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Effect of rosemary and ginger infusion on some hematological and biochemical criteria in diabetic rabbits.

Thesis

Submitted to the Council of the College of Veterinary Medicine at University of Karbala as a Partial Fulfillment of The Requirement for the Degree of Master in the sciences of Veterinary Medicine/ physiology.

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا

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سورة الاسراء الآية ﴿٨٥﴾

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I certify that this thesis **(Effect of rosemary and ginger infusion on some hematological and biochemical criteria in diabetic rabbits)** has been prepared by ***Noor Ali Abdul Imams*** under my supervision at the college of Veterinary Medicine, University of Kerbala in partial fulfillment of the requirements for the Degree of Master in the Sciences of Veterinary Medicine in Veterinary Physiology, Biochemistry and Pharmacology.

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Declaration

I hereby declare that this thesis is my origin work except for equations and citations which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Kerbala or other institutions.

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/ / 2022

Dedication

To the messenger of mercy, the Prophet Muhammad “Allah

blessing and peace be upon him and his pure family” and

To infallible Imams "peace be upon them "…

In my homeland, Iraq, which is bleeding martyrs…

He was Snedy, and he stood with me at all stages of my studies in order to reach this level... My father.

To our hope in this life. You are the candle that lights my way... my mother.

To My sisters &brothers, you are the bright moons, you are a blessing from the sky. There are no words to express my thanks to them…

(Huda , Hussein, Batool, Ahmead ,Fatima).

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Noor

Abstract:

The purpose of this study is to evaluate the therapeutic effect of rosemary, ginger infusion and their combination in rabbits experimenting with diabetes mellitus. Fifty healthy mixed-sex rabbits, ten rabbits control,forty rabbits induction of diabetes mellitus by alloxan and ensure the occurrence of diabetes mellitus and randomly distributed and raised over 4 weeks.(GI)controls negative administration orally of distilled water. (GII) positive control administered alloxan. At the dose of the marginal ear vein (100 mg kg.bw), single injection. (GIII) administered rosemary infusion orally daily in one dose (200mg/kg. Bow) in diabetic rabbits alloxan diabetic rabbits. (GIV) administration of ginger infusion orally daily on a dose basis (250 mg/kg body weight) in alloxan diabetic rabbits. (GV) administers rosemary infusion orally daily in one dose (200mg/kg. bw) (2ml) and ginger infusion orally daily on a dose basis (250 mg/kg body weight) (2.5ml) in alloxan diabetic rabbits.

At the end of the study blood was collected to assess blood sugar, HB1AC, Hematological parameters (RBC, PCV, HB, platelets and WBC), serum to evaluate the liver enzyme parameters (AST and ALT), lipid profile (cholesterol, triglycerides and HDL) and anti-oxidant parameters (GSH and MDA). The pancreas, liver and kidneys were isolated as part of the histopahological study.

The results showed a significant increase ($p \leq 0.05$) in glucose, HB1AC, liver enzymes (AST&ALT), albumin, lipid profile (cholesterol and triglycerides) and antioxidants (MDAs) significant decrease (HDL&GSH) in G(II). In G (III، IV, V) Showed significant increase ($p \leq 0.05$) in hematological parameters, albumin, GSH, Decreased glucose, HB1AC, hepatic enzymes, lipid profile and (MDA) to the control group in the G(V). No important difference of (HB) between the control group and the other treated groups.

Histological changes in pancreas, liver and kidney in diabetic rabbits showed congestion, inflammation, necrotic changes while in alloxan induced diabetes treated with rosemary and ginger infusion showed improved pathological abnormalities and reduce the immigration of inflammatory cells. In diabetic rabbits treated with two

herbal infusion showed normalized the histological structures and improvement of these organs.

In Conclusions Administration of the alloxan in rabbits causes decrease in hematological parameters, albumin, GSH, increase in glucose, HB1AC, hepatic enzyme ,lipid profile and MDA.Administration of rosemary , ginger infusion and their combination in alloxan diabetic rabbits causes an increase in hematological parameters, albumin, GSH, Decreased glucose, HB1AC, hepatic enzymes, lipid profile and MDA.

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List of Abbreviations

Abbreviation	Meaning
RBC	Red blood cells
HB	Hemoglobulin
PCV	Packed cell volume
WBC	White blood cell
Plt	Platelet
µmol/L	Micromole per litter
H&E	Hematoxylin and Eosin stain
mg/dL	Milligrams per deciliter
ROS	Reactive oxygen species
mmol/L	Millimoles per litter
µl	Microliter
Ml	Milliliter
EDTA-tube	Ethylene Diamin Tetraacetic Acid
ALT	Alanine aminotransferase
AST	Aspartate Aminotransferase
TC	Total cholesterol
TG	Triglyceride
HDL	High density lipoprotein
MAD	Malondialdehyde
GSH	Glutathione
DM	Diabetes mellitus
LADA	Latent autoimmune diabetes of adulthood
NIDD	Non-dependent diabetes mellitus
NIH	National institues of health
GAD	Glutamic acid decarboxylase
T1DM	Type one diabetes mellitus
T2DM	Type two diabetes mellitus
ICA	Islet cell antibodies
ROS	Reactive oxygen species
GIT	Gastrointestinal tract

ACCORD	Action to control cardiovascular risk in diabetes
ALX	Alloxan
CVD	Cardovasculardiseases
RE	Rosemary extract
Z.officinale	Zingiber officinale
R.officinale	Rosemary officinale
ETC	Electrone transport chain
FBG	Fasting blood glucose
HB1AC	Hemoglobulin AC
ETC	Electron transport chain
&	And
CVD	Cardiovascular diseases
(AEs)	Aqeous extracts
STZ	Streptozotocin

Chapter One: Introduction

1.1. Introduction:

Diabetes Mellitus (DM) is a metabolism-related diseases due to abnormalities in insulin production, the action of insulin, or both. Diabetes mellitus is a metabolic disease characterized by chronic occurrence hyper-glycemia with various degrees of changes in the metabolism of carbohydrates, fats and proteins. D M is probable to be one of the most ancient human illnesses known. Diabetes mellitus has become a serious and chronic metabolic disorder due to a complex interaction of genetic and environmental factors (WHO., 2017). Globally, diabetes is considered one of the main health challenges today. The WHO predicts that Diabetes is the seventh most common cause of mortality in 2030. From 2010 to 2030, the prevalence of adult diabetes will increase by 69% in the developing world and by 20% in the developed world. Diabetes mellitus (DM) not only causes hyperglycemia, but also a number of Complications include hyperlipidemia, high blood pressure and atherosclerosis (Sheriff *et al*.,2019).

Rosemary (*Rosemarinus officinalis* LS) is a persistent aromatic perennial shrub in the Lamiacea family, Usually referred to as rosemary, native to the Mediterranean Sea's north and south coast. It's a commonplace plant. Rosemary is a common spice and aromatizer in food manufacturing. Rosemary has been a traditional spice-based medicinal resource for centuries. Rosemary made up of dried leaves and flowers is an attractive organic source of biological activity phytochemical compounds because a variety of phenolic compounds are present. It is one of the most marketed plant extracts, it is used as a cooking grass. Aromatize and as antioxidants in manufacturing products and beauty products. Rosemary has antioxidant and pharmacologic properties in relation to the presence of phenolic compounds. Food for humans as well as animals (Labban *et al*.,2014 and Mena *et al*., 2016).

Ginger (*Zingiber officinal*) of the family Zingiberaceae. It is originally from Southeastern Asian and utilized in numerous countries worldwide. As a species for many years all over the world and seasoning to add flavor to food. The ginger root was also used as a traditional herbal remedy. The health promotion perspective for ginger is attributed to its richness of Phytochemistry. It has been reported that ginger also has anticancer, anticoagulant, anti-inflammatory and antioxidant features, as it may recover superoxide anion and hydroxyl radicals. Ginger was among the most

common herbs used in traditional Chinese, Ayurvedal, European and American (Kayode and Kayode., 2019).

The goals of the study: -

1. To investigate the effect of rosemary, ginger and combination of their infusion on complete blood count, glucose, Hb1Ac, lipid profile, AST, ALT, MDA, antioxidant GSH in diabetic rabbits.

2. To Study Histopathological changes of the pancreas, liver and kidneys in diabetic rabbits treated with rosemary, ginger and the combination of their infusion.

Chapter Two: Review Of The Related Literature

2.REVIEW:

2.1. Diabetes Mellitus (DM):

A word "diabetes" comes from the Greek term to obtain above it, a reference to micturition heightened (polyuria). It's a common characteristic the illness. "Mellitus" is the Latin word for honey, which means glucose in the urine of diabetic people. It is generally termed diabetes. In addition, there exists a rare condition called diabetes insipidus (water diabetes) where Kidneys emit a lot of water. Similar to diabetes mellitus, excessive micturition is a symptom, but both endocrine disorders are not linked. Diabetes mellitus was the most commonly reported endocrine disease associated with metabolic disorders (Akarte *et al*., 2012). This condition results in different pathological and physiological effects in different organs (Anfenan., 2014).

Diabetes mellitus is a metabolic disease where chronic hyperglycemia occurs with varying levels of alteration of the metabolic rate of carbohydrates, fats and proteins. It has become a serious and chronic metabolic disorder that is the result of a complex interplay of genetic and environmental factors (WHO., 2017). Diabetes is not a unique illness, but rather a set of metabolic disorders with an increase in blood sugar, that happens because of defects in insulin secretion its call to action or both. hypoglycemic complications are the main reason for morbidity and death for diabetics. Diabetes results in retinopathy, neuropathies and nephropathies (Ahmad *et al*., 2012).

It is chronic increase in blood sugar, which causes complications in the eyes, kidneys, heart, vessels and nerves . Diabetes has a number of complications, including ketoacidosis, a recurrent infection of weight loss, and cardiovascular disease. Diabetic complications include an increase in gluconeogenesis and ketogen (Kumar *et al*., 2011) and increased risk of cardiac failure and stroke . Diabetes is a complex set of metabolic symptoms, and is diagnosed with chronic high blood sugar along with changes in the metabolism of other biomolecules (proteins, lipids) that are associated with weight loss, polyuria, polyphagia and polydipsia (Javed *et al*., 2012). Complications from diabetes are associated with higher levels of free radicals, higher levels of fat peroxidization products, and lower levels of antioxidants (Ramakrishna and Jailkhani., 2008). The forms of peroxylic radicals and increased lipid peroxidation

leads to dyslipidemia and hyperglycemia in diabetes mellitus, a key pathway for developing microangiopathy. Hyperlipidemia was also reported to be the cause of increased lipid peroxidation in diabetes mellitus (Soliman., 2008).

Diabetic patients have diminished antioxidant defenses with lower levels of antioxidants like vitamin C and E or decreased activity of antioxidant enzymes like catalase, superoxide dismutase and glutathione peroxide . Lately, emphasis was placed on the relationship between the generation of free radicals, including reactive oxygen species (ROS), pathogenesis and advance of diabetes mellitus. Mechanisms that make it easier for free radicals to appear in diabetes mellitus may include metabolic stress because of changes in energy metabolism, inflammatory mediators and weaken antioxidant defenses (Madkor *et al*., 2011).

Hyperglycemia raises oxidative stress by the overproduction of reactive oxygen species resulting in an imbalance of free radicals and the cell's anti-oxidant defense system. Oxidative stress has been reported to have an effect metabolism of carbohydrates, lipids and proteins. Lipid abnormalities caused by uncontrollable hyperglycemia and insulin resistance in patients with diabetes are among the main risk factors for coronary heart disease, stroke and peripheral vascular disease (Madkor *et al*., 2011).

The primary indication diabetes mellitus refers to hyperglycemia, that is attributable to insufficient secretion of the pancreas or weak insulin stimulation of glucose by the target cells. It kills in silence and affects millions of people worldwide (Chaudhary and Tyagi., 2018).

2.2. Major classification of diabetes mellitus:

According to the diagnostic criteria, genetics and etiology. Diabetes mellitus classified into type I, type II, gestational diabetes and other types.

2.2.1. Type I Diabetes:

"Juvenile diabetes or insulin-dependent diabetes" (IDDM) the outcome of the pancreas's inability to produce insulin and demands that patients use insulin (Nasri *et al*., 2015). It is a chronic autoimmune disorder linked to the selective suppression of insulin production β-pancreatic cells. Type one diabetes, which is autoimmune where the immune system inadvertently destroys the beta cells of the pancreas that produce insulin(Ozougwu *et al*., 2013) .

In general, it grows more rapidly than the rest. Commonly diagnosed among children and youth, and occasionally among young adults. For life, the patients are required to routinely administer insulin. However, these words are incorrect as children can develop other types of diabetes, adults may present with type 1, and other forms of diabetes may need insulin treatment. A type one change which happens later in life, in general after the age of 30, referred to as adult latent autoimmune diabetes. Occasionally, patients suffering from autoimmune diabetes, promote insulin resistance as a result of weight growth and genetics. That condition shall called dual diabetes (Riza., 2009).

2.2.2. Type II diabetic:

Type two is also known as adult diabetes. The secretory defect of gradual insulin based upon insulin resistance. Individuals with diabetes are frequently insulin-resistant (Singh *et al*., 2016). Type two D M, diabetes "Adult diabetes or non-insulin-dependent diabetes mellitus "is causing insulin resistance one condition where the cells are unable to utilize insulin appropriately (Nasri *et al*., 2015).

Globally, it affects five to seven per cent of the global population. The disease is typically controlled through nutritional treatment, exercise and hypoglycemic agents (Bastaki., 2005). This is more important frequently reported type diabetes mellitus and has a strong association with a family history of diabetes, aging, obesity and physical activity deprivation (Baynest., 2015).

Metabolic disorder, which generally implies being overweight and insulin-resistant. In these patients, pancreatic insulins first produces, but the body is struggling to use that hormone, which controls glucose. In the end, the pancreas

cannot generate sufficient insulin to meet the needs of the body. The illness can take years or even decades. It is generally preceded by pre-diabetes, where glucose (glycemic) levels are above normal, but not sufficient for a diagnosis of diabetes. Type 2 diabetes was previously referred to as adult diabetes and insulin-dependent diabetes mellitus.Some patients need insulin therapy (Samreen., 2009).

2.2.3. Gestational Diabetes:

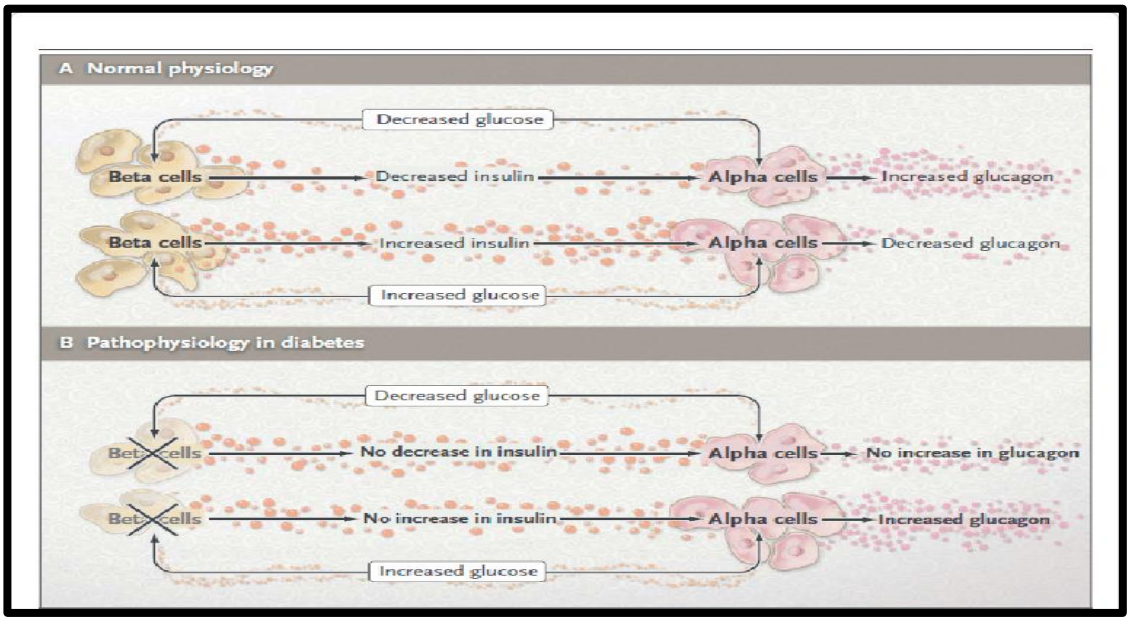
High blood glucose levels occurs in pregnant women without prior diagnosis of diabetes. This type of diabetes is possible occurring before type 2 diabetes develops (Nasri *et al*., 2015). It is common for pregnant women's development of diabetes. The effect of insulin in the mother's body, culminating in insulin resistance. These hormones can reduce, In pregnancy a lot of hormones are produced. Women who develop diabetes mellitus while pregnant and women whose type 2 asymptomatic diabetes mellitus has not been diagnosed are classified as gestational diabetes mellitus (Baynest., 2015).

