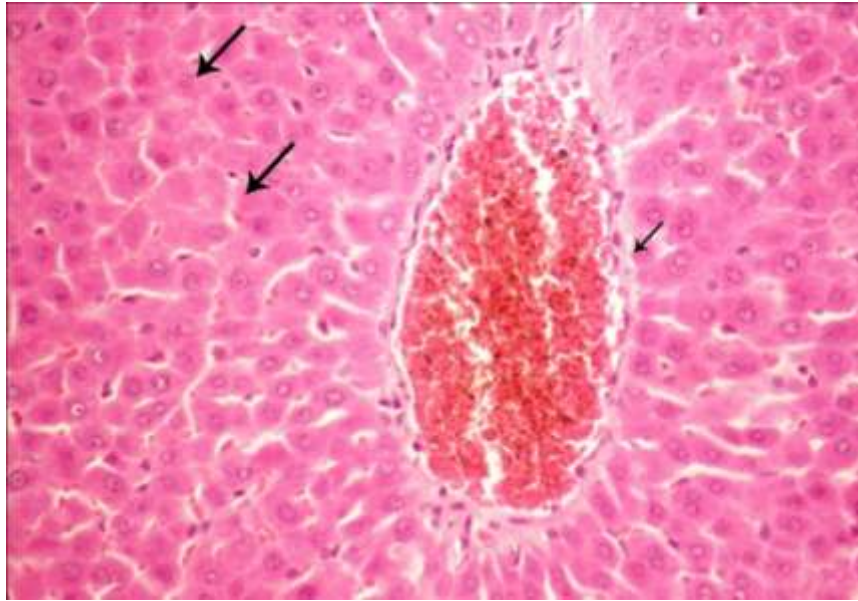
Figure 4.13. Liver of diabetic rats showing marked dilatation and congestion of central vein (small arrow), with necrosis of sporadic hepatocytes (large arrow).

(H&E stain x 400)
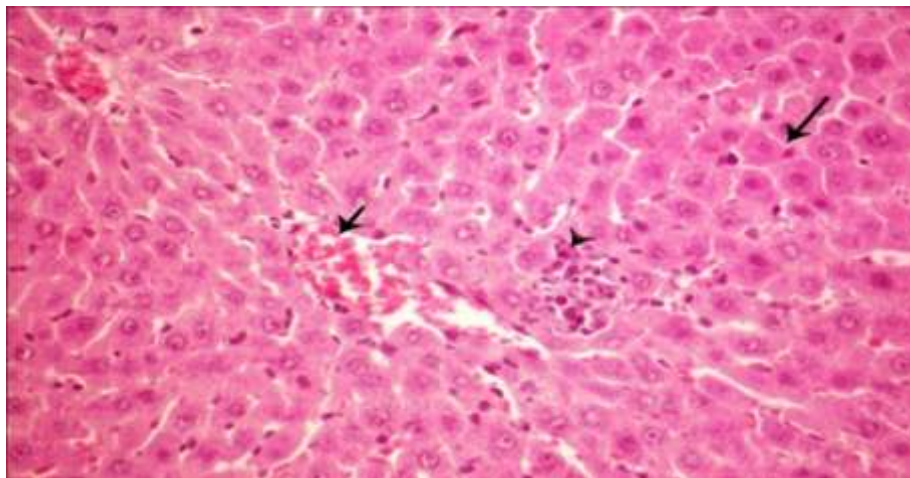
Figure 4.14. Liver of diabetic rats showing congestion of central vein (small arow), kupffer cells activation (large arrow), necrosis of sporadic hepatocytes and focal hepatic necrosis replaced by mononuclear infiltration (arrow head).

(H&E stain x 400)
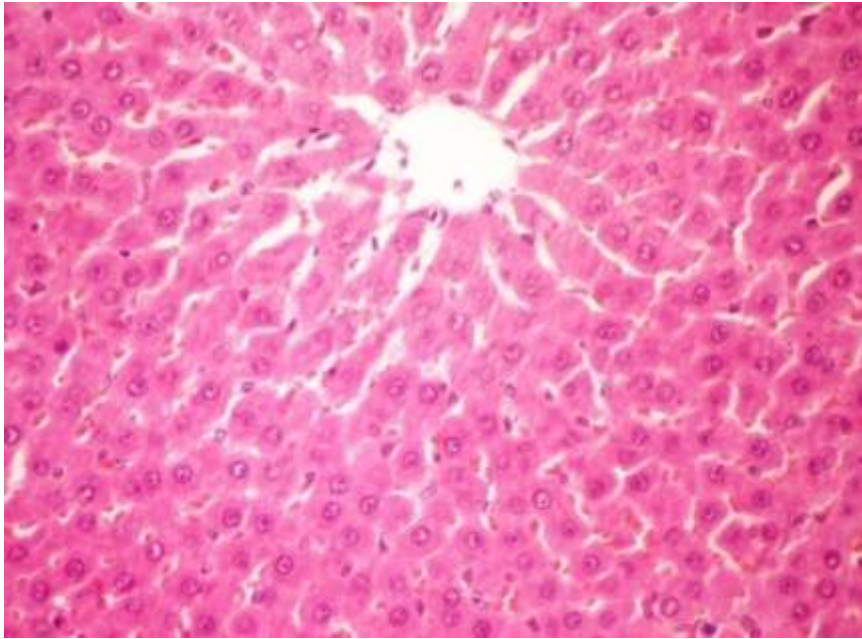
Figure 4.15. Liver of diabetic rats treated with curcumin showing no histopathological changes.
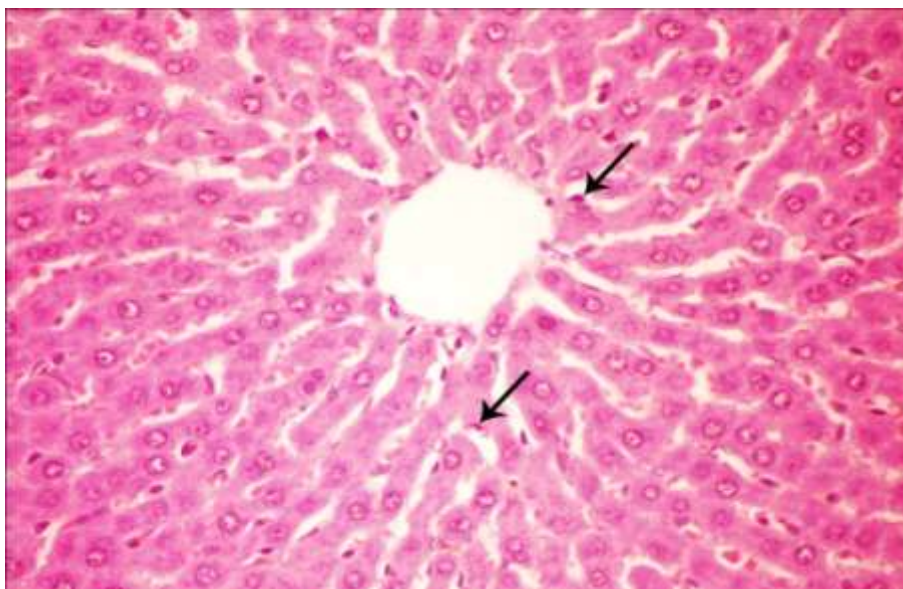
(H & E stain   x 400)

Figure 4.16. Liver of diabetic rats treated with curcumin showing kupffer cells activation.
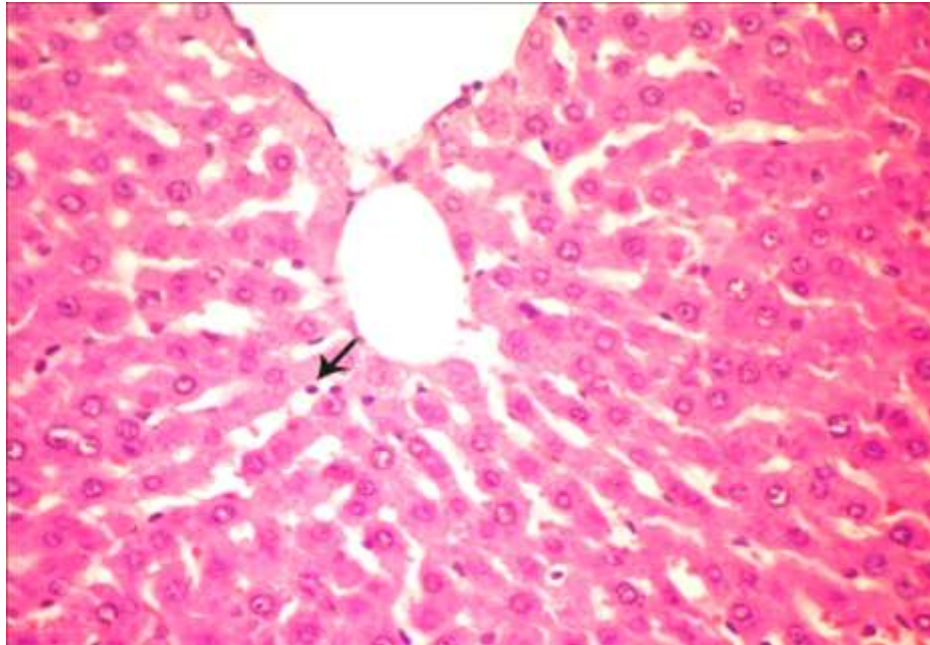
(H & E stain x 400)

Figure 4.17. Liver of diabetic rats treated with ginger showing kupffer cells activation.
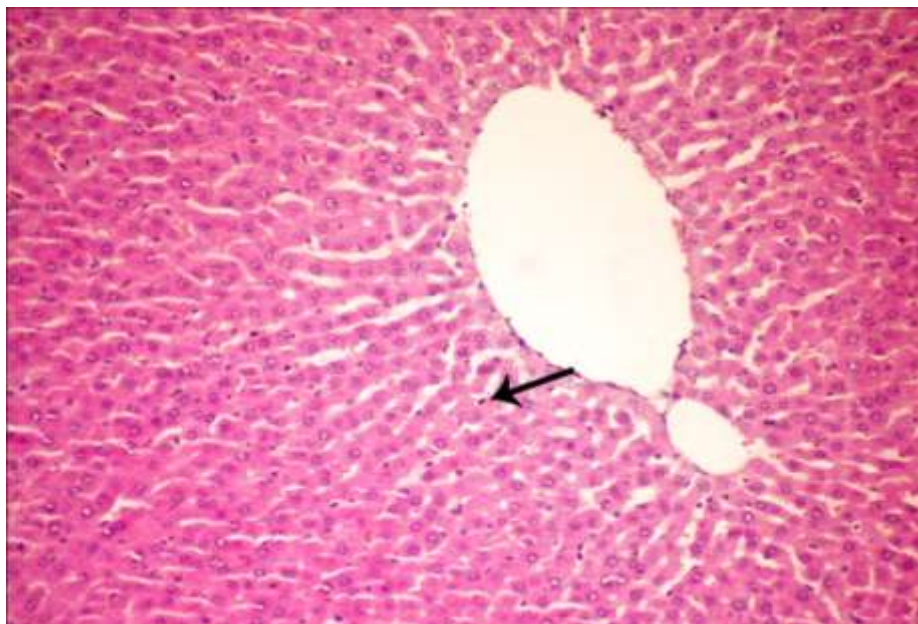
(H & E stain x 400)

Figure 4.18. Liver of diabetic rats treated with ginger showing kupffer cells activation.