Any women without diabetes during pregnancy can develop it because the metabolic temporary disorder that, generally within the last third month. Shifts Hormonal plays a role in this disease, as does too much. Hormonal changes contribute to this disease, as well as obesity and a familial history of diabetes. Based on the American Diabetes Association., approximately 4% of expectant mothers have gestational diabetes. Gestational diabetes may lead to the baby and mother problems like pre-eclampsia, premature childbirth, macrosomy (oversized child), jaundice and in an infant breathing trouble. It usually terminates at the time of pregnancy, but in later life for both mother and child increases the risk of type 2 diabetes (Riaz., 2009).

2.3. Causes of Diabetes Mellitus:

Diabetes mellitus a hereditary genetic disorder has multiple alleles on different chromosomes. The defect is a decrease in insulin secretion or the action of insulin, which leads to a rise in blood sugar (Kota *et al*., 2014 and Oshkondali *et al*.,2019). Millions of people worldwide are affected. Chronic hyperglycemia associated with this disease causes injury to organs in the kidneys, eyes, nerves and blood vessels. It is very important to control glycemic status in order to avoid this complication (American Diabetes Association, 2016).

Patients with this disease increase significantly daily as a result of changing lifestyles, such as reduced physical activity (Shaw *et al.*, 2010). Diabetes comes with many risks, such as family history, race, high blood pressure, signs insulin-resistant, history of vascular disease, inactive lifestyles, and so on. Thus, it can be prevented through lifestyle change, including nutritional therapy, fitness, behavioral therapy, weight loss and monitoring (American Diabetes Association, 2016). Genetic and family history, Family medical history has various influences, Bodily weight and type, gender, Amount of physical activity, Food, Other diseases, Hormones, Drugs, Chemicals, Environment, Virus ,Smoking, Alcohol (Riaz.**,** 2009).



Figuer (2-1): Panel A shows the physiological effect of a decrease in insulin coupled with a low glucose concentration in stimulating alpha-cell glucagon secretion, and Panel B shows the pathophysiological effect of beta-cell failure and the resulting loss of a decrease in insulin secretion and loss of an increase in alpha-cell glucagon secretion, despite a low glucose concentration. Adapted from Cryer (Forbes and Cooper., 2013).

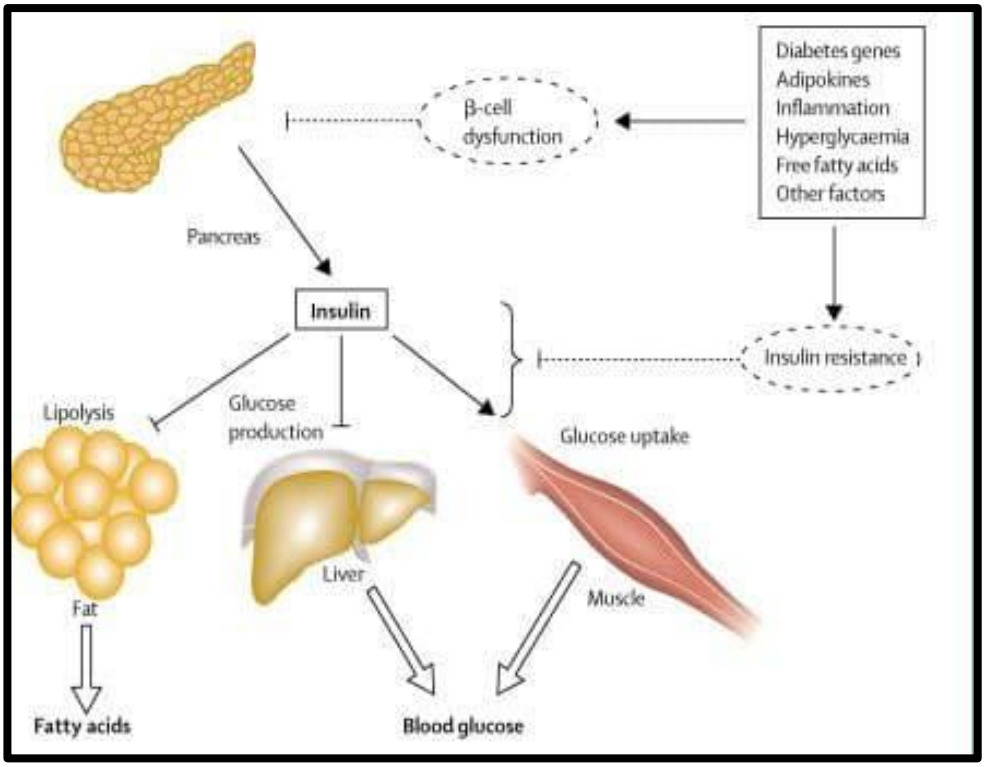


Figure (2-2): Pathophysiology of Hyperglycemia and increased circulating fatty acids in type 2 Diabetes. Adapted from Pittas (Sekikawa *et al*., 1993).

2.4. Complications of diabetes mellitus:

Hyperglycemia is a condition associated with diabetes mellitus and is associated with most complications of diabetes as the major cause. Hyperglycemia is an abnormal increase in blood glucose levels and type 2 diabetes is an outcome of insulin insensitivity. Long-term hyperglycemia results in increased production of reactive oxygen species (ROS) and shifts in endogenous antioxidants. Post-prandial hyperglycemia may induce enzyme-neutral glycosylation of different proteins and biomolecules, resulting in the appearance of chronic complications. Consequently, the postprandial plasma glucose monitoring levels are essential in treating early or managing diabetes mellitus, most notably type 2 diabetes and a reduction in chronic vascular complications (Ortiz-Andrade *et al*., 2007 and Riaz.**,** 2009).

Hypoglycemia has been recognized as a medical requirement leading to impairment of of cerebral function. Changes depend on how severe and how long the hypoglycemia is. Insulin-induced hypoglycaemia has been one of the main complications in treating type 1 diabetes mellitus. Hypoglycemia can set off a series

of events which may be extremely detrimental to the heart. Increased mortality due to increased insulin treatment in the Controls on the cardiovascular risk of diabetes trial was attributed to hypoglycemia. Studies have shown that hypoglycemia leads to pro-inflammatory changes (Gogitidze-Joy *et al*., 20103).

The brain has a dominant role in activating defense systems to counterregulate glucose. Measurements of glycogen levels in humans and rodents under various glycemic conditions showed that acute hypoglycemia depletes glycogen stores throughout the brain to try to maintain normal functioning. The brain's glycogen reserves start to decline when the brain's glucose drops to almost undetectable levels. Nevertheless, following hypoglycemia, glycogen reserves in the whole brain increase considerably above reference levels, known as supercompensation . Apart from the clear short-term as well as long-term effects of neuroglycopediatrics . The brain is also susceptible to systemic inflammation because circulatory cytokines can get into the brain. , microglia and neurotoxic sequencing of events. Hyperglycemia is defined as high plasma levels, like whole cholesterol, triglycerides, low-density lipoprotein, very low-density lipoprotein, and low-density lipoprotein (Abbott *et al*., 2014).

Patients with DM are at increased risk of complications such as peripheral vasculation, retinopathy, nephropathy, neuropathy, coronary artery disease. The exact reasons behind type 2 diabetes must always be clear (Shoback and Gardner., 2018; Tamadon *et al*., 2015). Most common lengthy complications are associated with lesions in the blood vessels. The risk of heart disease is approximately twice as high. The most widely spread "macrovascular" diseases are peripheral vascular diseases angina pectoris, heart attack and stroke (shoback and Gardner., 2018).

Diabetes mellitus is harmful to the capillaries leading to microangiopathies Causes vision-related symptoms, including reduced vision and blindness. Diabetic nephropathy usually results in changes in renal tissues, more and more protein loss in urine and chronic kidney disease. Diabetic neuropathy often gives rise to itching, numbness and pain in the feet. In addition, it increases the risk of skin lesions as a result of changes in feeling. Blood vessel complications in the legs are associated with the risk of diabetes-related foot problems. Diabetic foot ulcers, which can be difficult to treat and may require occasional amputation (Tavafi *et al*., 2013 and Rouhi *et al*., 2013).

In comparison to people who don't have diabetes, people suffering from the disease have about 1.5 times more cognitive problems and medicinal plants. Hypoglycemic activities have been shown to neutralize the complication . Nutritional Factors also appear to have an impact on the evolution of type 2. Trans fats and Saturated fats add to this risk as well. Failure to exercise greatly increases the risk of cases (Shoback and Gardner., 2018).

2.5. Inductive Diabetic Mellitus In Rabbitts Methods:

Many chemical compounds are used on a global scale to induce diabetes in animals. Alloxane monohydraté and streptozotocine are among those commonly used due to their low cost. However, other chemicals are less poisonous, but more expensive. Currently, the established animal models of diabetes are primarily those with beta-Cell destruction of chemical drugs. More importantly, alloxane (ALX) and streptozotocin-induced modelling is most commonly used. Alloxane in its monohydrate form has long been used as a chemical agent for diabetes induction. Utilization of various modes, doses and pathways of administration (Seham., 2019)

It has been observed that the drug is diabetogenic when given parenteral, that is to say intravenous, intraperitoneal or sub-cutaneously. Moreover, the dosage of alloxane needed to induce diabetes depends on the animal species, route of administration and nutrition status . As well, alloxane was found was not poisonous for human beta cells, even at extremely high levels. This can be attributed to various mechanisms of glucose uptake in humans . Streptozotocin and alloxane have been used for the production of experimental multiple sclerosis in animals such as mice, rats and rabbits , although streptozotocin is the most widely used chemical in rodents (Etuk.,2010 and Bacevic *et al*., 2020).

Alloxane is considered to be the choice drug for Inductive DM in rabbits. A variable Alloxan dose between 50 and 200 mg/kg was declared necessary to cause DM in rabbits. It is interesting to note that even with the same dose of alloxane given to rabbits of the same sex, and similar maturity and weight, as well as the same route of administration (intravenous injection) inconsistencies in survival rate reports are available (Bacevic *et al*., 2020)

2.6. Alloxan monohydrate:

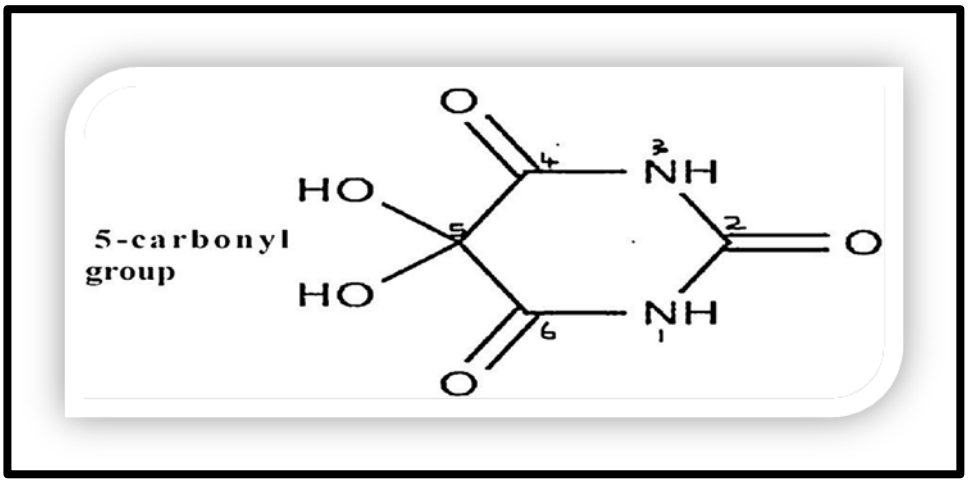


Figure: (2-3) chemical structure of Alloxan (Macdonald Ighodaro *et al*., 2017).

Alloxan (2,4,5,6-tetraoxypyrimidine;2,4,5,6-pyrimidinetetrone) is a pyrimidine oxygen derivative. Alloxane an organic substance, derived from urea, a cancer-causing cytotoxic analogue of glucose (Lenzen., 2008). Alloxane is the most common method of diabetes induction in rabbits because it is structurally similar glucose, cytoskeletal structure integrity, lysosomes, DNA and mitochondria would lose and β cell decay leading to an insulin deficiency production when alloxane is absorbed through of β-cells (cheekati *et al*., 2017).

Alloxan initially induces the forming reactive oxygen species (super oxide radicals, hydrogen peroxide and hydroxide radicals) that act as mediators of cellular damage (Lenzen, 2008). The Liebig and Wohler discovered the enclosure as well in 1828. Allantoin is a commodity from Atlanta and oxaluric acid originates from oxalic acid and urea present in the urine. Furthermore, Dunn, Sheehan and McLetchie first described the alloxan model to induce diabetes in rabbits in 1943 . Alloxan a beta cytotoxicglucose is used extensively to develop an animal model of type 1 diabetes mellitus (IDDM) is the drug of choice in rabbit (Masood *et al*., 2013).

Alloxane is rapidly absorbed into pancreatic beta cells through the GLUT2 receptor . Its beta-cytotoxic action causes the suddenly releasing insulin, resulting in severe hypoglycemia and even death if blood glucose is not given. Rabbits are becoming more and more utilized as experimental models of diabetes and alloxane has been the preferred chemical for developing diabetes models in rabbits . Selectively

destroying insulin-producing beta-pancreatic islets. Alloxan induces a multiphase glycemic reaction when injected into a laboratory animal, with corresponding reverse in plasma insulin levels and sequential changes in ultrastructural beta cells, causing the death of necrotic cells (Macdonald Ighodaro *et al*.,2017).

Alloxane is a hydrophilic, unstable chemical that resembles glucose that is responsible for its selective uptake and accumulation through the pancreatic beta cell. The similarity of the shape makes it possible to carry it into the cytosol by the glucose-carrying agent (GLUT2) in the beta cell's plasma membrane (Elsner *et al*., 2002). Alloxane was attributed to enabling inhibition of glucose-induced insulin secretion selectively by inhibition of glucokinase. Inhibition of glucokinase diminishes glucose oxidation and ATP generation more suppressive of glucose-induced insulin secretion (Macdonald Ighodaro *et al*.,2017).

2.6.1. Alloxan's mechanical action:

Alloxan As a toxic agent of islet βcells, alloxan damages βcells by producing a superoxide-free radical to damage cell DNA, activate ADP-Ribose polymerase activity, decrease coenzyme content , impair mRNA function, reduce insulin before βcells synthesis, and induce insulin deficiency finally. The alloxane-induced animal model for diabetes is similar to human type I diabetes. Although the diabetic alloxane animal model is widely used to evaluate the effectiveness and safety of antidiabetic medications and alloxan is cheap, it is hard to understand the safe dose of alloxan in the previously adopted method. As a result, the application of the alloxan-induced diabetic animal model is impaired because of its low success rate and high mortality rate (Li *et al*., 2005).

Alloxane induces the formation of reactive oxygen species (superoxide radicals, hydrogen peroxide and hydroxide radicals), which damage the cells . In addition, these cell lesions can lead to autoimmune reactions against β-cell Second, alloxan disrupts micro-tubule formation and destroys those that have already produced . Third, alloxane is supposed to inhibit enzymatic transfers bound to the enzyme n-acetyl Glucosamine, that are very abundant in beta cells Because of these reasons, alloxane is a powerful and diabetogenic agent and is widely used for the inducement of diabetes in laboratory animals . Chemical induction is one of the most effective

methods of inducing investigational diabetes mellitus by alloxane. For these reasons, Alloxane is a powerful diabetes-causing agent and is widely used to induce diabetes in laboratory animals . It is a well-known diabetogenic agent and is used to induce type I diabetes in laboratory animals (Cheekati *et al*.,2017).

Alloxan is an uretic derivative that causes selective necrosis of pancreatic islet βcells. Moreover, it has been used extensively to generate experiments on diabetes in animals such as rabbits, rats, mice and dogs, different levels of disease severity by varying the amount of alloxan taken . Since There is a general understanding that alloxane selectively destroys insulin-producing beta cells in the pancreas. It is therefore used to induce diabetes in test animals (Macdonald Ighodaro *et al*.,2017).

The toxic effect of alloxane on pancreatic beta cells implies oxidation of essential sulfydryl (HS-groups), inhibition of the enzyme glucokinase, generation of free radicals, and interferes in intracellular calcium homeostasis. The underlying mechanism implies selective absorption of the compound as it is structurally similar to glucose and the most effective absorption of pancreatic beta cells (Dhanesha *et al*., 2012).

2.7. Medicinal herbs with antidiabetic activities:

Medicinal plants are now in high need for primary health care in developing countries because of its usefulness, security and reduced adverse impacts(Bhargava *et al*., 2012). A World Health Organization report says 80 percent of people in developing countries depend on herbal therapy for their primary and primary healthcare 85% medicinal plants is produced by plants (Ghasemzadeh *et al*., 2015). It is well known that herbal supplements are used as food additives for improving human and animal health, by virtue of their antioxidant properties and their safety. Some of the most effective natural anti-oxidants, such as phenolic compounds, flavonoids and phenylpropanoids, are present in natural food additives (Elwan *et al*., 2020).

A variety of the herbs were employed to treat and prevent several chronic conditions. Like diabetes, hyper-cholesterolemia and triglycemia; one such herb is rosemary, which has organic antioxidant mechanisms (Labban *et al*., 2014). Herbal medicines were used to cure diabetes mellitus before insulin therapy was invented

1921 . Herbal medicines are excellent sources of hypoglycemic compounds and are used in conjunction with existing therapies to treat diabetes . Plant extract affects the level of glucose in the blood by various mechanisms :some plants have compounds analogous to insulin, others may inhibit insulin activity (Hussain *et al*., 2013) and other mechanisms could enhance the regeneration of the pancreas beta cells (Hosseini *et al*., 2015).

More than 400 herbal medicinal products have been used to treat diabetes mellitus and antidiabetic agents have been found . Diabetes mellitus has a rapidly increasing incidence and is expected to increase by 2030. In addition to the current therapeutic options available, there are many herbal medications, which have been recommended for its treatment. Medicinal herbs have been used for many years to treat diabetic due to the benefit generally with little or no side effects. The majority of these plants are antioxidants and thus prevent or cure curable diseases. Aside from the ability to control the toxicity of toxic drugs and others (Nasri *et al*., 2015).

One of the possible benefits vegetable and fruit components are antioxidants and as a consequence, their contribution to reducing systemic oxidative stress (Hulbert et al., 2005 and Rafieian-Kopaei *et al*., 2013). It has been proven that vegetables and fruits contain high levels of antioxidants, potentially reducing risk for diabetes, particularly type II. Vegetables and fruits are also important sources of omega-3 polyunsaturated fatty acids, alpha linolenic acid (Hajivandi and Amiri., 2014 and Nasri *et al*., 2014). Herbal medicines have also played a major role in managing DM on a global scale. Medicinal plants have a lot of history treating illnesses. In traditional medicine, approximately 800 herbs are used to treat DM (Hung *et al*., 2012 and Rafieian-Kopaei *et al*., 2013).

In stressful conditions, free radicals have been shown to be over-produced, causing oxidative stress and anti-oxidant defenses . This oxidative stress is a common cause or exacerbation of curable chronic diseases such as diabetes hypertension, cardiovascular,cancer,Shirzad,cognitive diseases, and pain or exacerbation of additional diseases such as infective diseases (Baradaran *et al*., 2014 and Tamadon *et al*., 2015).

Although, in some cases, synthetic antioxidants have also worked to reduce DM. however, contrary to the natural antioxidants, synthetic antioxidants usually produce

side effects such as toxicity . It is therefore essential to prepare natural products with antioxidant activities for preventing and treating diseases associated with free radicals (Baradaran *et al*., 2014 and Nasri *et al*., 2014). Apart from plants that have been introduced here, many other plants have an antioxidant activity (Bahmani *et al*., 2014 and Rafieian-Kopaei *et al*., 2014). These plants have attracted a lot of attraction due to their protection or healing properties against most treatable diseases like cognitive deficits, memory problems, cancer and cardiovascular diseases attributable to their antioxidative activities. Therefore, they could also work with the DM.The herbs were used for ethnomedical purposes in diabetes treatment for several centuries. In recent years, research on herbal medicines for diabetes management has attracted scientific attention (Ali *et al*., 2006).

2.8. *Rosemary Officinalis:*

2.8.1. Scientific classification of *Rosemary*:

Kingdom: Plantae

Division: Magnoliophyta.

Class: Magnoliopsida.

Order: Lamiales.

Family: Lamiaceae.

Genus: Rosmarinus L.

Species: officinalis (Kompelly et al., 2019) .

Figuer (2-4): Rosemary plants (Kompelly *et al*., 2019).

2.8.2. Synonms:

Romero, Alecrim, Rosemary, Rosmarin, Rosmarino and δενδρολι΄βανο (Begum *et al*., 2013)

2.8.3. *Rosemary* Description:

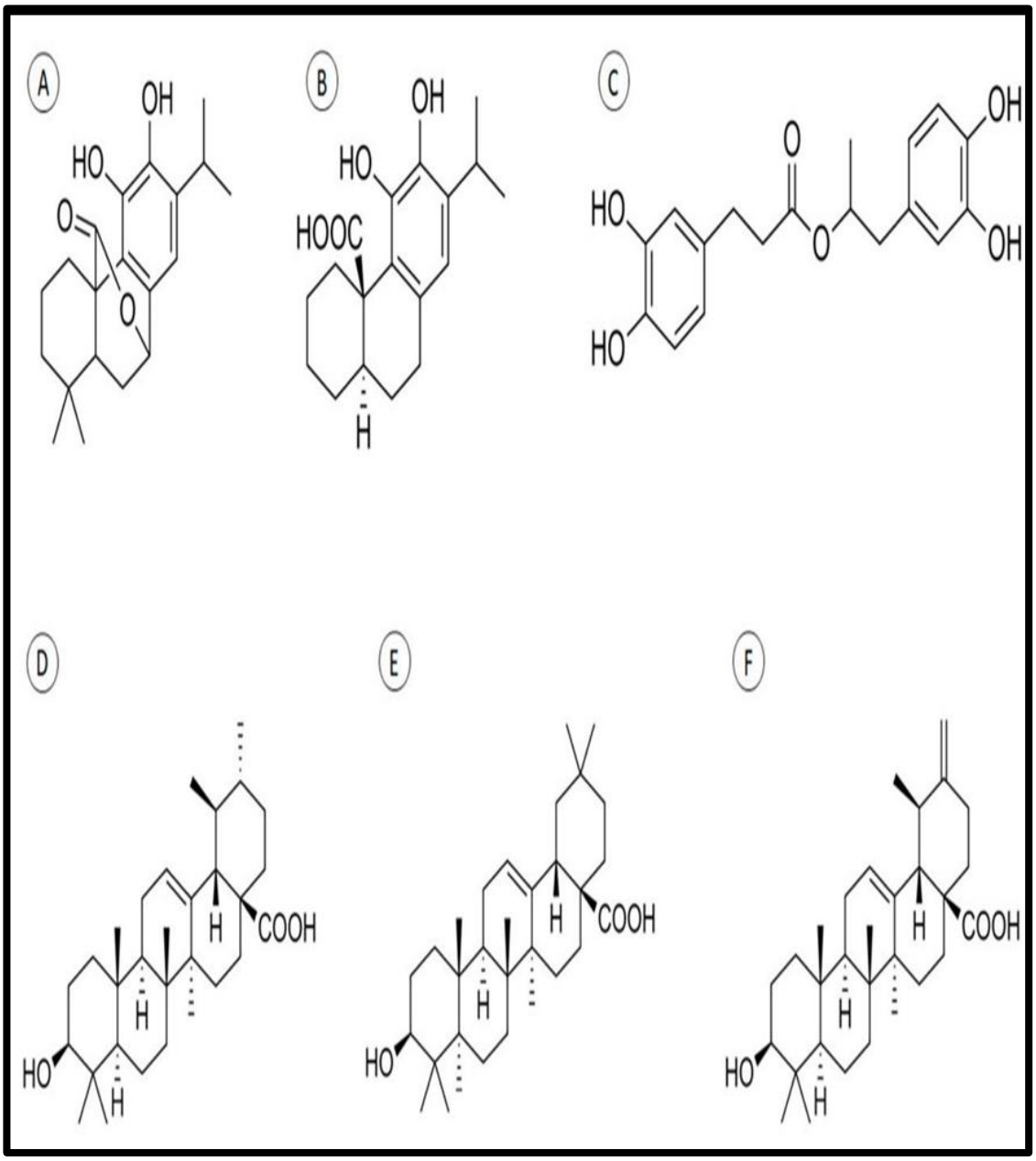
Rosemary is a potent plant of the Lamiacea family, originating in the Mediterranean area. It is derived from Ros' Latin (dew) and Marinus' (sea) meaning «dew of the sea». He was named New Year's Grass in International Herb Association, 2001. Rosemary is seen as grass from faithfulness, because Elizabethan lovers wore a branch of rosemary as its sign. Today, the demand of the plant market is increasing because it is used in several commercially available products. Rosemary is made up of pine leaves that are the heart of all the medicines and other advantages that are derived from the use of its oil. Rosemary is composed of over 20 rosemary species. The leaves are leathery, opposed, strongly curved, fringed and have a prominent midrib. The length of the leaf is between 1.0 and 2.5 cm and its width is 4 cm. The upper side of the leaf is green and the lower side is slightly woolly due to multiple trichomes. The margins are whole and strongly revolted at the obtuse apex, at the tapered base and not petiolated (Kompelly *et al*.,2019).

2.8.4. compositions of *rosemary*:

There are differences in the chemical composition of these secondary metabolites significantly according to environmental All rosemary extracts and essential oils are packaged with biologically active compounds that make the plant unique . For example, phenolics carnosic acid, carnosol and rosemary acid are the cause of the strong anti-oxidant activity of oil and rosemary extract. Rosemary extract (ER) contains a variety of polyphenol categories, including phenolic acids, flavonoids and phenolic terpenes. It is made up dried leaves and flowers and is a particularly appealing source of biologically active plant chemicals, because it contains miscellaneous phenols, including console, carnosic acid, rosmanol, 7-methyl-epirosemanol, isorosmanol, rosmadial, and caffeinated acid (De Macedo *et al*., 2020).

Rosmarina (Lamiaceaea family), commonly known as rosemary, is among the most popular perennial culinary herbs in the world over. Fresh and dried rosemary leaves have been used for their distinctive aroma within the food, cook or consume small amounts, such as tea. Rosemary essential oils dominated by 1,8-cineole, α-pinene, camphene, α- terpineol, and borneol as the principal constituents They also cause different pharmacological effects of the of global antioxidants. Far and away the

largest group of rosemary compounds this have attracted a great deal of attention over the past few years, however, is a single class of polyphenolic diterpenes (kompelly *et al*.,2019) .



Figuer (2-5): Chemical structure of some Rosmarinus officinalis secondary metabolites: carnosol (A), carnosic acid (B), rosmarinic acid (C), ursolic acid (D), oleanolic acid (E), anmicromeric acid (F), (De Macedo *et al*., 2020).

2.8.5. Pharmacological Properties:

Fresh leaves, dried up have been widely used in both seasoning and traditional medicine. It has historically been used in medicine for the treatment of renal colic and dysmenorrhea, promote hair growth and reduce symptoms of respiratory problems. Nowadays, rosemary extract is often used as aromatherapy to deal with anxiety problems and increase alertness . Rosemary formulations range from raw leaves to rosemary extracts. Traditional extraction methods such as hydrodistillation, decoction and maceration, as well as other methods, including liquid extract under pressure, enzyme extraction, nanofiltration and solid-state extraction have been used to extract polyphenols from plant materials. This important antioxidant activity does more than just make rosemary a food effective, conservative, but it too represents almost every other therapeutic capacity of rosemary, including its anticancer and antidiabetic processes. So, it was not until recently that their conservation mechanisms were examined. Which presents a major interesting for the industry today (Kompelly et al., 2019).

Recent research has shown that rosemary extracts are highly antibacterial, antifungal and antioxidant, these factors all work together to make the plant a highly effective foodborne pathogen inhibitors. Notably, Rosmarino has proven hepatoprotective, antispasmodic, anticarcinogens, antitumorics, antimicrobials, anti-inflammatories and antioxidants. He has also demonstrated antidiabetic, neuroprotective and other activities. The extract relaxes the smooth muscles of the trachea and intestinal tract, as well as the choleretic, hepatosurgical and antitumergenic activity. In addition, the rosemary constituents have therapeutic potential to treat and prevent pulmonary asthma, spamogenic disorders, diabetes mellitus, peptic ulcers, inflammatory disorders, liver toxicity, arterial sclerosis, ischemic heart disease, cataracts, cancer and impaired sperm motility. It is famous as an herbal medicine due to its high antioxidant activity and its use in traditional medicine as a cure for diabetes mellitus. Moreover, several studies have shown that rosemary extracts its antinociceptive, anti-inflammatory, anti-poptotic and neuroprotective characteristic (Kompelly *et al*., 2019).

Aqueous rosemary extract, as a medicine having strong antioxidant properties to remove free radicals generated, strengthen the anti-oxidizing system and inhibition of

oxidative stress. Rosemary extracts have a large ability to recover different types of reactive oxygen and nitrogen species, mostly free radicals. While rosemary extracts are largely used as natural antioxidants to improve the lifetime of perishable foods. This last instance, The European Union has endorsed rosemary extract as a safe and efficient natural dietary preservative (De Macedo *et al*., 2020).

Pharmacologically validated usages of rosemary include antibacterials, anticancinogenic, antidiabetogenic , anti-inflammation and antinociceptive effects, antioxidant (Bakirel *et*, antithrombogenic, antiulcerogenic , improving cognitive deficits , antidiuretics, and liver protective effects. This is a group of low the molecular-weight aromatic compounds known as essential oils that play a vital role in the plant's fragrance and cooking properties also cause various pharmacological effects of generalities antioxidants and antimicrobials known for several essential oils, and additional impacts, including anticarcinogenous activities. The other secondary metabolite group rosemary is a polyphenolic compound, including flavonoids (e.g., homoplantaginin, nepetrin, hesperidine and derivatives, etc.) and phenolic acid derivatives (e.g., romarinous acid) (Kompelly *et al*.,2019).

Rosemary is an aromatic herb that is commonly used as a flavoring food, has also been commonly used in traditional medicine. Rosemary was used as an agent light stimulant and analgesic and it's effective herbs for the treatment of headaches, poor circulation, inflammation. This plant has a strong antioxidant activity. It contains certain antioxidant components which have been shown to serve as a defense against oxidizing agents produced by oxidative stress. This includes carnosol, carnosic acid, ursol acid, rosmarinic acid and caffeinic acid, therefore effectively participate in lipid peroxidation . Reported that console, a natural polyphenol rosemary leaf, exemplifies effective antioxidant activity against free radicals (Kompelly *et al*.,2019).

2.8.6. Antidiabetic activity of *rosemary*:

The development diabetes is often associated with high oxidative stress; of the pancreas are sensitive especially to reactive oxygen species, resulting in a reduction in insulin secretion and an increase in blood glucose levels. The information has encouraged new treatments for diabetes concentrate on natural antioxidants, especially those found in plants. Unsurprisingly, numerous Studies have shown that Rosmarinus

officinalis is a promising anti-diabetic agent. Rosemary's anti-oxidant properties exert a number of anti-diabetic and hypoglycemic processes. In a study, romarin extraction reduced normoglycemic glycemia, hyperglycemia and diabetes bunnies. By inhibiting the peroxidation of lipids and activating antioxidant enzymes, so does the extract helped secrete insulin. It has also been found that rosemary relieves a delayed healing, a serious complication of diabetes. This anti-diabetic activity is due to the improvement of the antioxidant status of the body after administering rosemarin (Hamidpour *et al*.,2017).

2.8.7. Antioxidant Activity Of *Rosemary*:

The antioxidant activity of rosemary is directly associated with the chemical compounds found in essential oils and extracts of the plant. Whereas mechanisms of synergism among many components of oil probably contribute to the antioxidant activity, phenolic diterpenes carnosic acid, carnosol and there was rosemary acid found to be the most powerful antioxidants . *Rosmarinus Officinalis* has an anti-oxidant effect via a number of metabolic pathways. In addition, oil and rosemary It has been demonstrated that the extract destroys and protects against free radical . Rosemary is also able to prevent lipid peroxidation, a destructive process due to oxidative stress. As well as decreasing the quantity of reactive species within the body. There is evidence that rosemary increases the action of antioxidant enzymes (Hamidpour et al.,2017).