(H & E stain x 200)
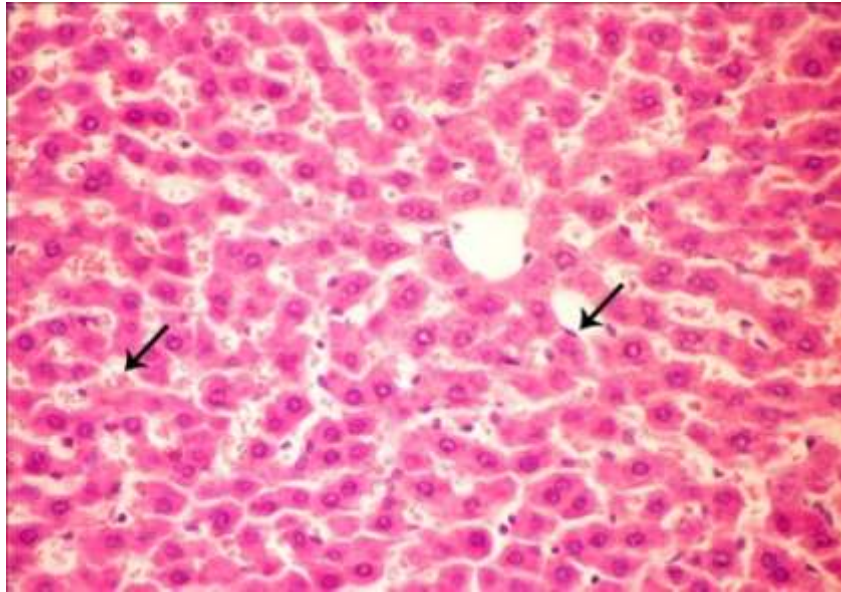
Figure 4.19. Liver of diabetic rats treated with ginger showing kupffer cells activation and slight congestion of hepatic sinusoids.

(H & E stain x 400)
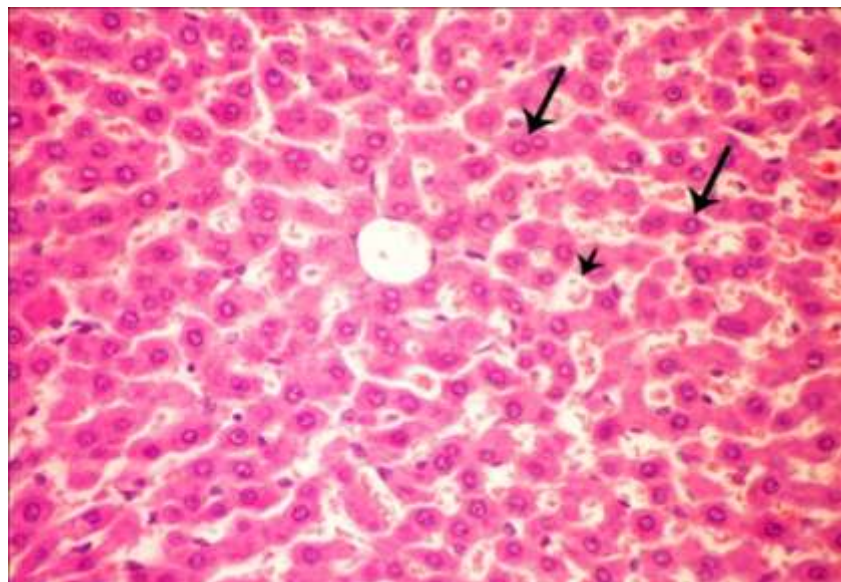
Figure 4.20. Liver of diabetic rats treated with ginger showing slight dilatation and congestion of hepatic sinusoids (small arrow), with binucleation of hepatocytes (large arrow).
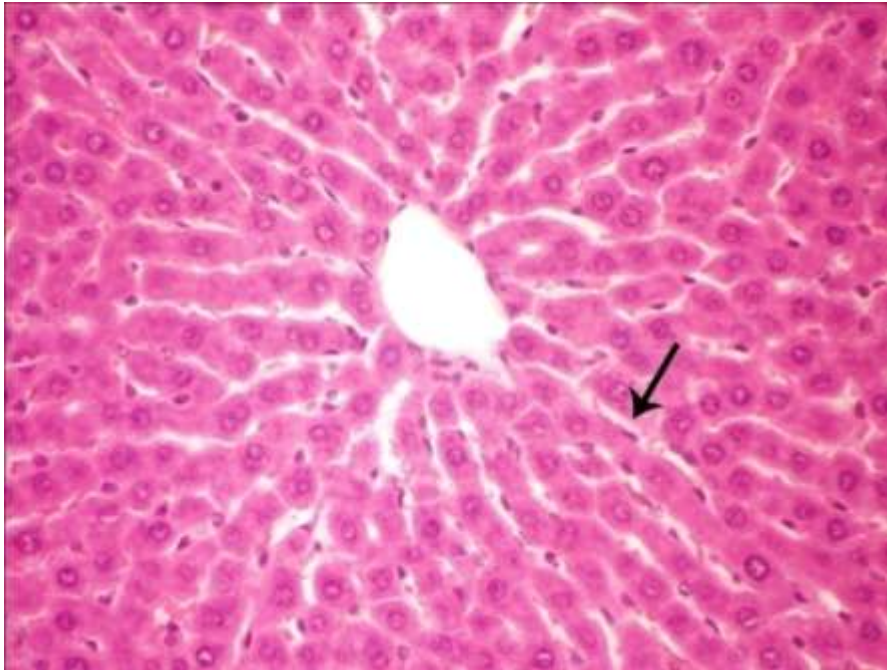
(H & E stain   x 400)

Figure 4.21. Liver of diabetic rats treated with both curcumin and ginger showing kupffer cells activation (large arrow).

(H & E stain x 400)

Figure 4.22. Liver of diabetic rats treated with both curcumin and ginger showing no histopathological changes.

(H & E stain   x 400)

Figure 4.23. Liver of diabetic rats treated with both curcumin and ginger showing no histopathological alteration.

(H & E stain   x 400)

4.5.2. Pancreas:

The histological appearance of the pancreatic islet cells of normal control rats showed no histopathological changes (Fig. 4.24 and 4.25). Microscopic examination of the pancreatic sections of diabetic untreated group revealed necrosis of β-cells of islets of langerhan's (Fig. 4.26 , 4.27 and 4.28), and atrophy of islets of langerhan's (Fig. 4.29), as well as cystic dilatation of pancreatic duct and congestion of pancreatic blood vessel (Fig. 4.30).

However, examined sections of diabetic rats treated with curcumin showed no histopathological changes (Fig. 4.31), and other sections revealed slight congestion of blood vessel with normal pancreatic acini and normal β- cells of islet of langerhan's (Fig. 4.32). While some examined sections of diabetic rats treated with ginger showed no histopathological changes (Fig. 4. 33), and other sections revealed slight vacuolation of sporadic β-cells of islets of langerhan's (Fig. .4.34). On the other hand, examined pancreatic islet tissues in diabetic rats treated with both curcumin and ginger revealed no histological changes (Fig. 4.35 and 4.36), except few leucocytic cells infiltration in some sections (Fig. 4.37).
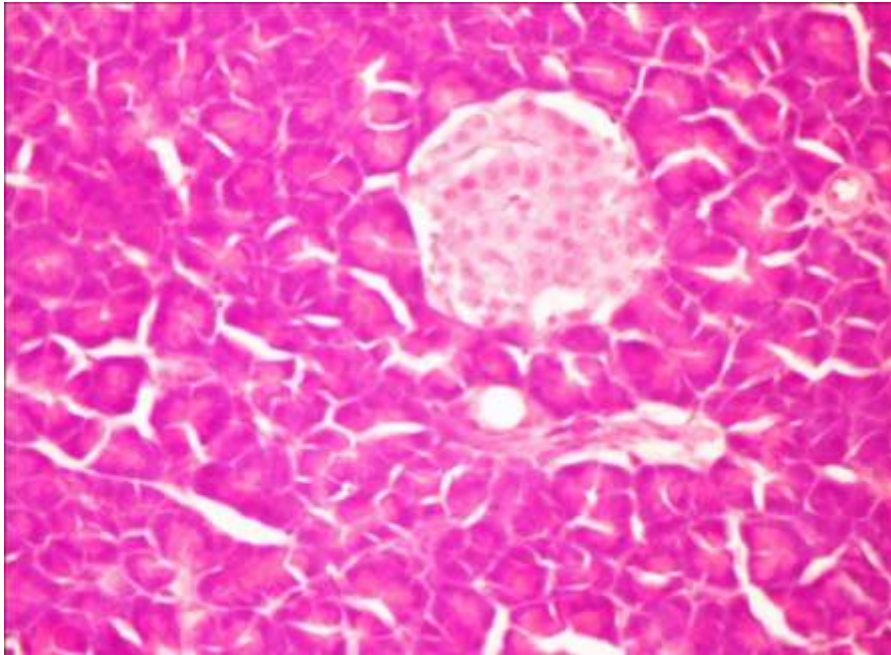
Figure 4.24. Pancreas of control negative (-ve) rats showing no histopathological change.
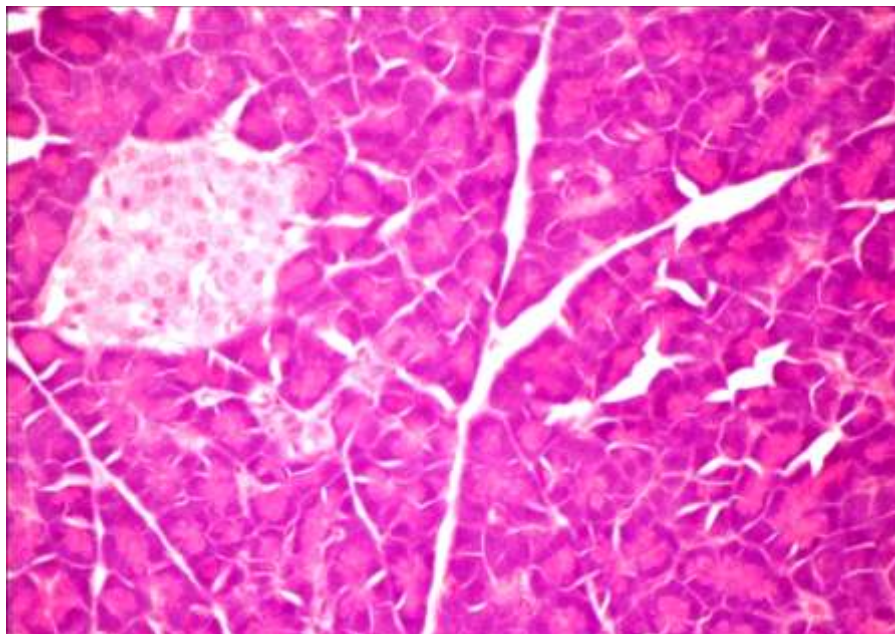
(H & E stain   x 400)

Figure 4.25. Pancreas of control negative rats showing no histopathological change.
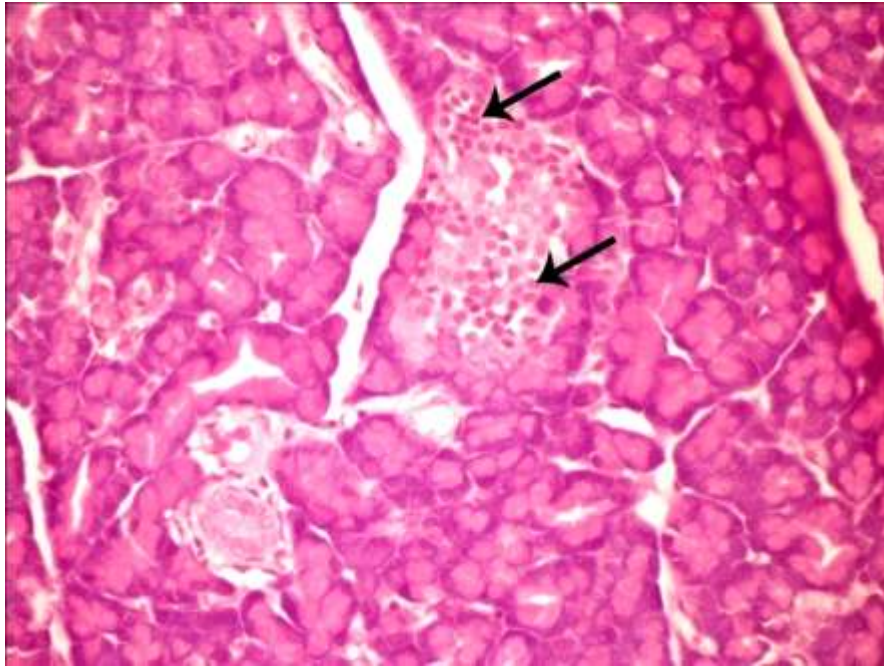
(H & E stain   x 400)

Figure 4.26. Pancreas of diabetic rats showing necrosis of *β*- cells of islets of langerhans (large arrow).
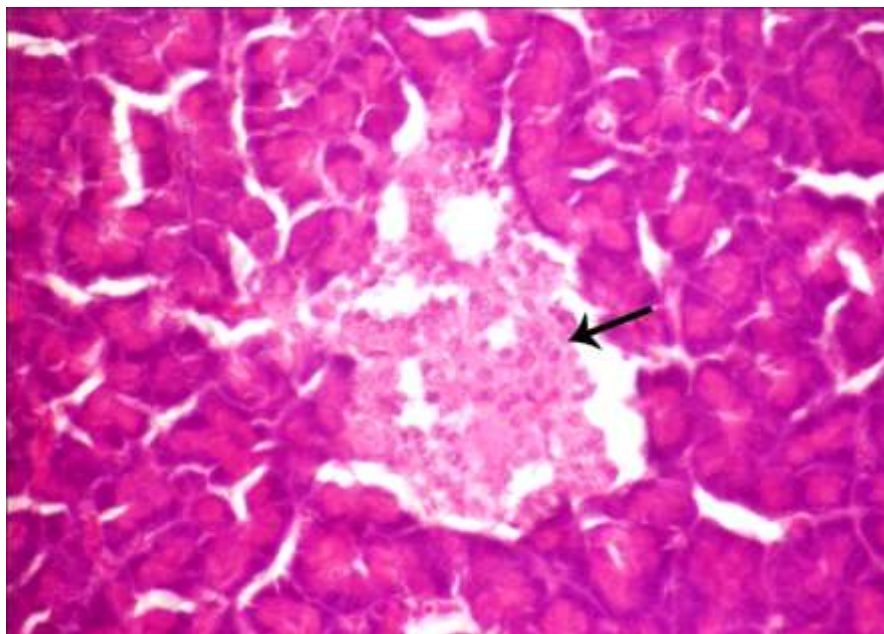
(H & E stain x 400)

Figure 4.27. Pancreas of diabetic rats showing necrosis of *β*- cells of islets of langerhans (large arrow).
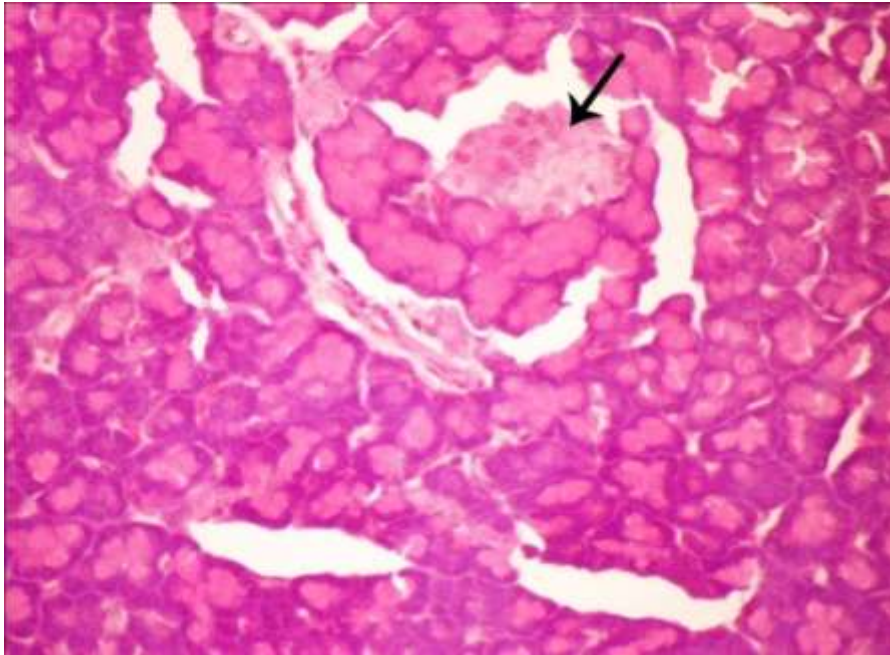
(H & E stain x 400)

Figure 4.28. Pancreas of diabetic rats showing necrosis and atrophy of islets of langerhans (large arrow).
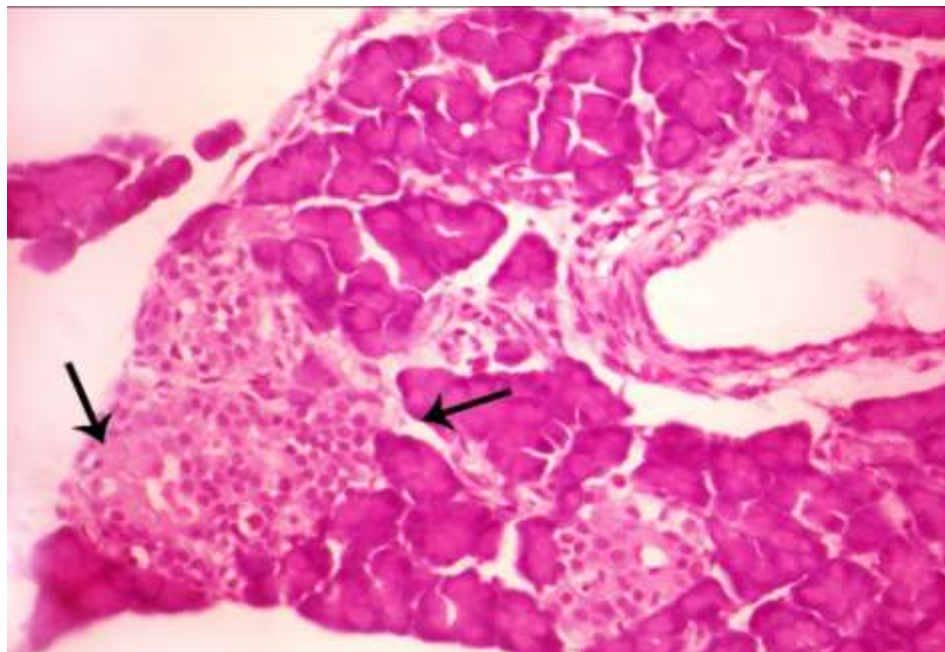
(H & E   stain x 400)

Figure 4.29. Pancreas of diabetic rats showing necrosis of *β*- cells of islets of langerhan's (large arrow).
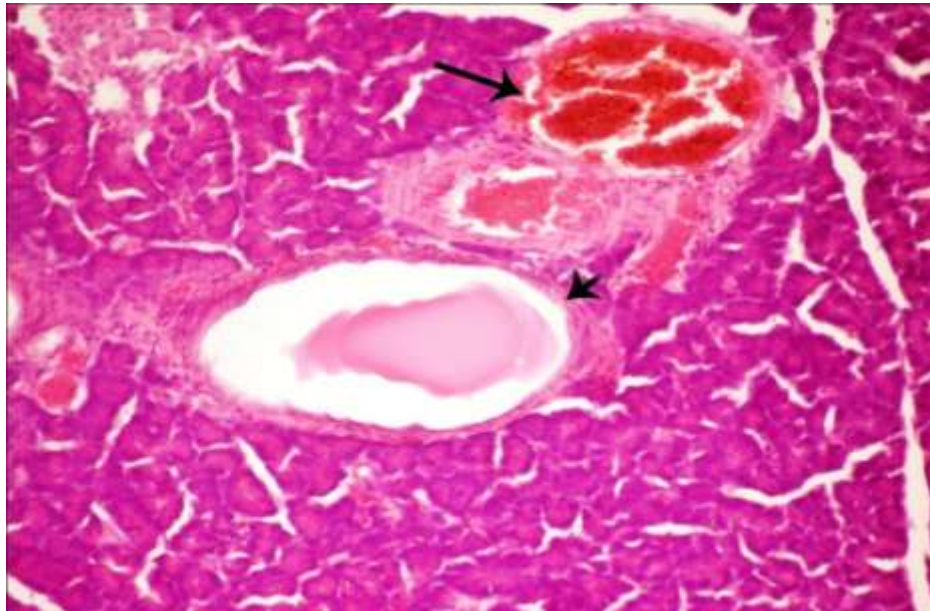
(H & E stain   x 400)

Figure 4.30. Pancreas of diabetic rats showing cystic dilatation of pancreatic duct (small arrow) and congestion of pancreatic blood vessel (large arrow).