Oxidative stress is a factor contributing to its development numerous diseases.In effect, its antioxidant activity is a fundamental part of virtually every other therapeutic application of rosemary. Of the natural antioxidants, rosemary is widely recognized as a species with the greatest anti-oxidant activity. We know about Rosemary extracts are active in medicine and in the food industry because of its high antioxidant and phenolic oils to protect against oxidative degradation in foods containing oil and lipids . Rosemary has a long-standing reputation as having antioxidant molecules, like rosmarinic acid, console, rosmaridiphenol and those molecules were found in the soluble fraction in ethanol (De Macedo *et al*.,2020).

2.9. *Zingiber officinale.*

2.9.1. Scientific classification of *ginger*:

Kingdom: Plantae.

Division: Magnoliophyta.

Class: Liliopsida.

Order: Zingiberales.

Family: Zingiberaceae

Species: Zingiber officinale var. Roscoe (*Abbasi *et al*., 2019*).

Figuer (2-6): Dried rhizome (Abbasi *et al*., 2019).

2.9.2. Synonms:

Ginger, Zanjabee, Shangwez, Hotiyoon, Adrakam, Adrak, Sonth, Zanjabeel ,sunth and Ada (Abbasi *et al*., 2019).

2.9.3. *Zingiber officinale (Ginger)* Description:

Ginger is a rhizomatous perennial herbaceous plant that can grow as high as 90cm in culture. The rhizomes are aromatic leaves, dense, lobed, pale yellow, simple, alternate, narrow and oblong, lance-shape. The herb grows several clusters of lateral shoots, which begin to dry as the plant matures. The leaves are long and 2-3 cm wide with overlapping bases, the blade progressively shrinks to a point. Solitary inflorescence, pedunculate lateral radicles oblongcolumnar heads. The flowers are rare, quite small, with upper calyx, gamosepalous, with three teeth; cracking on one side, corolla of three oblong to semiequal Lancelot greenish segments (Mishra *et al*., 2012). It's from Southeast Asia. Ginger is a tropical herb that thrives in hot, humid climates. The crop is grown in China, Nepal, USA, India, Bangladesh, Taiwan, Jamaica, Caribbean, Nigeria and Indonesia. India is a major producer of Zingiber (Abbasi *et al*., 2019).

Zingiber officinale Roscoe (genus Zingiberaceae), known worldwide by the name of ginger commonly used as a spice and diet as well as the medicinal agent in traditional Indian, Asian and Arabian medicine in the form of a fresh paste, dry powder, candies (crystallized ginger) and slices of syrup. Zingiberacea includes approximately 80 to 90 species of perennial aromatic herbs with fresh rhizomes and tuberous roots (Račková *et al*., 2013).

It is a perennial with sharp green narrow leaves resembling grass and yellowish green flowers with violet markings . For over 5000 years ago, ginger was used to cook and treat numerous ailments. It is originating in Southeast Asia . Ginger is a crawling perennial with a dense tuberose rhizome that extends below ground. Within the first year, a green stem, erected in the form of a reed, grows from this rhizome at a height of approximately 60 cm. The plant has narrowed, lanceolate to linear-lanceolate leaves, about 15-30 cm long, that disappear annually (Retrieved., 2013 and Srinivasan.,2017).

Ginger, (which comprises 47 generations and 1400 species). It has a commercial crops that are not restricted to Asia, as they are case globally. World ginger production amounted to3. 3 million tons during 2016. Ginger can be consumed in the form of fresh, dehydrated or treated produce. Fresh rhizome is widely used as a spice and

nutritional seasoning, either as a powder, extract, supplement or medicine (Mbaveng .,2017 and Nemati et al., 2021).

2.9.4. Compositions of *Ginger*:

In addition, ginger rhizome has also been used in medicinal traditional plant-based as well. The benefits health of ginger depends on its richness plant chemistry . Brought together fresh ginger under two main categories, volatile and non-volatile. Volatile compounds include sesquiterpenes and monoterpenooids which give ginger its distinctive flavor and taste. In contrast, non-volatile compounds are ginger, shogaol, paradol and ziner . Bioactive molecules in ginger, such as ginger, have been shown to be an antioxidant in multiple modules (Dugasani *et al*., 2010).

Minerals in ginger include iron, calcium and phosphorus, as well as vitamins like thiamine, riboflavin, niacin, and vitamins A& C. The composition varies in relation to the type, variety, agronomic conditions, drying methods and storage conditions . Chemical make-up a standard component in ginger are volatile oil, starch and resin. Its smell comes from the presence of its volatile oil, gingerlos the principal of which is [six] - gingerol a greasy liquid and the most abundant element among gingerols, zingerone less prickly is also manufactured from gingerols in the drying process and spicy Bran taste is due to the presence of resin and zingérone, shogaols, gingers. Ginger contains acid resinous substances as well. The essential oil is light yellow, fragrant and non pungent. Ginger essential oil has a variety of terpenes and sesquiterpenes such as zingiberene. Volatile oil has a content zineberene (35%), turmeric (18%) and Farnesene (10 per cent), with less Bisabolena and b-sesquiphellandrene (Srinivasan.,2017 and Abbasi *et al*.,2019).

The root of ginger not only, rich in nutrients, amino acids, fatty acids, vitamins and minerals, but the product also includes compounds like ginger, diol ginger, Dione ginger, and shagaol, which is a powerful stimulator of the intestinal mucosa and digestion. Gingers and Shagaols are responsible for an acid appearance of fresh and cured ginger, respectively. Ginger rhizomes contain crude fiber (three to eight percent), ash (eight percent), water (nine to twelve percent) and volatile oil (two to three %) fats (three to six percent), proteins (nine percent), carbohydrates (sixty to seventy percent), (Nemati *et al*.,2021).

2.9.5. Pharmacological Properties:

Ginger has obvious potential in the treatment of many diseases, such as degenerative diseases (arthritis and rheumatism), digestive health (indigestion, constipation and ulcer) Cardiovascular disease (atherosclerotic disease and high blood pressure), diabetes mellitus as well as cancer. In addition, it possesses anti-inflammatory and antioxidant properties for controlling the aging process. It also has antimicrobial potential which may be helpful in treating infectious diseases (Nicoll *et al*., 2009). Generation of free radicals or reactive oxygen species (ROS) in the course of metabolism beyond the antioxidant ability of one oxidative stress occurs within the biological system. Which plays a vital role in heart disease, Neuro-degenerative diseases, canceroma and aging (Vipin *et al*., 2017).

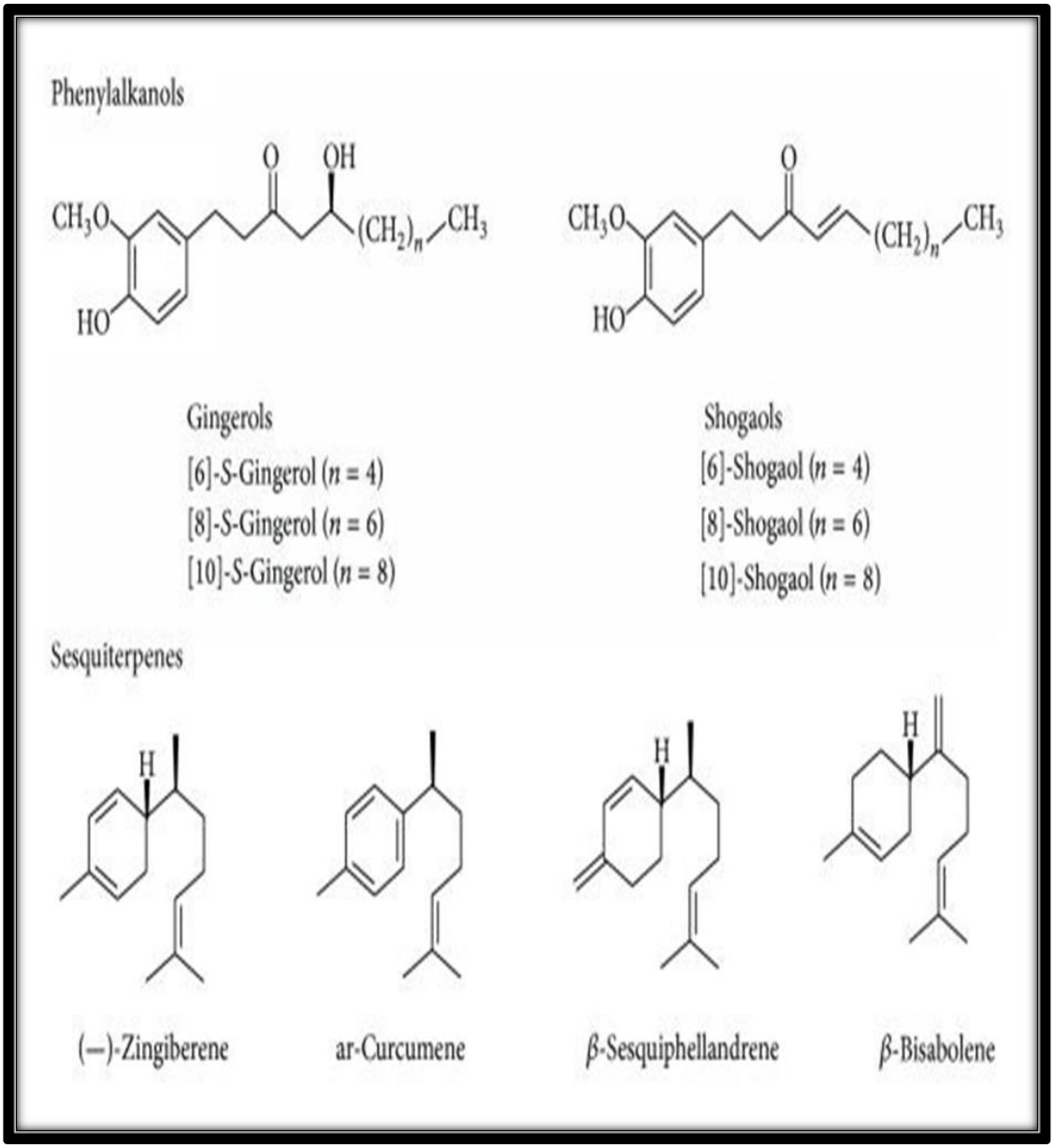
Ginger is a rhizome underground Zingiber plant member of Zingiberaceae and is amongst the most popular spices around the world. There is a lengthy history of use like a medicinal plant drugs to treat a range illnesses, including nausea and vomiting, constipation, indigestion (dyspepsia), pain and cold disorders, Most recently, it has been noted that ginger also has anti-cancer, anticoagulation, anti-inflammatory and antioxidant properties, because it can eliminate superoxide anion and hydroxyl radicals.Containing various phenolic components and they presented many bioactivities like, anti-anxiety, antinausea, anti-inflammation, decreased glucose levels and beneficial health outcomes reducing damage caused by free radicals and improving cardiovascular condition. In addition, Ginger is used for its diverse healing and mitigating properties and treating various symptoms such as animal mycotoxicosis , vomiting, soreness, indigestion and upper airway infection (Vipin *et al*., 2017 and Nemati *et al*.,2021).

In traditional medicines, Ginger has been employed for its wide array of medicinal properties such as anti-inflammatories, immunomodulators, anti-tumoric anti-apoptotic, antihyperglycemic, anti-lipid and anti-emetic, antimicrobial, hypoglycemic, antioxidant, hepatoprotective activities, etc. (Jafarzadeh *et al*., 2018), nephrol-protective activity, hepatic-protective activity, larvicide activity, pain-relieving activity (Wang *et al* .,2017) .

Flavonoids are important in plant bio-chemistry and play an important role in plant physiology acts as antioxidants, enzyme inhibitors, pigments and luminous displays. Phytochemical study of rhizomes of several species of Zingiber has been shown to contain bioactive compounds like gingerols, shogaols, diarylheptanoids, phenylbutenoids, flavanoids, diterpenoids and sesquiterpenooids .It has demonstrated that rhizomes are effective in treating a number of medical problems, including stomach problems, nausea, vomiting, epilepsy, sores in the throat, cough, colds, bruises, sores, hepatic problems, rheumatism, muscle pain, atherosclerosis, headaches, high cholesterol, ulcers as well as gastric discomfort. Furthermore, phenolic compounds, in particular ginger, in the root of gingerols have been demonstrated to have chemopreventive effects linked to their antioxidant and anti-inflammatory characteristic (Wang *et al* .,2017).

In the ayurvedic system, ginger and milk or water in batter form are used outdoors to treat childhood colic. Ginger and honey are combined for asthma bronchitis, cough, hiccough and common cold. Under the traditional Chinese system, it is believed that fresh ginger has a mild and warm temperament, while dry and toasted gingers are considered warm and hot, respectively. Ginger is used in the treatment of gastrointestinal problems in Western medicine. Henry VIII recommended that ginger be used to avoid plague. Ginger the bread produced by the Greeks is eaten after the meal as a digestive help . The blood-purifying, aphrodisiac, sex-stimulating, appetizing, antiflatulent, antispasmodic, anti-hemorrhoids, anti-emesis and anti-nausea are other traditional effects of ginger perspectives (Mahboubi., 2019).

Today, Ginger hydroethanol extracts are commonly utilized as analgesics, antidiabetic drugs, hepatosuppressants, nephropotectors and antioxidants . Ginger extract is an important inhibitor for catarrh and platelet aggregation. It is equally important for its cardiotonic effects and gastrointestinal actions. Ginger extracts are thermogenic and antibiotics in the wild and are important as digestive stimulating agents . Studies have shown that ginger can be used to grow naturally stimulant because it enhance immune function and contributes to the quality of meat animals. In a man-based study, ginger did not exhibit any teratogenic effect (Mbaveng., 2017 and Mahboubi.,2019).



Figuer (2-7): Chemical structures of components of Zingiber officinale (Roufogalis.,2014).

2.9.6. Antidiabetic Activites of *Ginger*:

Aqueous, methanol, ethanolic and Extracts of 6-gingerol were also found to reduce blood glucose considerably, in medicated diabetic animals and type 2 diabetic patients. However, but few scientific reports have been produced about the mechanisms of that effect. The possible mechanics of the diabetic effect of ginger can be modulated of antioxidant enzymes as well as inflammation cytokines thereby, prevent oxidative damage which exacerbates complications of diabetes, Increased output and susceptibility of insulin, acting as anti-glycatives and operating as an in vitro insulin survey. All of that was used to prevent complications with diabetes, but did not elucidate the effect of ginger on postprandial blood glucose mediated by digestive enzymes. In an in vitro study, amylase was separated from rats through ginger, whereas other A similar study, although more recently, found an inhibitory effect (Mahboubi., 2019).

2.9.7. Antioxidant Activity of *Ginger*:

Ginger is known to have a powerful antioxidant with an oil that has a protective effect on DNA. This effect has been found in certain cellular cultures . Ginger preventively affects lipidic peroxidation and inhibits or break a chain .Ginger modulates the genetic route, acts upon the tumor suppressor genes and modulates a number of biological activities. Ginger was utilized as a spice for thousands of years and its rhizomes and extracts contain phenolics like 6-gingerol and its derivatives. Has strong antioxidant activity. Many authors have also identifiable ginger as having antioxidant properties. The alkyl chain substituent of these compounds may contribute both a radical sweeping effect and inhibitory effects against peroxidation of liposome induced by the peroxyl radical. The activity of antioxidants could be due to radical scavenging activity(Duarte *et al*., 2016).

Aeschbach has found that generally is a good recuperator of peroxyl radicals generated by impulse radiolysis, suggesting that [6] -gingerol could be used as a "natural" substitute for "man-made" antioxidant food additives. Indicated that a functional drink consists of an extract rich in [6] gingerol that has a hypocholesterolemic effect in rats with spontaneous hypertension (Gunathilake et al., 2013). In addition, ginger water extracts may be incorporated into functional fruit-

based beverages intended for cardioprotection . Ginger leaves also exhibited higher antioxidant activity and phenolic levels compared to rhizomes and ginger stems. However, the antioxidant potency of rhizomes has been shown to be higher than that the leaves ((Duarte *et al*., 2016 and wang.,2017).

2.10. Oxidative stress:

Oxidative stress means disequilibrium amongst free radicals and antioxidant enzymes of their stabilizer inside the body. Reactive Oxygen species or free radicals can be generated from a normal cellular metabolism and respond with biomolecules such as proteins, lipids and DNA are responsible for cell lesions and resulting in degenerative alterations. Oxidative stress is defined as an unbalance among rising levels of reactive oxygen (ROS) and weak activity of antioxidant processes. Increased oxidative stress can damage cell structure and perhaps destroy tissues. However, is needed for adequate cell function, including the production of mitochondrial energy, was criminalized in physiological conditions such as aging, exercise, and within various of cancer, neurodegenerative diseases, CVD, diabetes, inflammatory conditions, and drug intoxication. But preventing antioxidants has been most of the time ineffective. These sources generate radicals without oxygen and increase of the cell breathing that leads to aerobic breathing in the form of free radical oxygen acts as an oxidative stress-inducing substance or molecule (Manisha *et al*., 2017).