(H & E stain x 400)
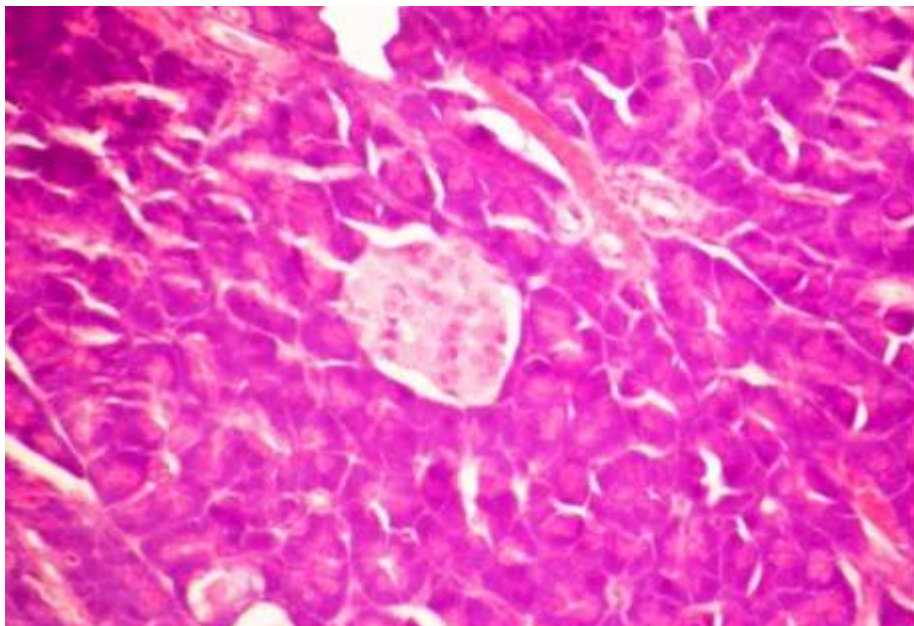
Figure 4.31. Pancreas of diabetic rats treated with curcumin showing no histopathological changes
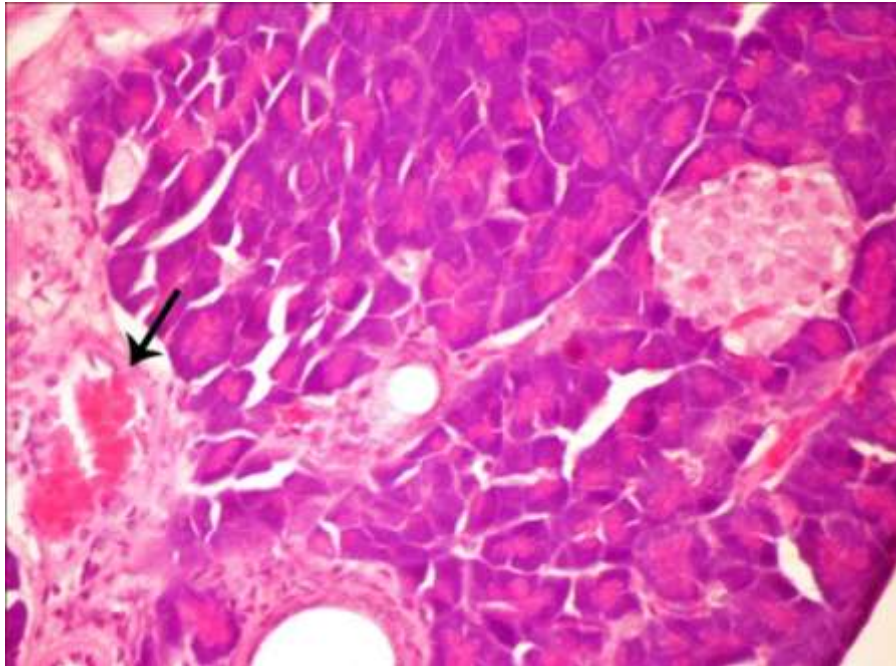
(H & E stain   x 400)

Figure 4.32. Pancreas of diabetic rats treated with curcumin showing slight congestion of blood vessel. Note normal pancreatic acini and normal *β* -cells of islet of langerhan's (large arrow).
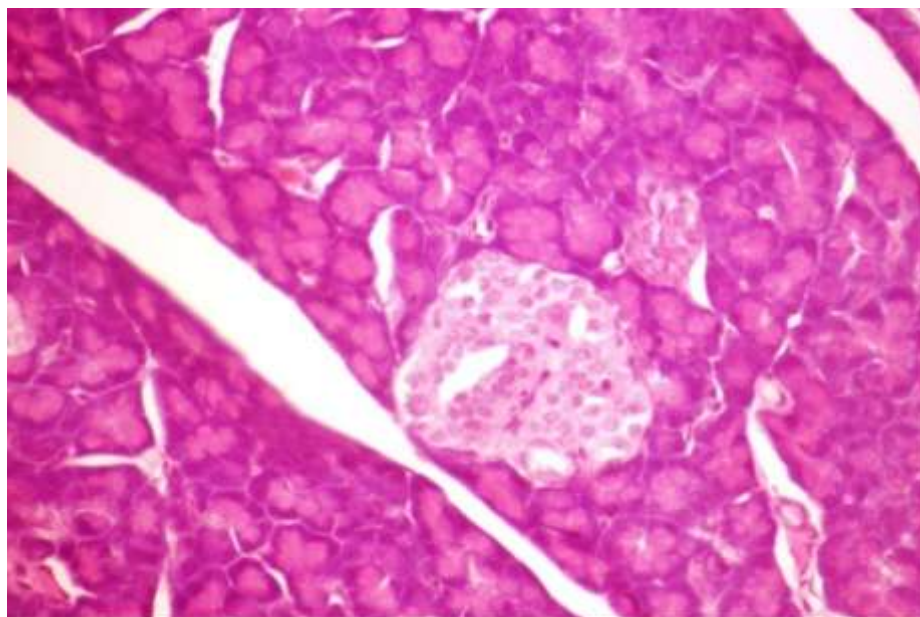
(H & E stain   x 400)

Figure 4.33. pancreas of diabetic rats treated with ginger showing no histopathological changes.
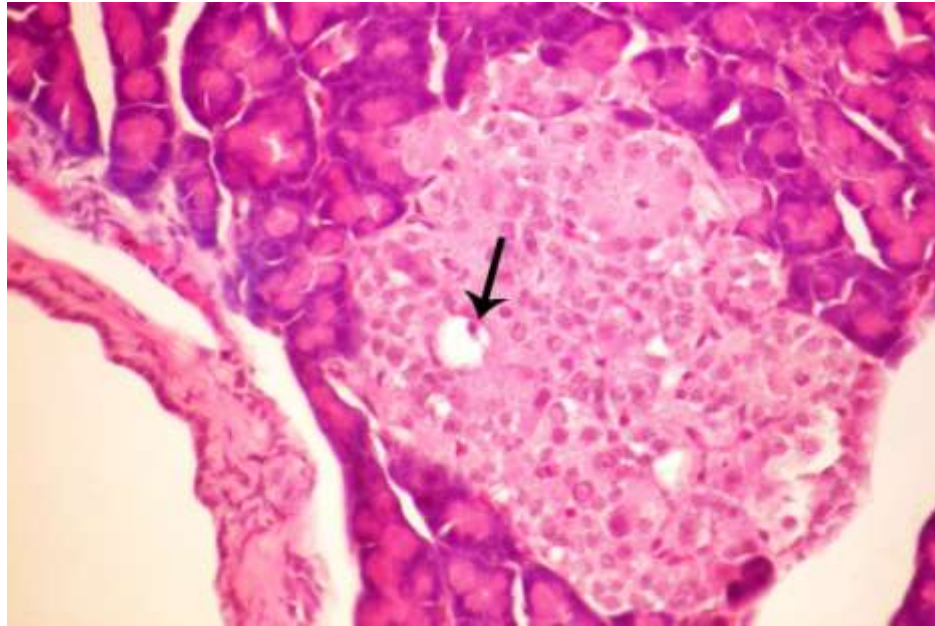
(H & E stain x 400)

Figure 4.34. Pancreas of diabetic rats treated with ginger showing slight vacuolation of sporadic *β*- cells of islets of langerhan's (large arrow).
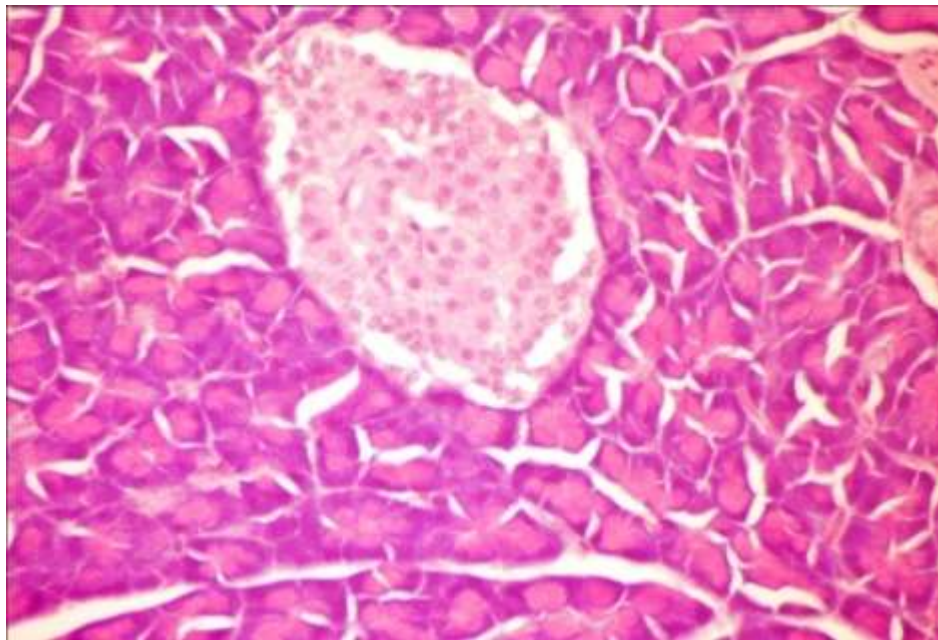
(H & E stain x 400)

Figure 4.35. Pancreas of diabetic rats treated with both curcumin and ginger showing no histological changes.
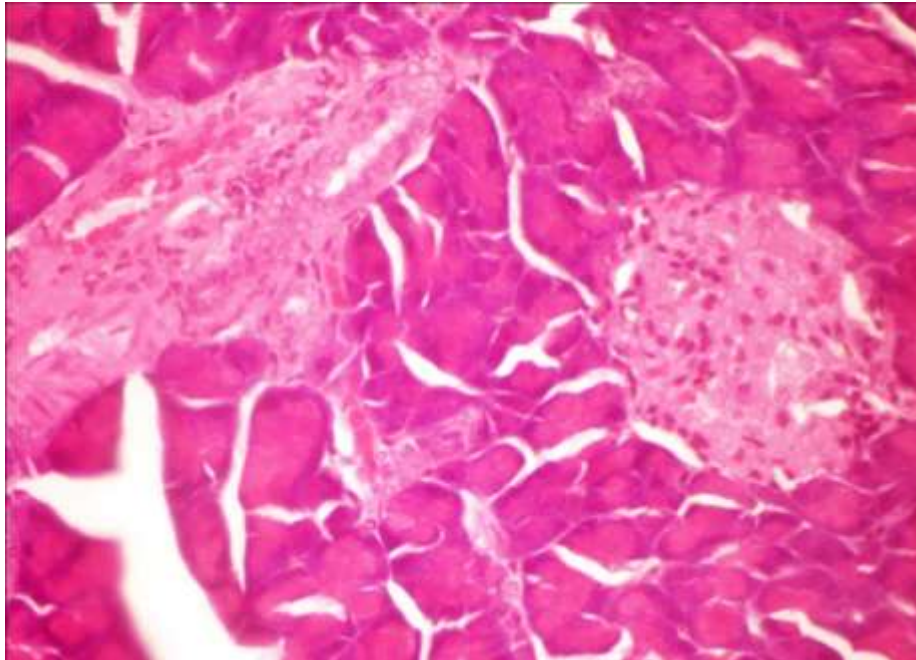
(H & E stain x 400)