Unequal stadium within manufacturing and eliminating the free radicals that cause stress. When more free radicals are generate in the human body these conditions have caused oxidative stress, which produces pathogen effects on the life cycle of living cells. Cause several age-related illnesses like cancer, neurodegeneration and senescente . If the ratio of oxidative stressors such as free radicals and antioxidants obtains disturbances, then the cell is passed through a stress, oxidative cell. Reactive oxygen species generated by the oxidative stress process include superoxide radicals, hydroxyl radicals and hydrogen peroxide(Fanjul-Moles and López-Riquelme., 2016).

The oxidative lesions of the DNA are mainly indirect and the supply radicals to DNA may cause the mutation so the cell can become cancerous. ETC mitochondria is a type of internal source factor for free formation radicals in internal cellular metabolism (Shinde *et al*., 2012). In addition to a lot such oxygen reagent

intermediates, some can act as secondary messengers of redox signalling. May interfere with normal cell signalling routes . During moderate exercise, oxygen intake increases from 8 to 10 folds, and the flow of oxygen through the muscle can increase from 90 to 100 folds. Even moderate physical activity can increase the production of free radicals and overwhelm antioxidant defenses, which leads to oxidative insult. The close link oxidative stress as well as lifestyle illnesses has become widely acknowledged (Manisha *et al*., 2017).

2.10.1. Oxidation of diabetes mellitus:

Oxidative stress has been a result of imbalance between the creation of free radical, often enhanced by dysfunctional mitochondria, and reduction in antioxidant defenses. A major source of reactive oxygen species is the mitochondrial electron transport chain in insulin secreting cells, insulin-sensitive peripheral cells, and endothelial cells. Oxidation is produced in diabetes conditions and is probably implicated in advancing the dysfunction of pancreatic beta cells in diabetes. Oxidative stress was caused by the pathogenesis and complications associated with type 2 diabetes. Metabolic disruptions contribute to oxidative stress and impaired the antioxidant defense system in patients suffering from type 2 diabetes. There seems to be an imbalance between oxidizing and anti-oxidizing systems in type 2 diabetics. These Patients are considered to have oxidation caused by long-term exposure to hyperglycaemia (Kassab and Piwowar., 2012).

2.11. Antioxidants:

Antioxidant are a category of chemicals that are naturally present in food that may prevention, reduction of oxidized within the physiologic system. The body is constantly generating radicals. It's because of the steady flow of oxygen. Radicals have responsibility for the cell lesions in the body and contribute to diverse types of health conditions, cardiovascular illness, diabetes mellosis ,etc. The Antioxidants being excellent scavengers of free radicals assist prevent or repair cellular damage from these radicals (Misra *et al*., 2014).

Antioxidants are molecules which prevent cell damage from oxidization of other molecules. Oxidation is a chemical reaction that transmits electrons between a molecule and a molecule oxidizer. Oxidative Free radicals are known for generating

their reactions. That is highly reactive species that contain one or more unmatched electron within their outer envelope. When They're formed, the ripple reaction begins. Antioxidants respond to stop that reaction through eliminating intermediate radicals & inhibiting alternative oxidation reactions through oxidation them (Hamidpour *et al*. 2017).

Herbs and animals are a great source of natural antioxidants. Alternatively, may also be synthesized by chemical process together with by a variety of agriculture wastes using a biological process. Depending on their solubleness are generally classified as water-soluble and fat-soluble. Generally, Hydrosoluble these include ascorbic acid, glutathione, and uric acid, perform functions within the cellular cytosol and blood plasma. Examples of fat dissolved that protection of cellular membranes from lipid peroxidation include αtocopherol, carotenoids and Ubiquinol. A further classification divides antioxidants in enzymes and enzyme-free antioxidants. In addition, there are certain antioxidants as micronutrients that cannot be produced by the body itself, such as vitamin E, beta-carotene and vitamin C, which should therefore necessarily added to the normal feeding (Nemati ., 2021).

Chapter Three: Methodology

3.Materials and Methods:

3.1. Chemicals:

3.1. The used chemicals, according to the chemical agents and their source are listed in table (3-1).

No.	Chemical agents	Source
1.	Alloxan powder	United State America (U.S.A)
2.	ALT (GPT) Colorimetric. Kit	SPECTRUM company, Germany
3.	AST (GOT) Colorimeteric. Kit	SPECTRUM company, Germany
4.	Eosin-hematoxylin stain	Merck, (Germany)
5.	Formalin 10%	TEBIA Company, (USA)
6.	Glucose kit	Germany
7.	Glutathione (GSH) Kit	Elabscience Biotechnology/ China
8.	HB1AC	Germany
9.	Kitamine	Noorbrok, (England)
10.	Lipid profile kit	Germany
11.	MDA	Elabscience Biotechnology/ China
12.	Xylazine	Noorbrok, (England)

3.2. Instruments:

3.2. The instrument used in this study with their sources are shown in table (3-2).

NO	Instruments	Source
1.	Analytical Sensitive Balance	Sartorius / Germany
2.	Auto home analyzer	GENEX X CHEM-S1/ USA
3.	Balance for animals	Shimadu Company\ Japan
4.	Centrifuge	HettichRotofix11/Japan
5.	Digital camera	ToupCam/ Chin
6.	Dissection set	China
7.	EDTA tube	Germany
8.	Electric centrifuge (80-2)	China
9.	Electric grinder	China
10.	ELISA biotech	USA
11.	Eppendorf tube	Bulbs /England
12.	Freezer	Denka /China
13.	Gel tube	Germany
14.	Gloves	Malaysia
15.	Incubator	Faithful /Malaysia
16.	Insulin syringes	India
17.	Light microscope	Lice / China
18.	Micropipette	Biobase/China.
19.	Rack for blood standing	China
20.	Spectrophotometer	Sesil, England
21.	Sterile Syringes	China
22.	Test tube	China

3.3. Experimental Animals:

The study is conducted through November 2021. Fifty in mixed health Local Rabbitt Race (Oryctolagus Cuniculus), weight (1300-1700) Kg. Normal environment light cycle conditions 12:12 h/day and animals fed with green fodder and water.

3.4. Inducing diabetes mellitus:

Diabetes mellitus was induced in rabbits fasting overnight through one injection of alloxane monohydrate (100mg/kg bw) in a marginal ear vein. Each 100 mg dose of alloxan was diluted in one ml of normal saline (Mir *et al*., 2013).

3.5. Preparation of aqueous romarin extract:

The extract has been prepared by soaking 5-10 g L 1dry leaves in boiling water during 10 minutes. After cooling to 25 degrees Celsius, the solution was filtered to remove the leaves before use (Shokrollali *et al*., 2011).

3.6. Aqueous extracts of ginger:

Ten grams of dry sample (powder) was crushed in the mixer with 100 ml (10%) of distilled water for 1 minute. The ground mixture was filtered into a thin cotton cloth and watery extracts (AEs) were used (Borhanshokrollali, 2015).

3.7. The experiment plan:

Fifty mixed rabbits were ten rabbits control,forty rabbits induction of diabetes mellitus by alloxan and ensure occurrence of diabetes mellitus and randomly distributed into five groups (10/group):

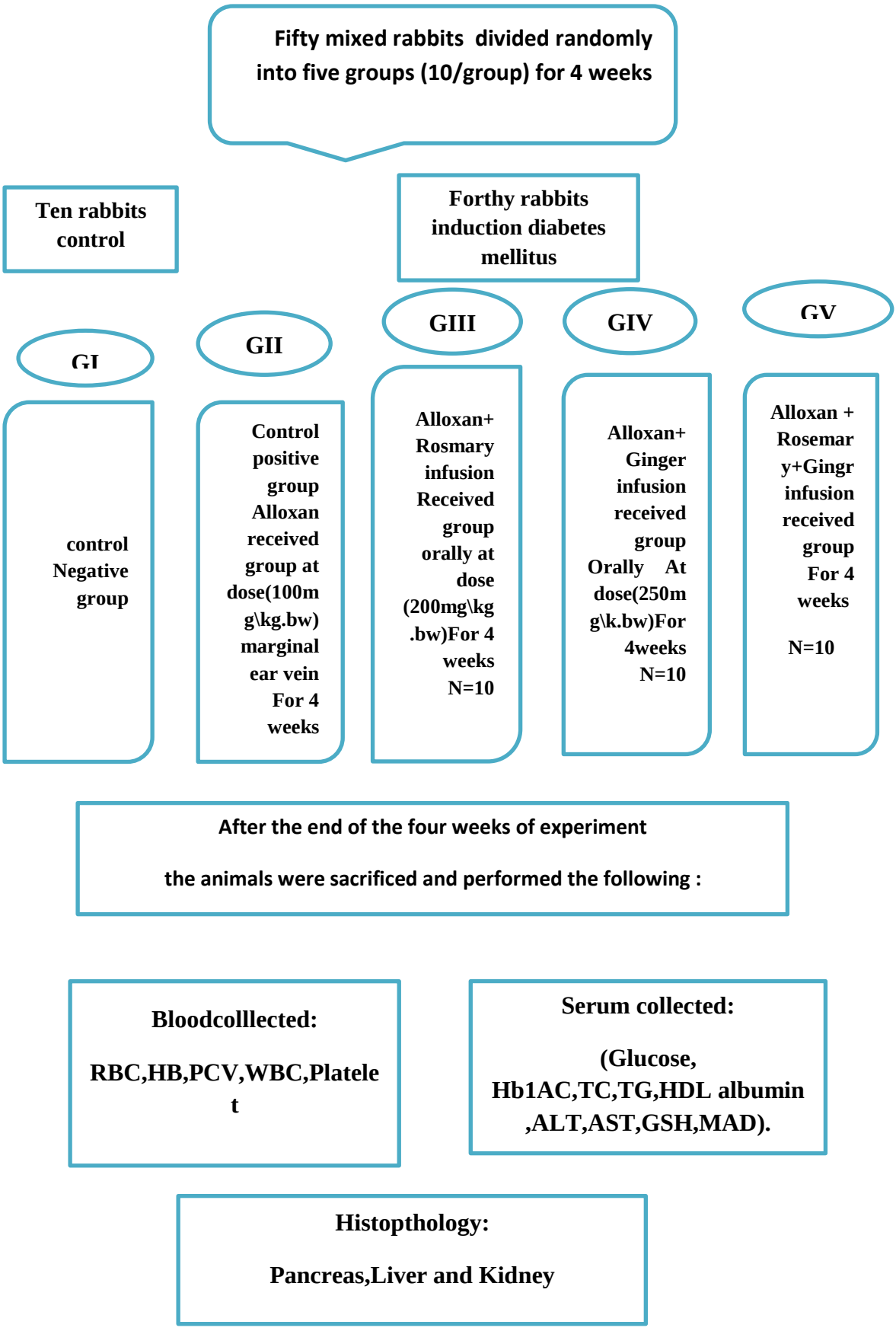
1- Group (G1) controls negative administration orally of distilled water.

2- Group (G2) positive control administered alloxan. At the dose of the marginal ear vein (100 mg kg.bw), single injection. (Mir *et al*., 2013).

3-Group (G3) administered rosemary infusion orally daily in one dose (200mg/kg. bw) (2ml) in diabetic rabbits (Najl., 2012) in alloxan diabetic rabbits.

4- Group (G4) administration of ginger infusion orally daily on a dose basis (250 mg/kg body weight)(2.5ml) in alloxan diabetic rabbits (Entedhar *et al*., 2016).

5- Group (G5) administers rosemary infusion orally daily in one dose (200mg/kg. bw) (2ml) and ginger infusion orally daily on a dose basis (250 mg/kg body weight) (2.5ml) in alloxan diabetic rabbits.



Figuer (3-1): The experimental design.

3.8. Blood and tissue specimen collection:

The animals were given anesthesia through injection (90 mg/kg) of ketamine and (40 mg/kg) xylazine. Blood samples were collected four weeks in a study of a cardiac puncture experiment. Was made with a 5 ml disposable syringe and 5 ml of blood was collected slowly and quietly. 2ml of blood collected in gel test tubes (to prepare serum) that leaves for 30 minutes at room temperature and then used to obtain serum centrifuge at 3000 revolutions per minute for 15 minutes to separate the serum and place in the Eppendorf tubes that have been stored in the freezer in -20C (Amin and Ahlfors 2008).2ml in anticoagulant test tube for CBC. At the end of the experiments, the rabbits were sacrificed following the abdominal lumen was opened and the pancreas, liver and kidneys were withdrawn and then added formalin (10%) as a fixator for histopathological preparation.

3.9. Hematologic parameters:

Was performed using a veterinary hematologic analysis (Scan HM5 Hematology System Abaxis Europe, United Kingdom).

3.9.1. Complete blood count:

It's a blood test used to measure all blood cells through an automated hematological analyzer (George-Gay and Parker., 2003). See Appendix (I).

3.10. Biochemical parameters:

3.10.1. Estimated blood glucose:

Principle:

D-Glucose + H_2O + O_2 _____ > Gluconic acid + H_2O_2.

POD

$2H_2O_2$ + 4-Aminoantipyrine + p-Hydroxybenzoic acid

_____> colored quinonic derivative + 4 H_2O.

(Trinder., 1969) as Appendix (II).

3.10.2. Estimate for HB1 CA:

Principle:

The percentage HbA1c whole blood can be directly determined by utilizing the interaction of antigen and antibody. Total hemoglobin and HbA1c have the same unspecific absorption rate of Latex particles. When we add mouse antihuman HbA1c monoclonal antibody (containing Multiple subunits), "Latex--HbA1c--mouse antihuman HbA1c monoclonal antibody--HbA1c--Latex" complex is formed. The amount of this complex is proportional to the amount of HbA1c absorbed onto the surface of latex particles. The amount of agglutination is measured absorbance. The HbA1c value is obtained from a calibration (Davidson *et al*., 2005) as Appendix (III).

3.10.3. Measurement of albumin concentration (g/DL):

principle:

Measurement of albumin is based on its binding to the indicator dye bromocresol green (BCG) at pH 4.3 to form a blue – green colored complex. The intensity of the blue – green colored is directly proportional to the concentration of Albumin in the sample its determined by monitoring the increase in absorbance at 623 nm, or 578 nm.

Albumin + BCG *PH* 4.3→Albumin_BCG complex

Serum albumin was estimated through calorimetric kits (Ghasemzadeh *et al*., 2010) as Appendix(VI).

3.10.4. Serum lipid profile estimate: refer to Appendix (IX).

3.10.4.1. Estimated total serum cholesterol level (mg/DL):

Principle:

Easter of cholesterol+H2O *Chol. estease* Cholesterol + Fatty acids Cholesterol +O2 *Chol. oxidase* Cholest-4-en-one+H2O2 H2O+4-Aminophenazone + phenol *peroxidase* Quinonimine Reagent: (Fasce., 1982) as Appendix(IX).

3.10.4.2. Serum concentration estimate of triglycerides (mg/DL).

Principle:

Triglyceride $lipoprotein\ lipase$ Glycerol + fatty acid Glycerol + ATP $Glycerol\ kinase,Mg$++ Glycerol-3-phosphate+ADP Glycerol-3-P+O2 $3{-}G{-}P{-}oxidase$ Dihydroxyacetonene-p+H2OH2O2+4-Aminophenazone+p+Chlorophenol$peroxidase$ Quinonimine+ H2 (Fossati and Prencipe .,1982) as Appendix (IX).

3.10.4.3. Estimation of the serum HDL cholesterol concentration (mg/dL):

Principle:

Cholesterol esters + H2O $Chol.esterase$ Cholesterol + fatty acid Cholesterol +½O2 + H2O $Chol.oxidase$ Cholestenone + H2O2 2 H2O2 + 4-Aminoantipyrine + DCFS $peroxidase$ Quinoneimine + 4H2O response described (Grove., 1979) as Appendix (IX).

3.11. Serum antioxidant estimations:

3.11.1. Serum malondialdehyde (MDA) assessment: (µM/l)

Principle:

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm (Muslih *et al*., 2002), illustrated in Appendix (VII).

3.11.2. Serum glutathione (GTH) assessment: µM/l.

Serum glutathione was measured using the Ellmans reactive method previously used by (Alzamely *et al*., 2001). This is shown in appendix (VIII).