Figure 4.36. Pancreas of diabetic rats treated with both curcumin and ginger showing no histological alterations.
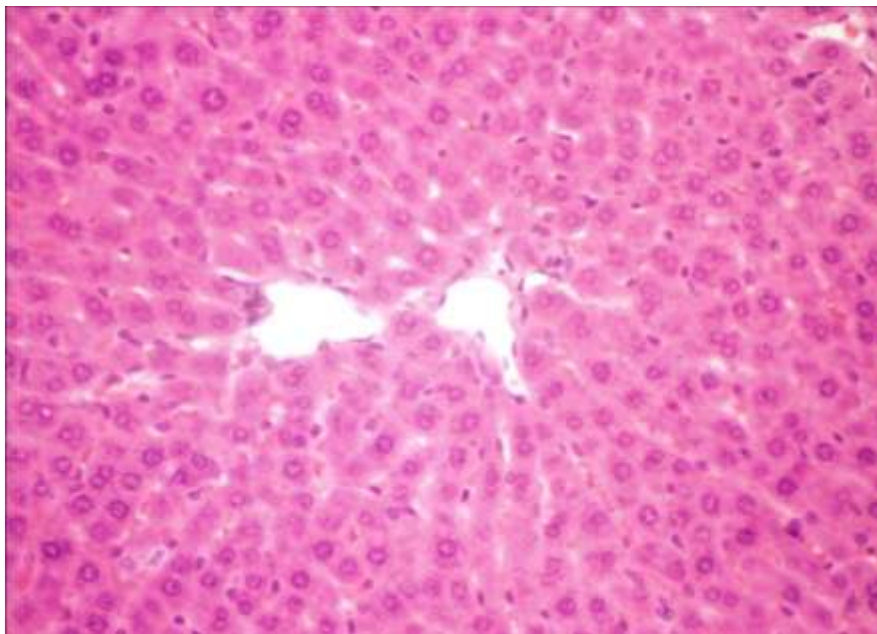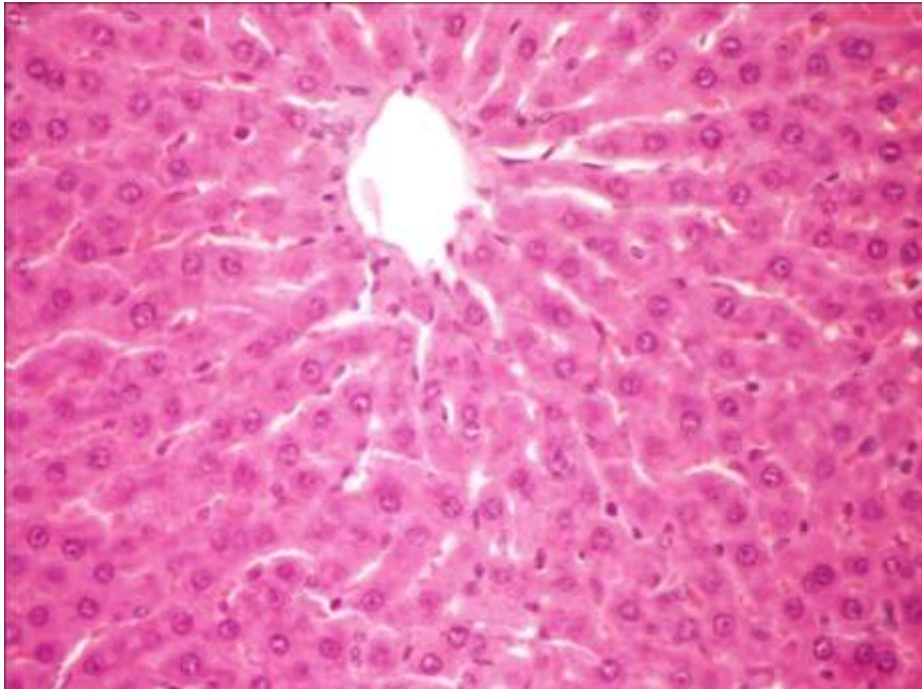
(H & E stain x 400)
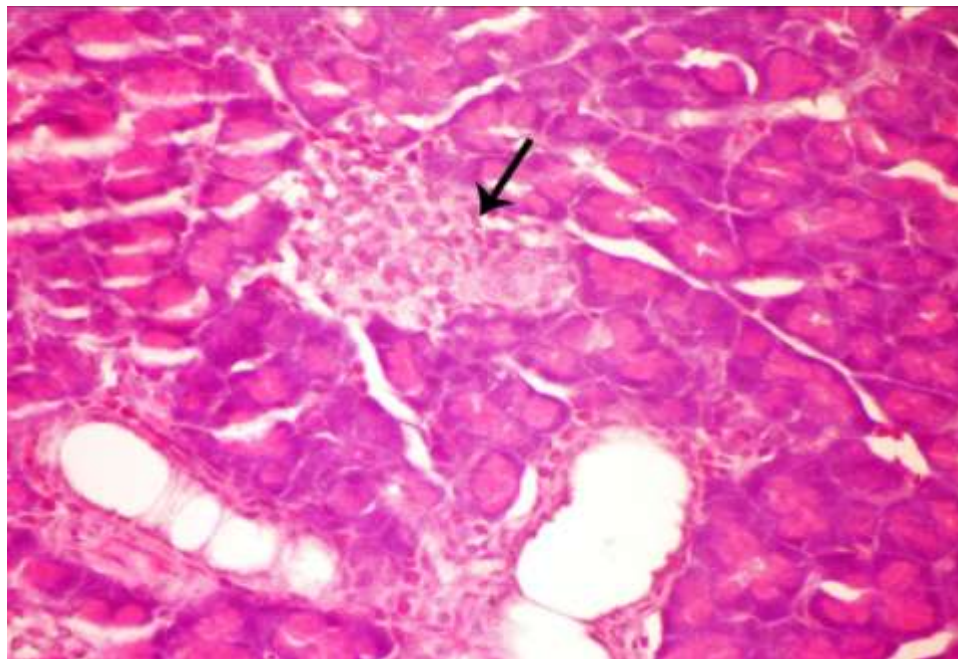
Figure 4.37. Pancreas of diabetic rats treated with both curcumin and ginger showing few leucocytic cells infiltration (large arrow).

(H & E stain x 400)

DISCUSSION

Chapter V

Discussion

Diabetes is recognized as one of the leading causes of morbidity and mortality in the world, while about 2.5 to 7% of the world's population has been diagnosed with diabetes mellitus, it is still expected to increase in future (**Abo *et al*., 2008**). In spite of the fact that synthetic drugs such as insulin-like substances are the most important therapeutic agents known to medicine, researchers have been making efforts to find insulin-like substances from plant sources for the treatment of diabetes (**Rafiq *et al*., 2009**). Recent scientific investigation and clinical studies had confirmed the efficacy of some medicinal plants and herbal preparations in the improvement of normal glucose homeostasis.

Herbal therapies have been used in patients with insulin-dependent and non-insulin-dependent diabetes (**Mukherjee *et al*., 2006**). The herbal drugs have been prescribed widely because of their effectiveness, fewer side effects and relatively low cost (**Venkatesh *et al*., 2003**). Several studies revealed the benefits of medical plants like curcumin or ginger which showed hypoglycemic effect and also delay in the development of DM (**Nammi *et al*., 2009, Sawatpanich *et al*., 2010 and El-Moselhy *et al*., 2011**).

In the current study, STZ- induced diabetic rats showed very highly significantly decrease in final body weight which also accompanied with increased on DFI and accordingly decreased in BWG% , as well as FER when compared with control group (-ve). The obtained results were in agreement with (**Suryanarayana et al., 2005 and Gupta et al., 2012**) who reported that, STZ-induced diabetic rats showed signs of loss weight compared with rats non-injected with STZ. Moreover, **Zafar and Naqvi (2010)** reported that STZ in a dose of 45 mg/kg induced significant reduction in the body weight of diabetic animals compared with non-diabetic. **Kota et al. (2012)** found that there were an association between hyperglycemia and decreased body weight of diabetic animals, DM induced reduction in body weight, and the body's inability to store or use glucose causes hunger and weight loss.

When insulin is deficient and the cells cannot metabolize glucose for energy, the cells compensate by increasing their metabolism of fats and proteins. Thus, the diabetic is usually thin, owing to the loss of fats and proteins from the body structure. The increased metabolism of fats releases into the blood large quantities of ketone bodies (e.g., acetone), which are intermediate products of fat breakdown, these excreted in the urine. Hyperinsulinic causes weakness, hunger, irritability and other symptoms of low blood glucose, also insulin shock can occur if blood glucose falls to a very low level (**Tharp and Woodman, 2008**).

Previous studies have reported that there was a decrease in body weight in diabetic rats treated with curcumin and increased in feed intake when compared with normal rats (**Soetikno et al., 2012a and Hussein and Abd El-Maksoud, 2013**), the same study also reported improvement in body weight and feed intake in diabetic

rats treated with curcumin compared with diabetic untreate rats. Our results agreement with the previous results and showed significantly decrease in body weight and increase in DFI and consequently decrease in BWG% and FER as compared with control (-ve) group, at the same time our results showed improvement in final body weight and DFI accompanied with increase in BWG% and FER when compared with untreated diabetic rats. The effect of curcumin treatment may be explained by its ability to inhibit angiogenesis in adipose tissue and decrease differentiation of preadipocytes (**Ejaz et al., 2009**). In addition, **Nasri et al.** (**2012**) and **Waer and Helmy (2012)** suggested that the gradual increase in the body weight was observed in the STZ diabetic rats treated with curcumin may be due to the retained levels of glucose and insulin levels because of the antioxidant effects of curcumin. Moreover, several studies have shown that curcumin control the leptin signaling in diabetic mice by reducing the phosphorylation levels of the leptin receptor and the induction of adiponectin, which improves body weight and related metabolic disease (**Tang et al., 2009 and Weisberg et al., 2009).**

In the experiment obtained results demonstrated that, ginger diabetic treated group showed significantly improvement and ameliorated reduction in final body weight, BWG%, FER and increased in DFI when compared with untreated diabetic rats. The present results in agree with the previous studies, which reported that STZ diabetic rats treated with ginger induced significant improvement in body weight and feed intake when compared with STZ diabetic untreated rats (**Ansari et al., 2008, Madkor et al., 2010 and Al-Aassaf, 2012**). Furthermore, STZ-induced diabetic rats treated with ginger in diverse doses of ginger reported that ginger is effective in reversing the weight loss observed in diabetic rats (**Abdulrazaq et al., 2011 and Asha et al., 2011)**, this is probably due to ginger contains over 20

phenolic compounds, which have been reported to display diverse biological activities such as antidiabetic, hypoglycemic and antioxidant **(White, 2007,Ali *et al.,* 2008, Islam and Choi, 2008 and Saraswat *et al.,* 2010).**

In the present study, STZ- induced diabetic rats showed very highly significantly increase in serum glucose concentration accompanied by a very highly significantly decrease in serum insulin level when compared with control group (-ve). A similar result was reported by **Yaghmoor and Khoja (2010)** who reported that STZ-induced diabetic rats in a dose of 60 mg/kg and had a negative effect in glucose concentration and insulin levels. Diabetes syndromes characterized by increased blood glucose, altered lipids, carbohydrate and an increased risk of diabetic complications and oxidative stress **(Davis, 2006 and Al-Assaf, 2012).**

In addition, **Kumar *et al.* (2012)** reported that rat injected with streptozotocin had been shown a marked raise in plasma glucose level and decrease in insulin level. The present results may be explained by streptozotocin action in *β*-cells is accompanied by characteristic alterations in blood insulin and glucose concentrations, two hours after injection, the hyperglycemia is observed with a concomitant drop in blood insulin, about six hours later, hypoglycemia occurs with high levels of blood insulin, and finally hyperglycemia develops and blood insulin levels decreas**.** This may be attributed to STZ (*N*-nitro derivative of glucosamine) is particularly toxic to the pancreatic, and injection with STZ leads to the degeneration of the Langerhans islets *β*- cells **(West *et al*., 1996**, **Ikebukuro *et al*., 2002 and Hayashi *et al*., 2006)**. Moreover, STZ induced destruction of *β*-cells of islets of Langerhans and causing degranulation and reduction of insulin secretion as proposed by **Zhang and Tan (2002)** and **Kavalali *et al.* (2002)**

In the current study, we observed that very highly significant increase in serum glucose concentrations while insulin level demonstrate very highly significant decreases in diabetic groups treated with curcumin, ginger or the both curcumin and ginger mixture as compared with control non-diabetic group (-ve). This result are conforming with the result of (**Na** *et al.,* **2011 and Soetikno** *et al.,* **2012a)** whose reported significant difference between STZ diabetic rats treated with curcumin at a dose level of 250 mg/kg for 7 weeks and 100 mg/kg for 8 weeks and non-diabetic control group. Similar results were reported by **Islam and Choi (2008)** and **Al-Assaf (2012)** who revealed significant difference between STZ-diabetic rats treated with ginger and normal rat.

On the other hand, treatment of diabetic rats with curcumin, ginger or their mixture exhibited remarkably ameliorated effect, there were very highly significant improvement in glucose concentration and insulin levels when compared with untreated diabetic group, and the mixture of both curcumin and ginger was more effective than curcumin and ginger alone, it exhibits remarkable glycemic control in diabetic group. This result agreement with **Seo** *et al. (***2008)** who revealed that curcumin improved homeostasis model assessment of insulin resistance and glucose tolerance, and elevated the plasma insulin level in db/db mice. Moreover, **Gupta** *et al***. (2012)** showed that curcumin revealed an anti-hyperglycemic effect and improved insulin sensitivity.

On the other hand, the present results were in conformity with the results of **(Islam and Choi, 2008, Jafri** *et al.,* **2010 and Al-Assaf, 2012)** who showed that improvement effect of ginger on glucose and insulin levels in STZ-induced diabetic rats when compared with untreated diabetic rats. **Saraswat** *et al***. (2010)** reported

that dietary ginger (3%) in the diet for 8 weeks in STZ- diabetic rat induced decrease in blood glucose levels while insulin was unaffected by ginger.

The hypoglycemic effect of curcumin may be attributed to curcumin supplementation ameliorates muscular insulin resistance by increasing the uptake and oxidative of fatty acids and glucose in skeletal muscles (**Na** *et al.***, 2011**). Moreover, **Best** *et al.* **(2007)** reported that curcumin induces electrical activity in rat pancreatic β-cells by activating volume-regulated anion channel, this effect led to depolarization of cell membrane potential, generation of electrical activity, and enhanced insulin release. Furthermore, curcumin protected islets against STZ-induced oxidative stress and corresponding islet damage and dysfunction by scavenging free radicals (**Pari and Murugan, 2007**). These results may in part explain the decrease serum glucose and increase insulin in diabetic rats fed curcumin in our study. Despite the positive results, some studies have shown conflicting results, where **Kowluru and Kanwar (2007)** reported similar results of blood glucose in diabetic rats and diabetic rats treated with dietary curcumin, and reported that curcumin did not prevent STZ-induced hyperglycemia. The underlying mechanisms by which curcumin can lower blood glucose is not fully defined (**El-Moselhy** *et al.***, 2011**).

In the present experiment, the hypoglycemic effect of dietary ginger were in conformity with the results of many studies, the hypoglycemic effect of ginger may be attributed to the bioactive and pharmacological compounds of ginger they may help in suppressing the free radical in diabetes,this will ultimately lead to decreased levels of blood glucose (**Ramudu** *et al.,* **2011a). Chakraborty** *et al.* *(***2012***)* revealed that ginger has been shown to modulate insulin release in rat pancreatic β-cells, thus enhanced plasma insulin levels in conjunction with lowered blood

glucose, this may be due to 6-gingerol, which is active component in ginger, which showed a protective effect on pancreatic *β*-cells and restored the plasma insulin level.

The key enzymes controlling carbohydrate metabolism associated with hyperglycaemia in diabetes are α-amylase and α-glucosidase, ginger extract showed the highest α-glucosidase and α- amylase inhibitory activities, the action of ginger extract against these two enzymes found to be correlated with the phenolic contents of gingerol and shogaol in these extracts (**Rani** *et al***., 2011**). In *vivo* studies on rats showed that after long-term (8 weeks) feeding with ginger, the activities of pancreatic lipase, amylase, trypsin, and chymotrypsin were significantly increased (**Platel and Srinivasan, 2000**).

In the present study, STZ- induced diabetic rats showed very highly significantly increase in serum TC, TG, LDL-C and VLDL-C levels accompanied by a very highly significantly decrease in serum HDL-C level when compared with control group (-ve). A similar result reported that STZ-induced diabetic rat in a dose of 30 mg/kg had a negative effect in lipid profile levels when compared with normal rats (**Kota** *et al***., 2012**). In addition, **Ramudu** *et al.* (**2011a**) found significant increase in TC, TG and phospholipids levels in STZ-induced diabetic rats against non-diabetic rats. These effect may be attributed to DM, which affects several lipid metabolism. Low insulin levels are associated with high levels of chylomicrons and very-low-density lipoprotein (VLDL-C) and lipoprotein lipase deficiency, which resulting in hypertriglyceridaemia (**Manley** *et al***., 2000, Holman, 2001 and Sacks** *et al.,* **2002**). Insulin affects many sites of mammalian lipid metabolism, it stimulates synthesis of fatty acids in liver, adipose tissues and in the intestine, insulin

deficiency has also been reported to increase the cholesterol synthesis and increase the activity of lipoprotein lipase in white adipose **(Suryawanshi *et al*., 2006)**.

While treatment of diabetic rats with curcumin, ginger or their mixture exhibited remarkably ameliorated effects, there were very highly significant improvement in TC, TG, LDL-C, VLDL-C and HDL-C levels when compared with untreated diabetic group, and the mixture of both curcumin and ginger was more effective than curcumin and ginger alone. These results are conforming to the result of **Rai *et al.* (2010)** who revealed that curcumin significantly lower TC, TG, LDL-C, VLDL-C levels and improved HDL-C level as compared with diabetic untreated rats. **Hussein and Abu-Zinadah (2010)** who stated that STZ-induced diabetic rats reported decreased in lipid profiles by oral administration of curcumin once daily for 7 weeks when compared with untreated diabetic rats. Moreover, the present results were in conformity with the results of **Madkor *et al.* (2010) and Al-Assaf (2012)** who showed improvement effect of ginger in TC and TG levels in STZ-induced diabetic rats when compared with untreated diabetic rats. **Ramudu *et al*. (2011a)** reported that oral administration of ginger for 30 days in STZ- diabetic rats induced decreased in TC and TG levels as compared with diabetic untreated group.

It has been proposed that curcumin and its metabolites function as peroxisome proliferator-activated receptor (PPARγ)-activating ligands, thus explaining their action as hypolipidemic agents **(Asai and Miyazawa, 2001).** In addition, there is substantial evidence to suggest that curcumin is effective in inhibiting lipid synthesis, storage, and stimulating fatty acids degradation. These effects mediated by regulating the activities of several key enzymes and the expression of transcription factors that regulate lipid metabolism **(Alappat and Awad, 2010)**. Curcumin hypolipidemic activities could be mediated through

cholesterol catabolism by the stimulation of hepatic cholesterol-7α-hydroxylase activity, and this step converts cholesterol to bile acid, which is important pathway in the degradation of cholesterol **(Jung *et al.*, 2006, Leelavinothan and Pidaran, 2007 and Wongeakin *et al.,* 2009)**, or might be due to its alkaloid components of curcumin **(Halim and Hussain, 2002)**.

In addition, numerous studies have indicated that curcumin reduces serum cholesterol concentrations by increasing the expression of hepatic low-density lipoprotein LDL receptors, blocks the oxidation of LDL, increased bile acid secretion and metabolic excretion of cholesterol, represses the expression of genes involved in cholesterol biosynthesis, and protects against liver injury and fibrogenesis in animal models **(Graham, 2009 and Shehzad *et al.,* 2011).**

The hypolipidemic activities of ginger may be explained by **Srinivasan and Sambaiah (1991)** who reported that, ginger stimulates the conversion of cholesterol to bile acids, an important pathway of elimination of cholesterol from the body. **Han *et al*. (2005)** found that *Z. officinale* increased the faecal excretion of cholesterol, suggesting that ginger may block absorption of cholesterol in the gut. Moreover, **Afshari *et al*. (2007)**, **Nammi *et al*. (2009)** and **Ramudu *et al*. (2011a)** mentioned that the hypocholesterolemic effect of ginger may be attributed to inhibition of cellular cholesterol synthesis, results in augmenting the LDL receptor activity, leading to the elimination of LDL from plasma thus modifying lipoprotein metabolism.

In the present study, STZ- induced diabetic rats showed very highly significant increase in MDA levels accompanied by a very highly significant decrease in serum SOD and CAT enzymes activity when compared with control

group (-ve). A similar results was reported by **(Morakinyo *et al*., 2011)** who reported that STZ- diabetic rats in a dose level of 45 mg/kg had a negative effect in MDA level and SOD and CAT enzyme activities, this may be attributed to increase in reactive oxygen species (ROS) which is involved in the development and progression of DM **(Suryawanshi *et al*.,2006 and Suryanarayana *et al*., 2007).** In addition, **Hussein and Abu-Zinadah (2010)** exhibited that rat injected with STZ has been shown a marked raise in MDA levels and decrease in SOD and CAT enzyme activities, these effect may be due to hyperglycemia increases oxidative stress through the over production of ROS, which is generally increased in diabetes and contribute to organ injury in systems such as the heart and liver. The toxic material produced by an activated phagocyte during reaction can cause maximal damage to the membrane because they are active in the lipid phase **(West, 2000).** The damaging effect of elevated toxic radical are due to an increase in the formation of superoxide radicals with in cells, which causes inactivation of antioxidant enzymes in hyperglycemic condition **(Suryawanshi *et al*., 2006).**

In the current study, the results reported that, there were very highly significant increase in MDA while SOD and CAT enzyme activities demonstrated very highly significant decrease in diabetic groups treated with curcumin, ginger or the both curcumin and ginger mixture as compared with control non-diabetic group (-ve). On another hand, treatment of diabetic rats with curcumin, ginger or their mixture exhibited remarkably ameliorated effects, there were very highly significant improvement in MDA, SOD and CAT enzyme activities levels when compared with untreated diabetic group, and the mixture of both curcumin and ginger was more effective than curcumin or ginger alone, it exhibits remarkable oxidative stress control in diabetic group. This results are conforming with the results of **(Hussein**

and **Abu-Zinadah, 2010 and Hussein and Abd El-Maksoud, 2013**) whose reported significant differences between diabetic rats treated with curcumin at a dose level of 50 mg/kg for 8 weeks and 80 mg/kg for 7 weeks and non-diabetic control group, meanwhile, curcumin revealed an improvement in TBARS and antioxidant enzyme activities in diabetic rats compared with untreated group. **Seo *et al.* (2008)** reported that curcumin normalizes erythrocyte and hepatic antioxidant enzyme activities (SOD, CAT and GP$_X$).