3.12. Study pathologic changes:

The pancreas, the liver and kidneys from each animal were quickly removed and preserved in the preparation of formaldehyde at 10% of the histolopathological study, according to (Mescher, 2010) as shown in Appendix (X).

3.13. Statistical Analysis:

The data from the statistical analysis were analyzed using Release 9.1 of the Statistical Analysis System (SAS., 2012). Was used to assess meaningful changes in group results. Five treatment methods were segregated using a "protected" Duncan assay ($p \leq 0.05$) (SAS Institute version 9.1., 2012).

Chapter Four: Results and Analysis

4.Results:

4.1. Effect of Rosemary, Ginger and their combination on Blood Parameters in diabetic rabbits.

The count of RBC, PCV volume were showed a significantly decreased ($p \leq 0.05$) in a (GII) in comparison with (GI,GIII,GIV,GV). There is a significant increase in (GIII,GIV,GV) compared to (GII) and non significant change between them and control group as table (4-1).

There are no significant differences observed Hb values in the control group and other treated groups.

The current results were showing a significant decrease ($P \leq 0.05$) of the WBCs count in group (GII) comparison with (GI,GIII,GIV,GV). A significantly increase ($P \leq 0.05$) of the WBCs in (GIII, GIV, GV) comparison to (GII), non significant change between them and a control group.

Table (4-1): Effect of Rosemary, Ginger and their combination on Blood Parameters in diabetic rabbits.

Groups / Parameters	GI	GII	GIII	GIV	GV
RBC (cell^10^{12}/l)	6.63 ± 0.10 A	5.48 ± 0.19 B	6.51 ± 0.10 A	6.61 ± 0.09 A	6.63 ± 0.02 A
HB (g\dl)	13.88 ±0.22 A	13.12 ± 0.30 A	13.84 ± 0.16 A	13.74±0.19 A	13.73± 0.14 A
PCV %	39.74 ±0.71 A	35.02 ±1.76 B	39.62 ±0.51 A	40.62±1.10 A	39.74 ±0.71 A
WBC 10^9/I	7.70 ± 0.24 A	5.82 ± 0.43 B	7.50 ± 0.20 A	7.74 ±0.27 A	7.70 ± 0.24 A
Platelet 10^9/I	295.40±2.31 C	235.00±8.46 D	406.20±6.62 A	381.00±5. 72 A	293.40±1.91 C

Different letters represent significantly difference .N=10

(P≤ 0.05) means ± SD.

GI. Control negative group GII. Alloxan positive group

GIII .Alloxan + Rosemary infusion Group in diabetic rabbits

GIV.Alloxan +Ginger infusion group in diabetic rabbits

GV. their combination group in diabetic rabbits

4.2. Effect of Rosemary, Ginger infusion and their combination on Blood in Glucose and HB1AC parameters in diabetic rabbits

4.2.1. blood glucose:

A significant increase ($P \leq 0.05$) in glucose values was noticed for (GII) of in comparison with (GI, GIII, GIV, GV) whereas, significantly decline ($P \leq 0.05$) in (GIII, GIV, GIV) compared with (GI and GII) and no significant change between GVand GI) as table (4-2).

4.2.2. HB1Ac:

As table (4-2). A significant increase ($P \leq 0.05$) in glucose values was noticed for (GII) of in comparison with (GI, GIII, GIV, GV) whereas, significantly decline ($P \leq 0.05$) in (GIII, GIV, GIV) compared with (GI and GII) and no significant change between GVand GI).

Table (4-2): Effect of Rosemary, Ginger infusion and their combination on Blood in Glucose and HB1AC parameters in diabetic rabbits .

Groups / Parameters	GI	GII	GIII	GIV	GV
Glucose (mg\dl)	85.80±3.42 C	239.80±934 A	188.20±4.55 B	185.60±4.15 B	84.40±5.33 C
HB1AC	4.56 ±0.14 C	8.96 ± 0.69 A	7.42 ±0.9141 B	7.34 ± 0.13 B	4.54 ± 0.14 C

Different letters represent significantly different .N=10

(P≤ 0.05) means ± SD.

GI. Control negative.

GII. Alloxan positive group group.

GIII .Alloxan + Rosemary infusion Group in diabetic rabbits.

GIV.Alloxan +Ginger infusion group in diabetic rabbits.

GV. Their combination group in diabetic rabbits.

4.3. Effect of Rosemary, Ginger infusion and their combination on Albumin and on liver function Parameters in diabetic rabbits

4.3.1. Albumin:

A significant decrease (P≤ 0.05) in albumin were noticed (GII) in comparison with (GI, GIII, GIV, GV) whereas, significantly increase (P≤ 0.05) in (GIII,GIV,GV) compared with (GI,GII) as table (4-3).

4.3.2. Aspartate amino transferase concentration (AST):

A significant elevation (P≤ 0.05) in AST concentration was noticed for group (GII) in comparison with (GI, GIII, GIV, GV) whereas, significantly decrease (P≤ 0.05) (GIII, GIV, GV) compared with (GII) as table (4-3).

4.3.3. Alanine Transferase concentration (ALT):

In table (4-3). The result showed significant higher (P≤0.05) in ALTconcentration. Were noticed for group (GII) in comparison with (GI, GIII, GIV, GV) whereas, significantly decrease (P≤ 0.05) (GIII, GIV, GV) compared with (GII) as table (4-3).

Table (4-3): Effect of Rosemary, Ginger infusion and combination of rosemary and ginger on Albumin and on liver function Parameters in diabetic rabbits.

Groups / Parameters	GI	GII	GIII	GIV	GV
Albumin (g\l)	3.78 ±0.31 B	3.70 ± 0.26 C	3.81 ±0.23 A	3.62± 0.37 A	4.00 ± 0.16 B
AST (U\l)	112.00±8.49 C	140.00±1.37 A	129.80±1.0 B	135.40±1.77 B	133.00±1.34 B
ALT (U\l)	66.60±14.93 C	130.80±2.88 A	110.00±1.67 B	105.40±1.07 B	102.40±0.74 B

Different letters represent significantly difference . N=10

(P≤ 0.05) means ± SD.

GI. Control negative group.

GII. Alloxan positive group.

GIII .Alloxan + Rosemary infusion Group in diabetic rabbits.

GIV.Alloxan +Ginger infusion group in diabetic rabbits.

GV. their combination group in diabetic rabbits.

4.4. Effect of Rosemary, Ginger infusion and combination of rosemary and ginger on lipid profile Parameters in diabetic rabbits.

In table (4-4).The result was shown significant higher ($P \leq 0.05$)in cholesterol (GII) in comparison with (GI, GIII, GIV and GV) whereas, significantly decrease ($P \leq 0.05$) in (GIII,GIV,GV) in comparison with (GI and GII).

Triglyceride concentration showed a significant increase ($P \leq 0.05$) in (GII) in comparison with (GI,GIII,GIV and GV) whereas, significantly decrease ($P \leq 0.05$) in (GIII,GIV,GV) in comparison with (GI and GII).

HDL concentration, significant decline ($P \leq 0.05$) in (GII) in comparison with (GIII, GIV, GV) whereas, significantly increase($P \leq 0.05$) in (GIII,GIV and GV) and no significant change between (GV and GI) as table(4-4).

Table (4-4): Effect of Rosemary, Ginger infusion and combination of rosemary and ginger on lipid profile Parameters in diabetic rabbits.

Group / Parameters	GI	GII	GIII	GIV	GV
Cholesterol (mmol\l)	62.20±0.37 C	74.43±0.50 A	65.80 ±0.20 B	62.60±0.67 B	64.40 ±0.87 B
Triglyceride (mmol\l)	92.12 ±0.90 C	97.55±1.87 A	73.48±1.03 B	76.24±1.37 B	78.70 ±1.83 B
HDL (mmol\l)	20.18 ±0.33 BC	18.70±0.91 C	23.94 ±0.52 A	21.70 ± 0.89 A	19.82 ±0.17 BC

Different letters represent significantly difference .

(P≤ 0.05) means ± SD.

GI. Control negative group

GII. Alloxan positive group

GIII .Alloxan + Rosemary infusion Group in diabetic rabbits

GIV.Alloxan +Ginger infusion group in diabetic rabbits

GV. their combination group in diabetic rabbits

4.5. Effect of Rosemary, Ginger infusion and combination of rosemary and ginger on serum Antioxidant activity in diabetic rabbits.

4.5.1. Estimation of Glutathione (GSH) concentration:

significantly decrease (P≤ 0.05) in GSH concentration in (GII) in comparsion with (GI,GIII,GIVand GV) whereas ,showed significant higher (P≤ 0.05)in group of (GI,GIII,GIVand GV) and no significant changes between them and (G1). As table (4-5).

4.5.2. Estimation of Serum Malondialdehyde (MDA):

According to result of MDA which show significantly increase (p≤ 0.05) in(GII) in comparsion with (GI.GIII,GIVand GV) whereas ,showed significant decrease (P≤ 0.05)in (GIII,GIVand GV) compred with (GIand GII) as table(4-5).

Table (4-5): Effect of Rosemary, Ginger infusion and combination of rosemary and ginger on serum Antioxidant activity in diabetic rabbits.

Group / Parameters	GI	GII	GIII	GIV	GV
GSH (µmol\l)	3.34± 0.42 A	1.44 ± 0.17 B	2.38 ± 0.26 AB	2.02 ± 0.25 AB	3.52 ± 0.33 AB
MDA (µmol\l)	0.24 ± 0.05 B	0.70 ± 0.08 A	0.52 ± 0.03 BC	0.46 ± 0.08 BC	0.46 ± 0.24 BC

Different letters represent significantly difference .N=10

(P≤ 0.05) means ± SD.

GI. Control negative group

GII. Alloxan positive group

GIII .Alloxan + Rosemary infusion Group in diabetic rabbits

GIV.Alloxan +Ginger infusion group in diabetic rabbits

GV. their combination group in diabetic rabbits

4.6. Histopathology Changes:

4.6.1. Pancreas:

Pancreas of a control group of rabbit showed the normal histology of pancreas figure (4-1). Pancreas of rabbit from group alloxan induced diabetes (4-2). The figure (4-3) revealed normal histological shed acne in alloxan induced diabetes treated with rosemary infusion . In figure (4-4) of rabbit pancreas showed improvement in alloxan diabetic rabbits treated with ginger infusion . The figure (4-5) showed improvement and normal sizes of pancreatic acini in alloxan diabetic rabbits treated with a combination of two herbal rosemary and ginger infusion .

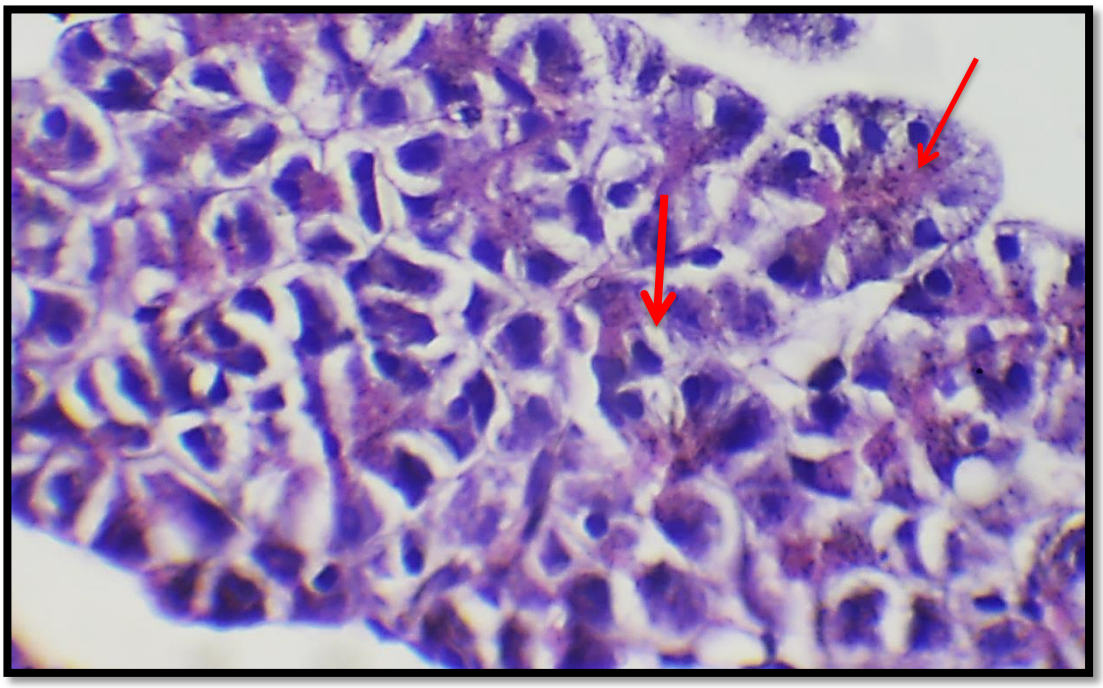


Figure (4-1) Photomicrograph of Pancreas of control rabbits, showed the normal structures of pancreatic acinar tissue (red arrow) represented by single layer of cuboidal cells acini.(40 X, H&E).

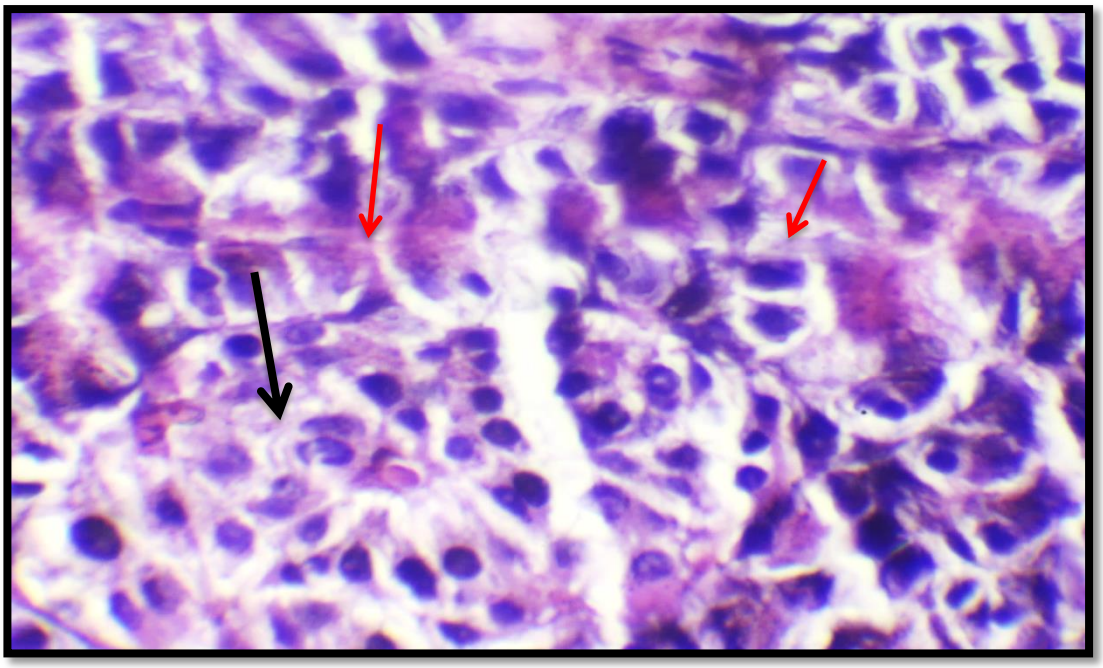


Figure (4-2) photomicrograph of pancreas of alloxan in diabetic rabbits showed sever congestion and necrotic changes of pancreatic acini (red arrow), marked damage of Langerhans islet cells (black arrow), (40X, H&E).

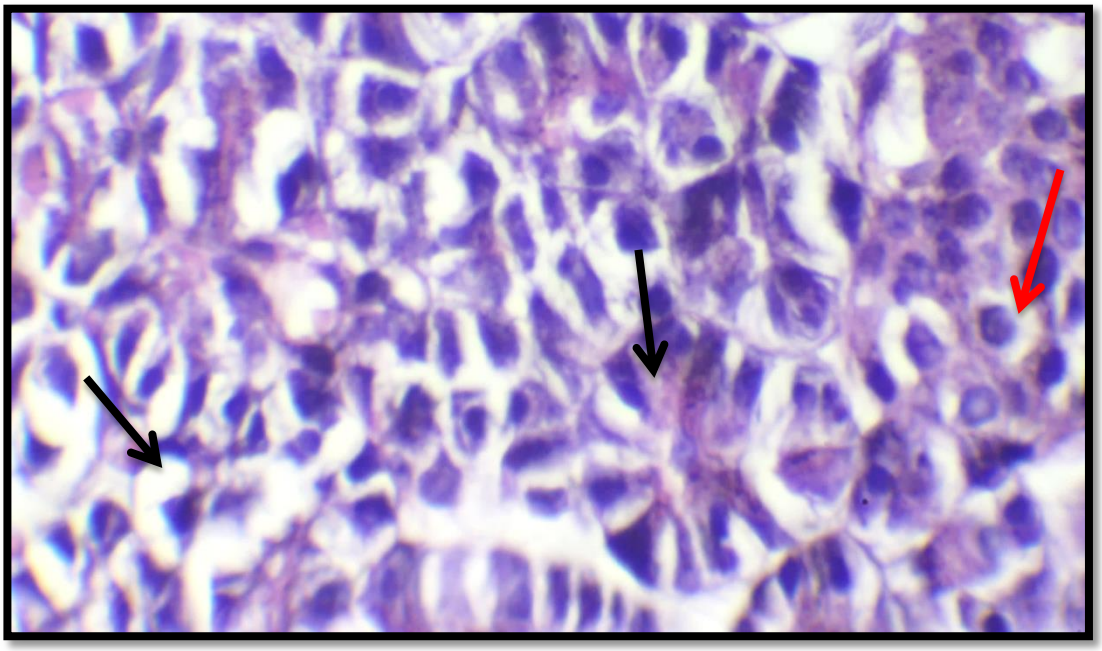


Figure (4-3) photomicrograph of diabetic rabbits pancreas treated with rosemary infusion showed degenerative changes (vacuolation) in acinar epithelia (black arrow), degeneration (cloudy swelling) of Islet cells (red arrow), (40 X, H&E).

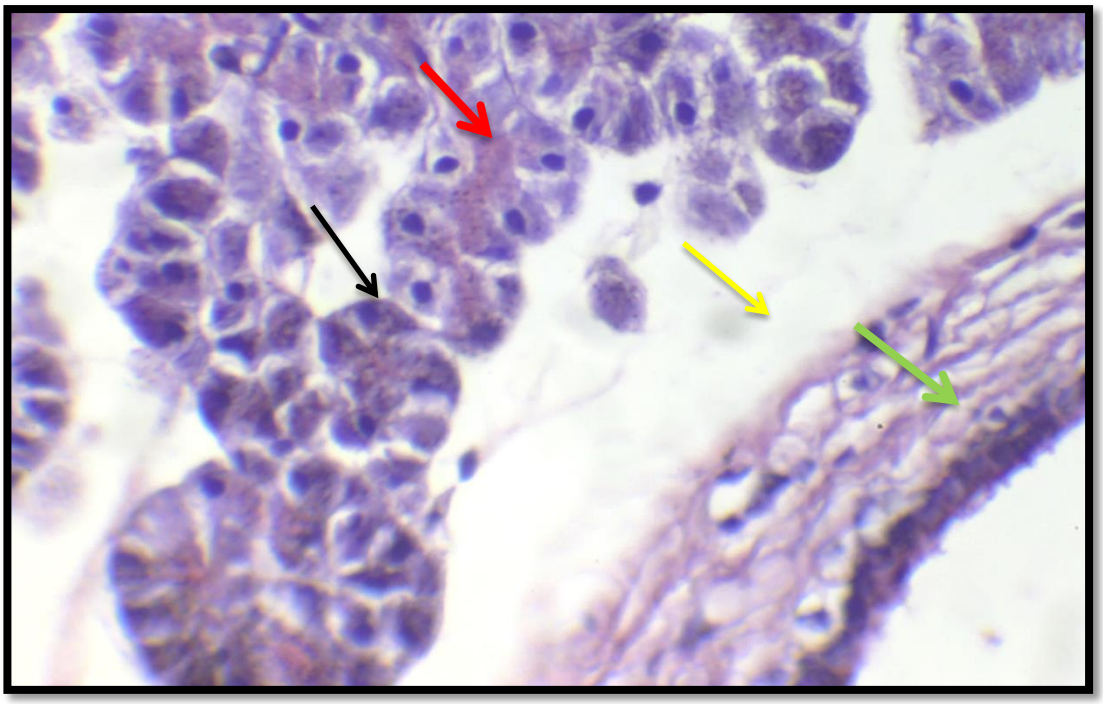


Figure (4-4) photomicrograph of diabetic rabbits pancreas treated with ginger infusion showed slight congestion (red arrow), degeneration of the acinar epithelium (black arrow) and abnormal pancreatic septa (yellow arrow) with normal intertubular duct wall (green arrow). (40 x, H&E).

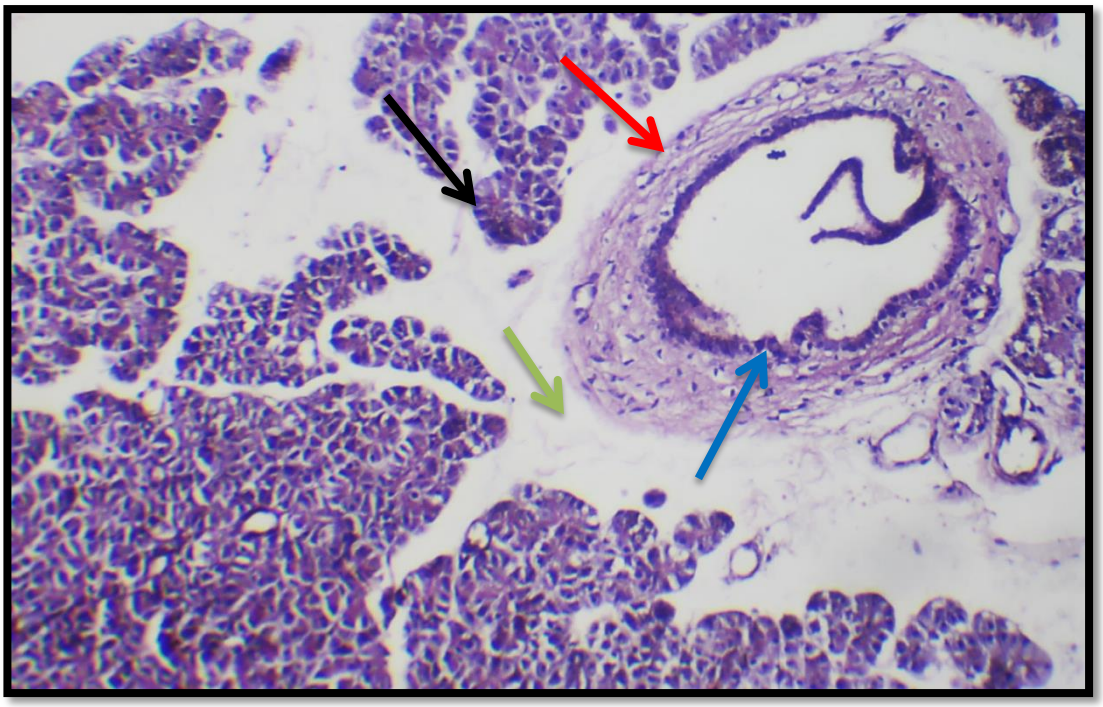


Figure (4-5) Photomicrograph of diabetic rabbits pancreas treated with a combination of both herbal infusion reveled normal pancreatic acinar tissue (black arrow), normal interlobular duct and ductile epithelium (blue arrow) with normal collagen surrounding the duct (red arrow) and close to normal connective tissue septa (Green arrow). (40 X, H&E).

4.6.2. Liver:

Liver tissue from the control groups(figure 4-6) showed a normal histological architecture. The figure (4-7) showed There is severe congestion of portal vein with sever inflammation and areas of fibrosis in group alloxan diabetic rabbits. The figure (4-8) revealed Significant inflammatory response, fibrosis and tissue depletion in alloxan-induced diabetes treated with the rosemary aqueous extract. In figure (4-9) there is area of slight changes indicate the action of extract curative activity in alloxan diabetic treated with ginger infusion on liver tissue. The histological section of rabbit liver showed improvement and semi normal hepatic lobules in alloxan diabetic treated with two herbal rosemary and ginger infusion(4-10).

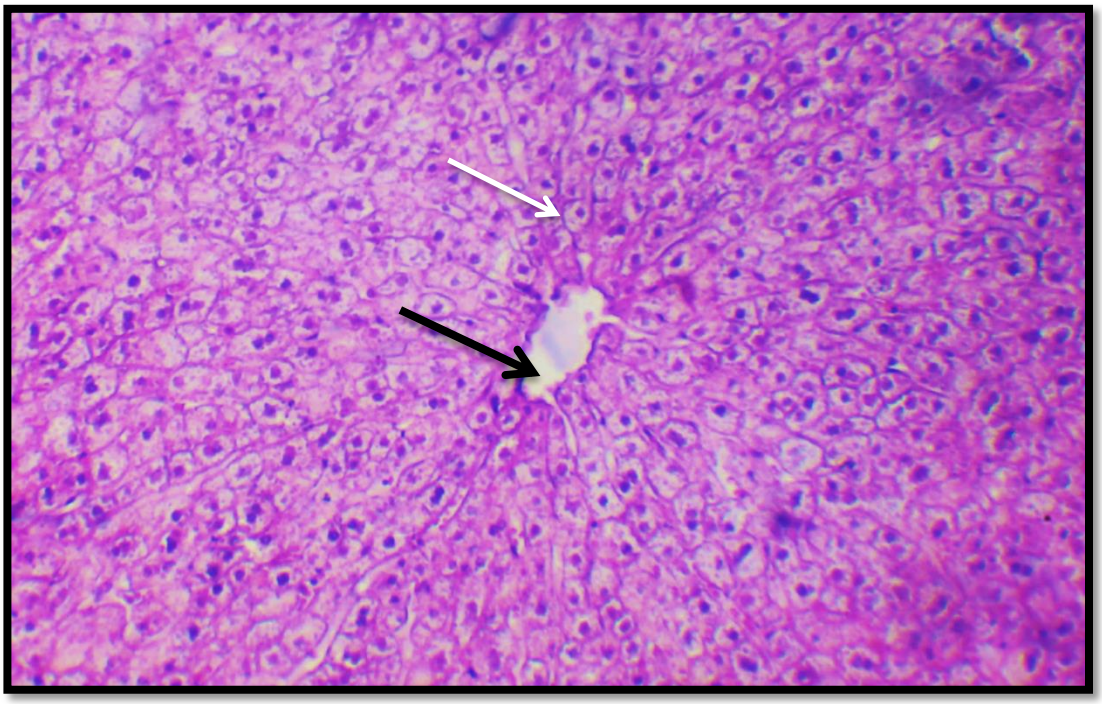


Figure (4-6) Photomicrograph of rabbits liver from the control group showed normal histology with slight congestion in central vein (black arrow), the normal arrangement of hepatic cords around the central vein (white arrow) . (40 X, H&E).

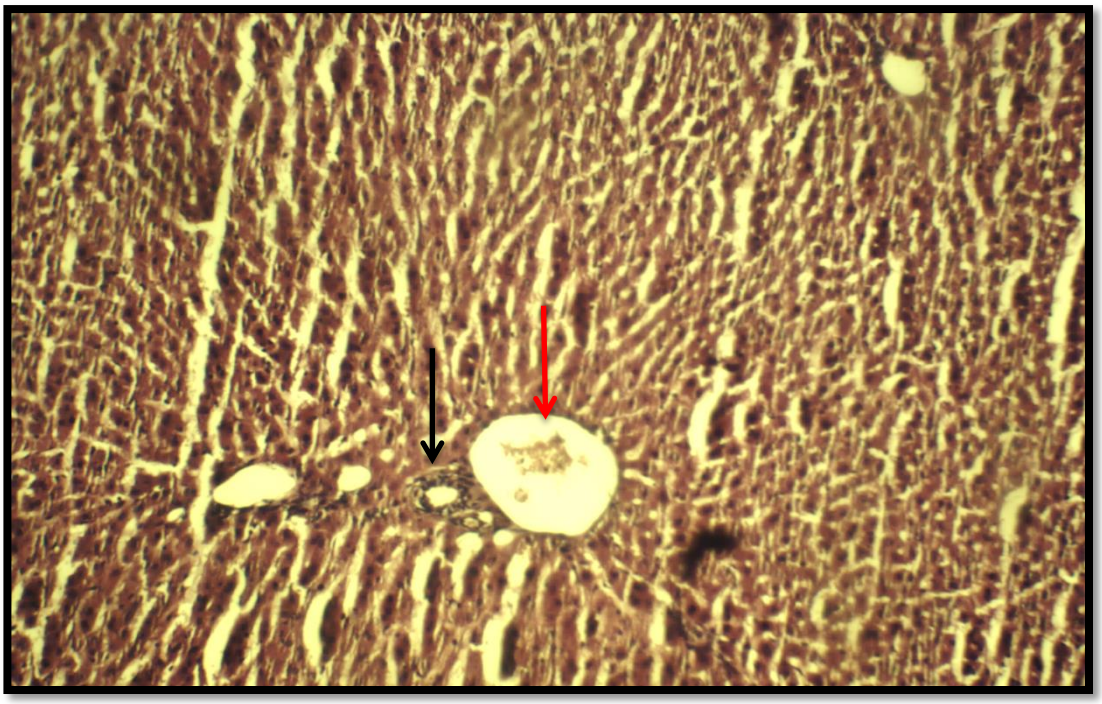


Figure (4-7) Photomicrograph of liver in alloxan diabetic rabbits group, there are severed portal vein congestion (red arrow) with sever inflammatory cell infiltration in portal area (black arrow). (40 X, H&E).

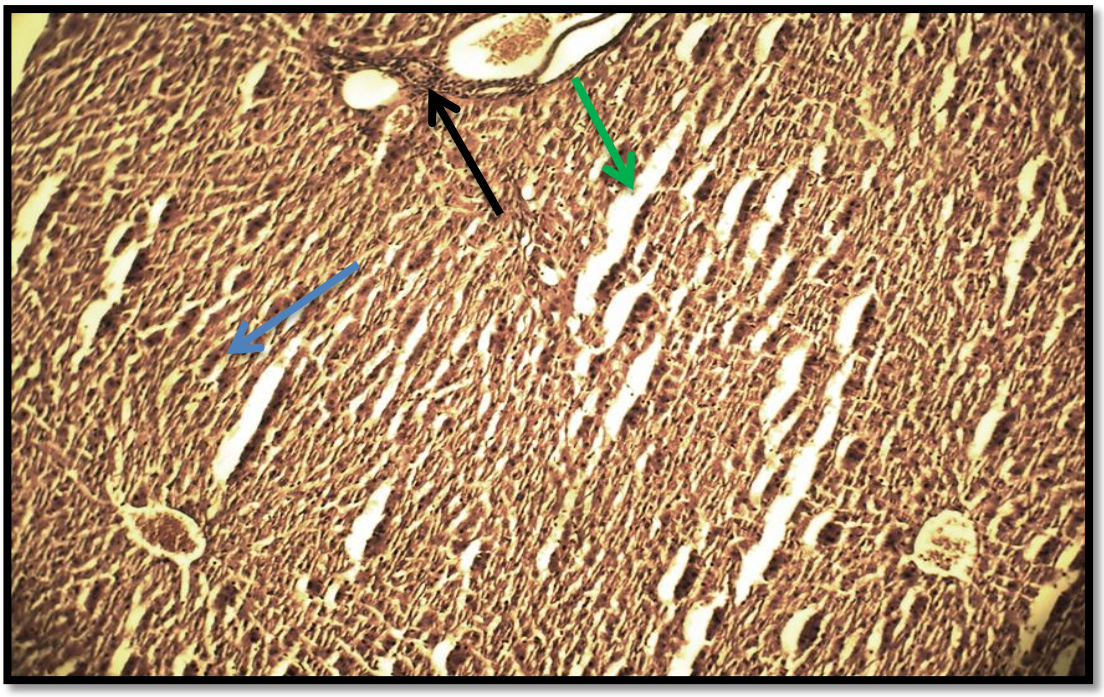


Figure (4-8) Photomicrograph of liver in previously alloxan diabetic animal and treated with rosemary infusion revealed, significant inflammatory response characterized by heavy infiltration of mononuclear cells (black arrow), areas of fibrosis on hepatic tissue (blue arrow) and marked tissue depletion represented by spaces between hepatic cord (green arrow) .(40 X, H&E).