Moreover, the present results are in accordance with **Morakinyo *et al.* (2011)** and **Al-Assaf (2012)** who revealed significant difference between STZ-diabetic rats treated with ginger and normal rat, while there were improvement effect of ginger on lipid peroxidation and antioxidant activity against oxidative stress in STZ-induced diabetic rat when compared with untreated diabetic rats. **Ansari *et al*. (2008)** reported that dietary ginger (5%) in the diet for 6 weeks in STZ- diabetic rats induced improvment diabetes oxidative stress and its complications. In addition, **Shanmugam *et al.* (2011**) reported that in STZ-induced diabetic rats fed with a diet containing (1 - 2%) of ginger powder for 30 days, the blood glucose level was significantly reduced. Moreover, in diabetic rats treated with ginger, the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP*x*) and glutathionereductase (GR) decreased in hepatic and renal tissues.

Several studies have shown that consumption of nutrient-rich antioxidant such as curcumin and ginger decreased diabetic complications and improves the antioxidant system (**Shanmugam *et al.,* 2011 and Hussein and Abd El-Maksoud, 2013**). Curcumin activity as an antioxidant and free-radical scavenger has been demonstrated from several studies, it prevents the oxidation of hemoglobin and

inhibits lipid peroxidation, this activity can arise either from the phenolic hydroxyl group or the methylene group of the β-diketone (heptadiene-dione) moiety **(Ligeret** *et al***., 2004, Suzuki** *et al***., 2005, Chen** *et al***., 2006, Anand** *et al***., 2008 and Gupta** *et al***., 2012).** Curcumin also reduces oxidative stress by reducing peroxide formation and increasing antioxidant enzyme activities **(Alappat and Awad, 2010)**. In addition, **Arun and Nalini (2002)** reported that curcumin lowers blood glucose in diabetic rats therefore, the nicotinamide adenine dinucleotide phosphate NADPH/ NADP ratio goes up resulting in increased activity of glutathione reductase, which in turn elevates the availability of GSH, the substrate for GPx, so the activity of GPx increases which in turn scavenges H_2O_2.

Treatment of diabetic rats with ginger attenuated the production of free radicals and peroxidation of lipids thereby preventing oxidative damage of cellular structures **(Morakinyo** *et al.***, 2011)**. Further, ginger has an ability to increase the intracellular activities of SOD, CAT and GSH enzymes and has synergistically combats oxidative stress by scavenging free radicals and/or augmenting endogenous antioxidant activities **(Shobana and Akhilender, 2000)**. This may be due to ginger contain many antioxidant compounds that may modulate the antioxidant enzymes in diabetic rats, especially gingerol and hyxahydrocurcumin which are responsible for significant inhibition of lipid peroxidation. In addition, the increased in activities of antioxidant enzymes may act as an added compensation mechanism to maintain the cell integrity and protection against free radicals damage **(Manju and Nalini, 2005 and Young** *et al.,* **2005)**. Preliminary clinical trials showed that the antioxidant effect of ginger was potent when it was used in combination with some herbs, significantly physiological improvement accompanied with reduction of serum

triglyceride and cholesterol in diabetic and hyperlipidemic patients have been recorded **(Kamal and Aleem, 2009 and Ugwuja *et al.,* 2010).**

In the current study, the results showed that, liver tissues in STZ-induced diabetic rats showing activation of kupffer cells, apoptosis of hepatocytes, marked dilatation and congestion of central vein with necrosis of sporadic hepatocytes , as well as congestion of central vein and focal hepatic necrosis replaced by mononuclear infiltration when compared with control (-ve) group. A similar result reported by **Hussein and Abu-Zinadah (2010)** who reported that, liver sections of STZ diabetic rats in dose of 65 mg/kg showed massive fatty changes, necrosis and broad infiltration of lymphocytes. Moreover, **Hashemnia *et al.* (2012)** reported that liver sections of untreated diabetic rats showed degenerative changes in the hepatocytes represented by disorganization of the hepatic cords, congestion of the central veins with mild hepatocellular necrosis and the sinusoids were infiltrated by mild nonspecific inflammatory cells, and the hepatocytes showed pyknosis, karyorrehexis, chromatolysis and cytoplasmic vacuolization. The damage effect of STZ could be attributed to the increased production of highly reactive intermediates of STZ, which are normally detoxified by endogenous GSH, but when present in excess can deplete GSH stores, allowing the reactive intermediate to react with and destroy hepatic cells **(Blum and Fridovich, 1985).** Moreover, STZ stimulates H_2O_2 generation, which cause DNA fragmentation and increase oxidative stress in liver and pancreas cells **(Bolkent *et al.,* 2008 and Nirmala *et al.,* 2009).**

In the present study, liver tissues in diabetic rat treated with curcumin showed apparent normal histological structure, except slight kupffer cells activation, as well as liver tissues in diabetic rats treated with ginger showed kupffer cells

activation, slight congestion of hepatic sinusoids with binucleation of hepatocytes. Meanwhile, in diabetic rats treated with both curcumin and ginger examined liver sections showed no histopathological alteration except kupffer cells activation in few sections.The present findings confirmed by **Murugan and Pari (2006)** who showed that curcumin improve the liver pathological changes and reduced the congestion and inflammation when compared with diabetic untreated rats. In addition, **Hussein and Abu-Zinadah (2010)** reported that the histological architecture of liver sections of rats treated with curcumin showed more normal patterns, with a mild degree of fatty change, necrosis and lymphocyte infiltration, almost comparable to those of control group. In addition, curcumin found to prevent liver lipid peroxidation in rats with STZ-induced diabetes and showed graduate restoration of hepatocytes and blood sinusoids, also most of the hepatocytes showed normal and regular pattern **(Soetikno *et al*., 2012b and Waer and Helmy, 2012)**.

The protective effect of curcumin against oxidative damage may be attributed to, curcumin is an unique antioxidant, which contains a variety of functional groups, including the β-diketo group, carbon–carbon double bonds, and phenyl rings containing varying amounts of hydroxyl and methoxy substituents, it contain also some alkaloid bases and phytosterols **(Halim and Hussain, 2002 and Wright, 2002).** The effect of curcumin is most likely mediated through its ability to inhibit cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS) **(Venugopal and Adluri, 2007)**, it prevented the activation of NF-$_K$B (nuclear factor kappa-light-chain-enhancer of activated B cells) in rats **(Nanji *et al.,* 2003)**, thus explained its ability to inhibit lipid peroxidation and liver injury**.**

Ramudu *et al.* **(2011b)** reported that ginger protected the liver tissues from STZ-induced oxidative damage, the results showed regenerated hepatocyctesin in diabetic rats treated with ginger. Moreover, treatment with 1 % of dietary ginger for 4 weeks showed improvementin the antioxidant status and have protective role in hepatic tissues **(Mallikarjuna et al., 2008).** The same results reported by **(Abd-El Aty and Morgan, 2011)**. The hepatoprotective activity of ginger extract may be due to its direct radical scavenging activity **(Ajith et al., 2007).**

In the present study, it was reported that microscopic examination of the pancreatic sections of diabetic untreated group revealed necrosis and atrophy of β-cells of islets of Langerhan's, as well as cystic dilatation of pancreatic duct and congestion of pancreatic blood vessels. This results are in accordance with the findings of **Gandhi and Sasikumar (2012)** and **Kulkarni (2012)** who reported that, STZ diabetic rats showed a significantly reduced in mean β-cells number. Moreover, the β-cells were smaller in size and markedly degenerated with necrosis of pancreatic islets. More evidence was provided by **Qadori (2011)** who reported that diabetic pancreatic tissue showed shrinkage of islets Langerhans in size, signs of necrosis of β-cells destruction and reduction in number of islets, and significant reduction was in β-cells diameter at the 2nd and the 4th weeks after injected with STZ.

Many studies found that in diabetic mice induced by STZ, the DNA in β-cells was damaged and subsequent induced inhibition of insulin biosynthesis and secretion **(Panchatcharam et al., 2006 and Kanitkar et al., 2008).** This effect may be explained by STZ stimulates H_2O_2 generationin pancreatic β -cells which causes DNA fragmentation. It was demonstrated that reducing sugars trigger oxidative modification and apoptosis in pancreatic β-cells by provoking oxidative stress

through glycation reaction (**Kaneto *et al*., 1996**). In addition, DM cause disturbs and imbalance between oxygen frees radicals (OFR$_S$) production and cellular defense mechanisms, this imbalance can result in cell dysfunction and destruction in pancreas tissues (**Kakkar *et al*., 1998 and Waer and Helmy, 2012**).

In the present study, the examined sections of pancreatic of diabetic rats treated with curcumin showed no histopathological changes, and other sections revealed slight congestion of blood vessel with normal pancreatic acini and normal *β*- cells of islet of Langerhan's. Also some examined sections of diabetic rats treated with ginger showed no histopathological changes, while other sections revealed slight vacuolation of sporadic *β*-cells of islets of Langerhan's. On the other hand, examined pancreatic islet tissues in diabetic rats treated with both curcumin and ginger revealed no histological changes, except few leucocytic cells infiltration in some sections.

Clear evidence of pancreatic islets growth to curcumin treatment respond in diabetic mice showed that curcumin might promote hormone or growth factors for pancreatic islets in diabetic mice neogenesis (**Sharma *et al*., 2006 and Weber *et al*., 2006**). **Best *et al*. (2007)** showed that curcumin induced electrical activity in rats pancreatic *β*-cells by activating the volume-regulated anion channel and have indicated that activation is the result of increased channel open probability, this effect were accompanied by potential depolarization of the cell membrane, the generation of electrical activity and enhanced insulin release. Moreover, curcumin retarded islet ROS (Reactive oxygen species) generation and inhibited apoptosis, indicating that it protects islets against STZ-induced oxidative stress by scavenging free radicals (**Meghana *et al*., 2007 and Waer and Helmy, 2012**).

Curcumin also decreased β-cells volume, presumably reflecting loss of Cl($-$), and hence water, as a result of anion channel activation. These findings are consistent with the suggestion that Cl(-) fluxes play an important role in regulating β-cells function, the stimulation of β-cells function by curcumin might contribute to the hypoglycemic actions of this compound **(Best *et al.*, 2007)**. Additionally, curcumin was found to induce heme oxygenase-1 expression, which has been reported to have cytoprotective effects in mouse pancreatic β-cells **(Pugazhenthi *et al.*, 2007).**

Ginger is a good source of antioxidant and therefore may be capable of preventing tissue damage by ROS, it can protect the liver and pancreas tissues from lipid peroxidation on STZ diabetic rats **(Usha and Saroja, 2000 and Bhandari *et al*., 2005).** The present results also agree with **Aggarwal (2010)** and **Chakraborty *et al*. (2012)** who revealed that ginger has been shown to modulate insulin release in rats pancreatic β-cells, the effect of ginger may be explained by 6-gingerol, which is active component in ginger, it showed a protective effect on pancreatic β-cells, inhibit and intervene cytodegeneration of pancreatic β-cells and hepatocytes and helped in scavenging the free radicals.

CONCLUSION

AND

RECOMMENATIONS

Chapter VI

Conclusion and Recommendations

This study demonstrated that the combination of both curcumin and ginger possesses significantly   reduction in hyperglycemic, hyperlipidemic and antioxidant effect in diabetic rats, as well as overcome most of the histopathology changes in liver and pancreas tissues , the majority of the cells tend to be normal. Therefore, it recommended that dietary curcumin and ginger or their mixture could be excellent adjuvant support in the therapy of DM and prevent its complications. Further research should be done to investigate the active components of curcumin and ginger and identify the type of phenolic compounds responsible for their hypoglycemic, hypolipidemic and antioxidant effects.

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ARABIC SUMMARY

تأثير الزنجبيل والكركم وخليطهما على سكر ودهون الدم للفئران المصابة بالسكر

العنود بنت عبد العزيز الفالح

الملخص

داء السكري هو حالة مزمنة تنتج عن نقص جزئي أو كلي في هرمون الأنسولين؛ مما يؤدي إلى ارتفاع نسبة السكر بالدم، و يعد من الأمراض كثيرة الانتشار في العالم، و نسبة المصابين به في تزايد مستمر. في المملكة العربية السعودية وجد أن نسبة الإصابة بداء السكري أصبحت في تزايد ملحوظ خلال العقدين الماضيين؛ نتيجة التغيير في السلوك الغذائي و نمط الحياة. ولذا تعد السيطرة على داء السكري و مضاعفاته عبئاً عالمياً يتطلب جهوداً مضاعفة للسيطرة عليه. تعد النباتات الطبية التقليدية بديلاً علاجياً مساعداً في السيطرة على داء السكري، وقد اتضح أن الكركم و الزنجبيل من النباتات التي أظهرت تأثيراً ايجابياً في السيطرة على داء السكري . إن الكركم (diferuloylmethane) من المواد الفعالة كمضاد للأكسدة، خافض للسكر ومضاعفاته، و خافض لمستويات الدهون في مصل الدم. كما ذكرت الدراسات أن الزنجبيل له فوائد علاجية عديدة من ضمنها خفض مستوى السكر، المساعدة في تحفيز إفراز الأنسولين خفض مستويات الدهون في الدم كما له تأثير مضاد للاكسدة .

تهدف هذه الدراسة إلى تقييم تأثير الكركم و الزنجبيل و خليطهما على مستوى جلوكوز وصورة لبيدات الدم، بالإضافة إلى مستوى الدهون فوق المؤكسدة و نشاط الإنزيمات المضادة للأكسدة في الفئران التي تم حقنها بمادة الإستريبتوزوتوسين المُحدِث للسكر. قسمت الفئران في

هذه التجربة (عددها 35 فأراً) من ذكور الالبينو، تتراوح أوزانها ما بين (180-195 جم) وبعد فترة التأقلم إلى مجموعتين رئيسيتين، المجموعة الأولى : المجموعة الضابطة السالبة (عددها =7) و تم تغذيتها على حمية قياسية، المجموعة الثانية : المجموعة المصابة بالسكري (عددها=28) ، والتي تم تقسيمها إلى 4 مجموعات فرعية: المجموعة الأولى ضابطة موجبة مصابة بالسكري (غير المعالجة) تم تغذيتها بحمية قياسية، المجموعة الثانية مصابة بالسكري معالجة بالكركم بنسبة 0,5% من محتوى الحمية القياسية، المجموعة الثالثة مصابة بالسكري معالجة بالزنجبيل بنسبة 3% من محتوى الحمية القياسية، و المجموعة الرابعة مصابة بالسكري معالجة بمزيج الكركم و الزنجبيل بنسبة 0,5% و 3% على التوالي من محتوى الحمية القياسية. تم إحداث مرض السكري من خلال حقن الغشاء البريتوني (الغشاء المبطن لتجويف البطن) بمادة الإستريبتوزوتوسين (بجرعة مقدارها 65 ملجم/كجم من وزن الجسم). الكركم المستخدم في التجربة تم الحصول عليه في صورة بودرة (يحتوي 95% مادة Currcuminoids)، بينما الزنجبيل المستخدم تم تجفيد جذوره بجهاز التجفيد، و من ثم طحنها (الناتج 13,6%) ثم تم تخزينها على درجة حرارة - 20 ºم حتى مرحلة الاستخدام.

أثناء التجربة تم تسجيل المتناول الغذائي اليومي لكل مجموعة من الفئران، وكذلك قياس أوزان الفئران مرتين أسبوعيا لكل المجموعات. تم تقييم القيمة الحيوية للمجموعات المختلفة من خلال حساب نسبة الزيادة في الوزن، ومعدل الاستفادة من الغذاء. في نهاية التجربة بعد مضي 8 أسابيع، تم ذبح الفئران بعد صيام ليلة كاملة . تم سحب عينات الدم من العين لكل فأر تحت تأثير التخدير باستخدام مادة الداي أيثيل ايثر. تم وضع عينات الدم مباشرة في جهاز الطرد المركزي لفصل مصل الدم، ومن ثم تخزينه على درجة -20ºم لحين استخدامه للاختبارات البيوكيميائية . تم استئصال الكبد و البنكرياس بعد عملية الذبح من كل فأر في المجموعات المختلفة، حيث تم وضعها في محلول الفورمالين (10 %) لفحصها هستولوجيا .

تم قياس تركيز الجلوكوز، مستوى الأنسولين، الكوليستيرول الكلي، الجليسريدات الثلاثية، الكوليسترول ذو الليبوبروتينات عالية الكثافة في مصل الدم ، ثم تم حسابيا تقدير مستوى الكوليسترول ذي الليبوبروتينات منخفضة الكثافة، و الكوليسترول ذو الليبوبروتينات منخفضة الكثافة جدا، و أيضا تم قياس مستوى نشاط الإنزيمات المضادة للأكسدة (السوبر أوكسيد ديسموتيز و الكتاليز) و الدهون فوق المؤكسدة (المالونداي الدهيد).

أوضحت النتائج أن الفئران المصابة بالسكري المُحدث بواسطة الإستريبتوزوتوثين أظهرت ارتفاعاً ذا دلالة معنوية بدرجة عالية جداً ($p<0.001$) في مستوى الجلوكوز، اللبيدات ، الدهون فوق المؤكسدة ، و هو مرتبط بحدوث انخفاض ذي دلالة معنوية بدرجة عالية جداً ($p<0.001$) في الكوليسترول ذي الليبوبروتينات عالية الكثافة، نشاط الإنزيمات المضادة للأكسدة ومستوى هرمون الأنسولين عند مقارنته بالمجموعة الضابطة السالبة. وقد أظهر العلاج بالكركم و الزنجبيل و مزيجهما تحسناً ذا دلالة معنوية بدرجة عالية جداً ($p<0.001$) في القياسات البيولوجية، مستوي الجلوكوز، الأنسولين، اللبيدات، الدهون فوق المؤكسدة، و نشاط الأنزيمات المضادة للأكسدة عند مقارنتها بالمجموعة المصابة بالسكري غير المعالجة. لوحظ أن مزيج الكركم و الزنجبيل أظهر فعالية عالية في معالجة مجموعة السكري عند مقارنتها بالمجموعات المعالجة الأخرى .

أوضحت الدراسات الهستوباثولوجية لأنسجة الكبد والبنكرياس عند الفئران المصابة بمرض السكري غير المعالجة ظهور تغيرات سلبية على الأنسجة ، بينما أدت المعالجة إلى التغلب على هذه الأعراض السابقة؛ حيث إن أغلب الخلايا أصبحت أقرب إلى الحالة السليمة، وهذا التحسن ربما يفسر التأثير المضاد لمرض السكري، وخاصة لمزيح الكركم والزنجبيل تحت الدراسة. و لذا تبين من هذه الدراسة أن خليط الكركم و الزنجبيل يمتلك القدرة على خفض مستويات الجلوكوز وتحسين مستوى الليبيدات في الدم، بالإضافة إلى دوره المضاد للأكسدة في

الفئران المصابة بالسكري. و لذلك فإنه يوصى باستخدام خليط الكركم و الزنجبيل للتخفيف من

الجهد التأكسدي الناجم عن السكري. كما يلزم عمل المزيد من الأبحاث لمعرفة الآلية العلاجية

للكركم و الزنجبيل، ولتحديد المركبات الفعالة المسؤولة عن الدور الإيجابي الفعال تجاه البول

السكري والجهد التأكسدي.
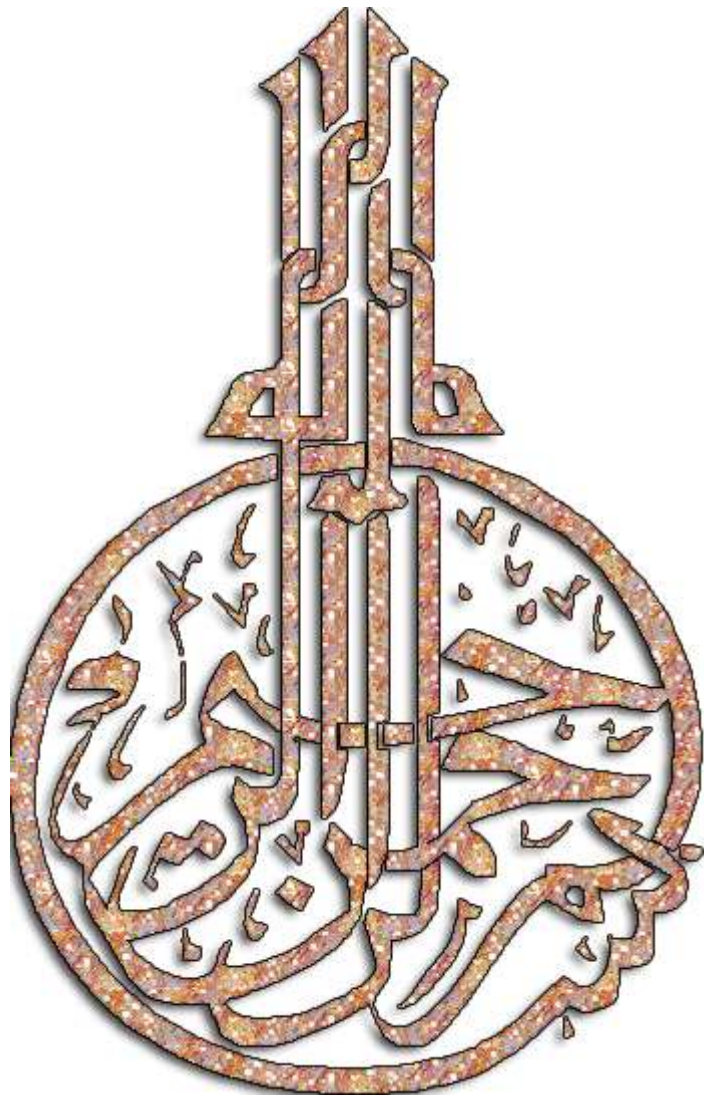
تأثير الزنجبيل والكركم وخليطهما على سكر ودهون الدم للفئران المصابة بالسكر

إعداد

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بإشراف

د.هاله عبد الرحمن حسن خطاب

د.نادية صالح عبود العمودي