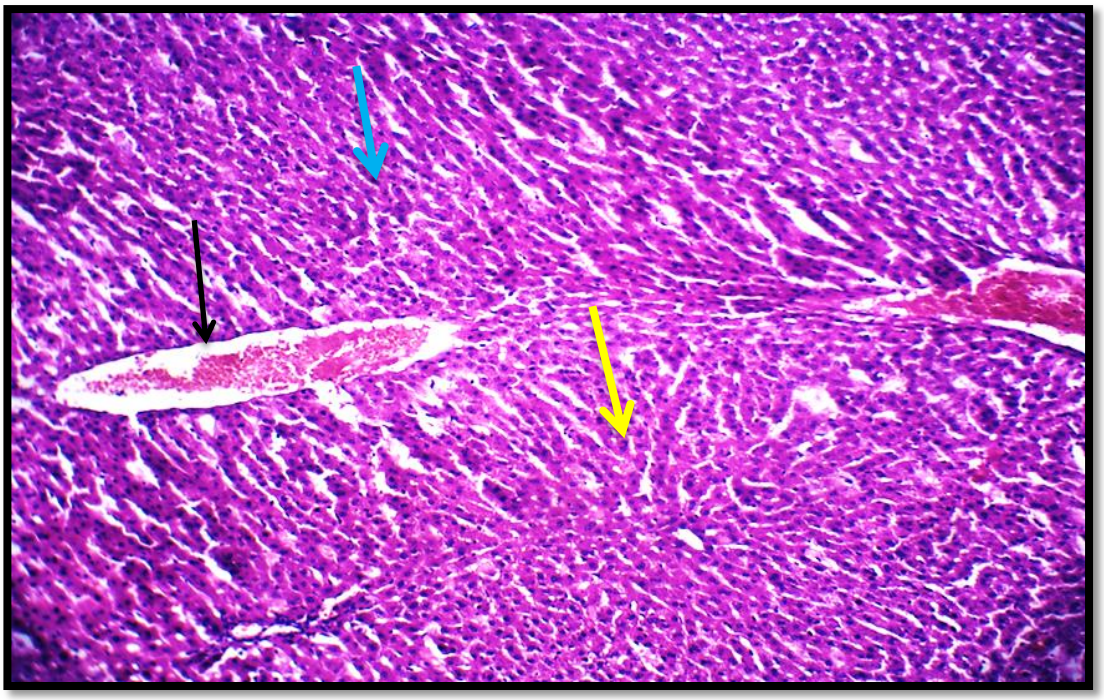


Figure (4-9) photomicrograph of diabetic animal liver treated with ginger infusion, showed severe portal vein congestion (black arrow), mild hepatocyte necrosis (blue arrow) with infiltration of inflammatory cells (yellow arrow) .(40 X, H&E).

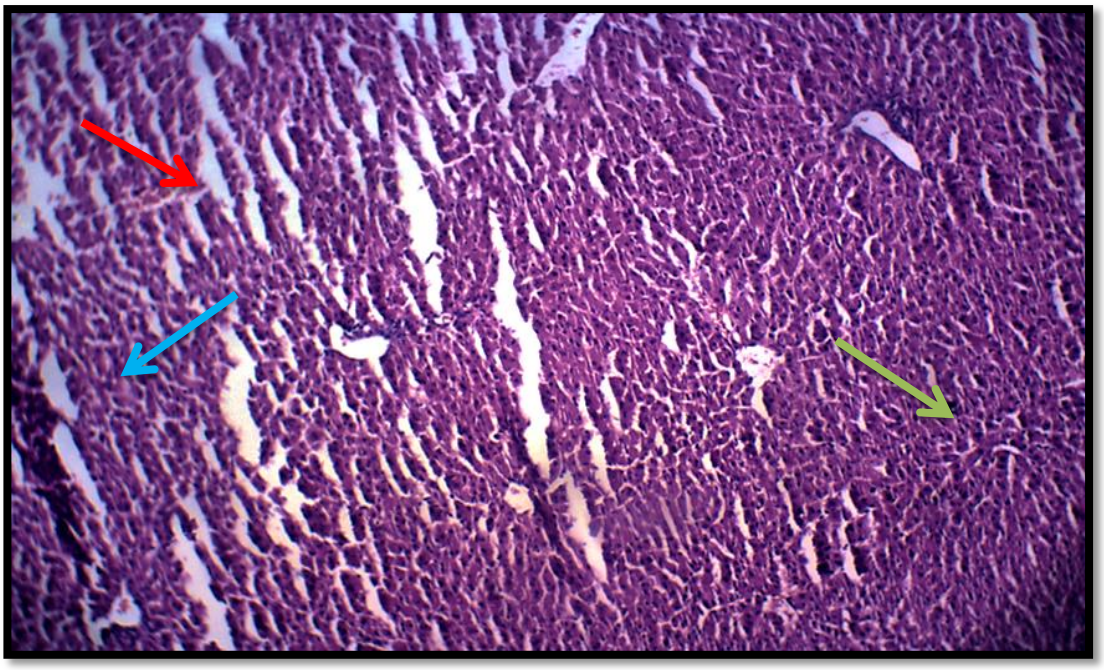


Figure (4-10) photomicrograph of rabbit liver treated with two herbal infusion (ginger and rosemary) showed improvement in the hepatic tissue structure (right side), slight necrosis and inflammation (Green arrow), whereas the left side revealed hepatic depletion (red arrow) and sever fibrotic changes (blue arrow). (40X, H&E).

4.6.3. Kidney:

The histological section of the kidney is shown in figure (4-11) from the control group, reveled normal renal histology. The histopathology section of most of renal tubules revealed dilatation with a thickness of epithelial lining due to hyperplasia, there is a diffuse inflammatory response in alloxan diabeti in figure (4-12).The figure (4-13) showed significant inflammation with severe dilatation of renal tubules necrotic renal epithelial lining showed nearly resembling normal renal tubules in the group treated with rosemary infusion . The figure (4-14) renal tubules showed characteristic changes to normal in the group treated with the ginger infusion . The figure (4-15) in group alloxan diabetic rabbits treated with rosemary and ginger infusion group showed normal tissue structure of renal tissue .

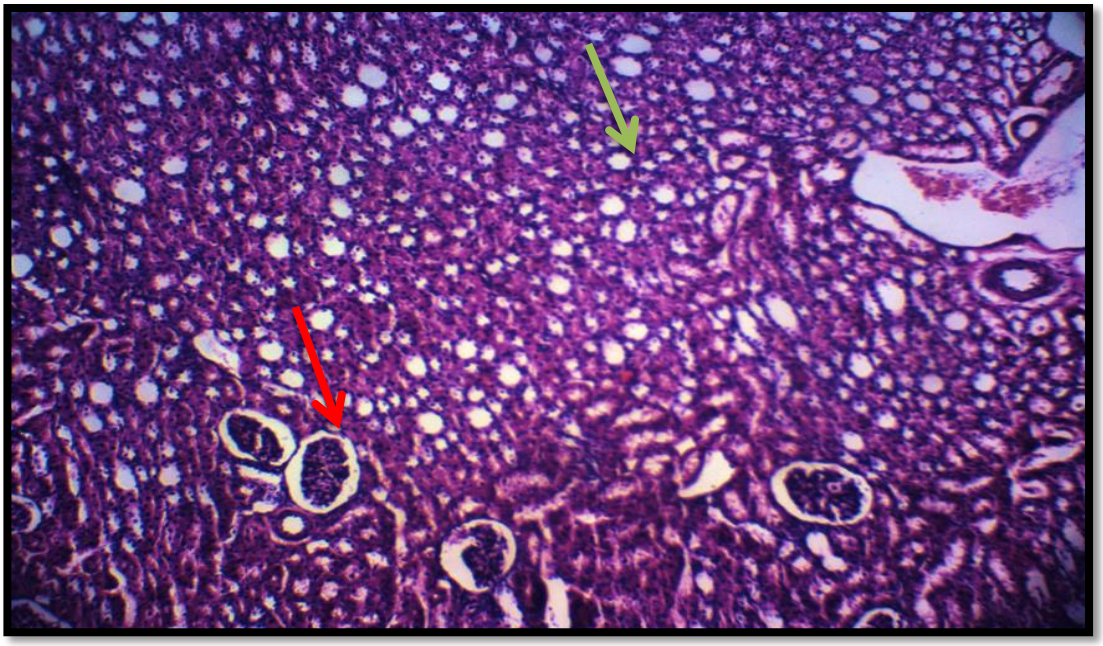


Figure (4-11) Photomicrograph of rabbits kidney of control group revealed normal renal histology, normal renal tubules (green arrow) with some slightly atrophied glomeruli (red arrow). (40X, H&E).

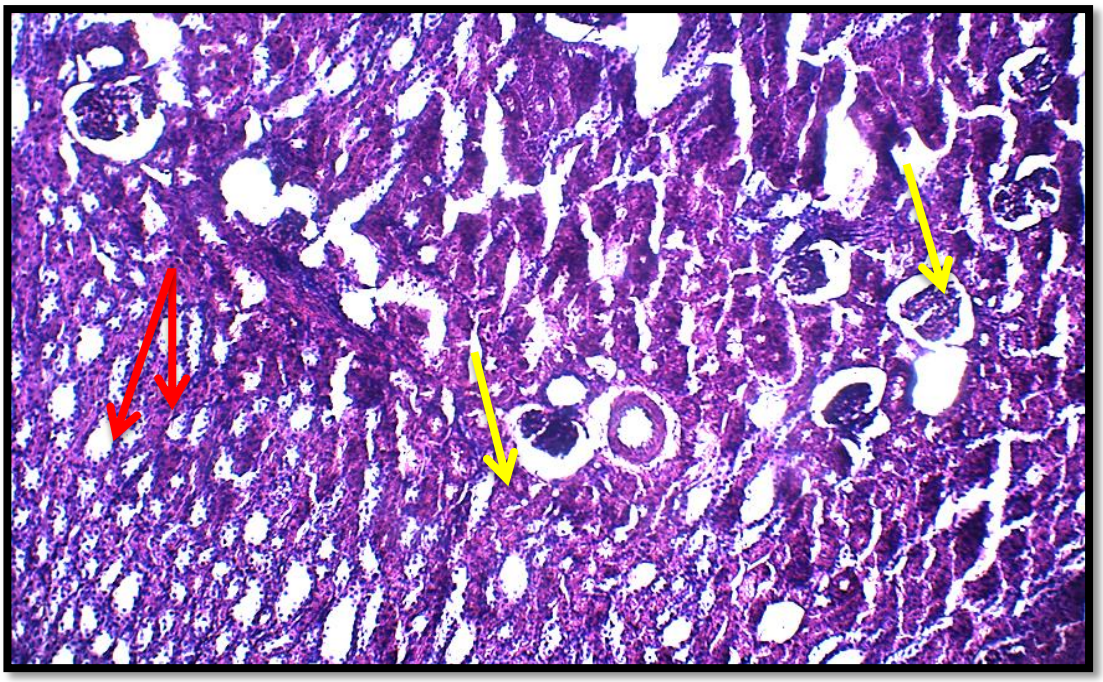


Figure (4-12) Photomicrograph of kidney in alloxan diabetic animal, showed renal tubules dilatation with hyperplasia of epithelial lining (red arrow) with diffuse inflammatory infiltration in renal tissue and glomerular tissue (yellow arrow). (40X, H&E).

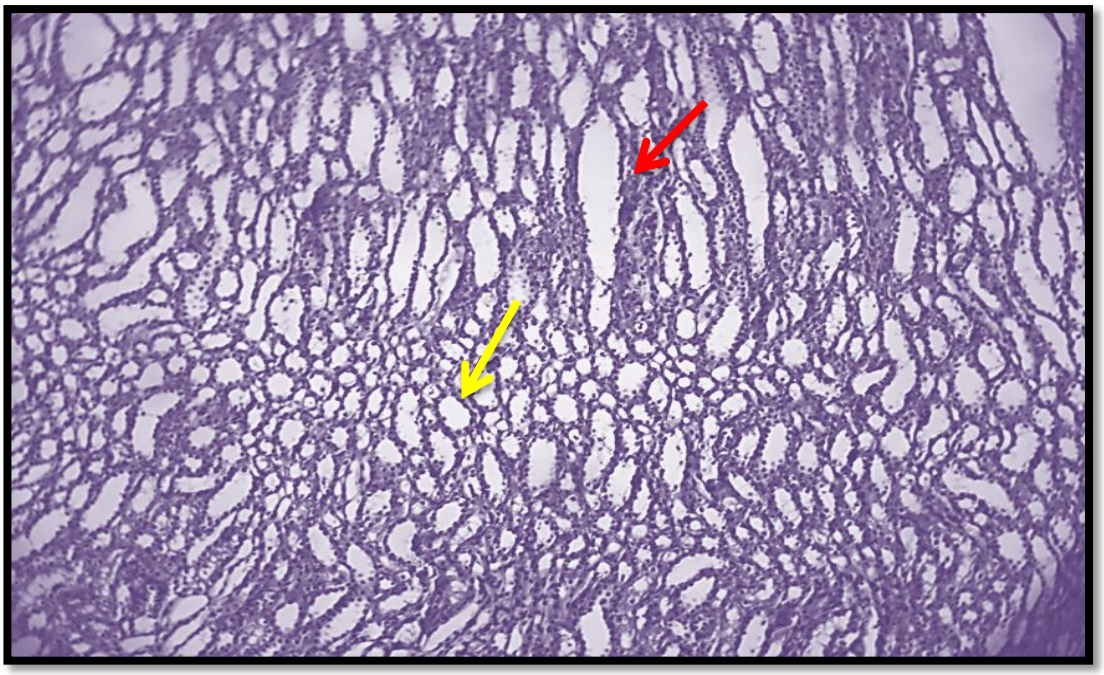


Figure (4-13) Photomicrograph of the kidney of a diabetic rabbits treated with rosemary infusion the upper portion showed significant dilatation and inflammation (red arrow) of renal tubules, necrotic renal epithelial lining, below showed nearly slight changes with necrosis and severe inflammation in renal tubules (yellow arrow). (40X, H&E).

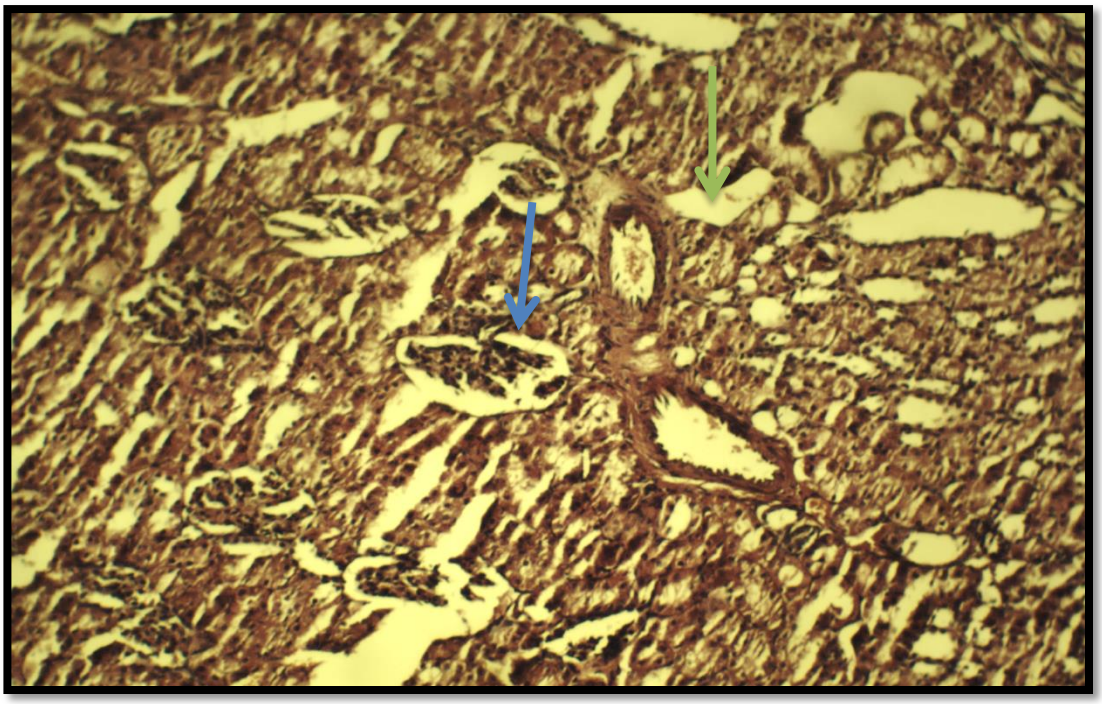


Figure (4-14): photomicrograph of diabetic animal kidney treated with ginger infusion showed necrotic epithelial tissue of renal tubules and the tubules undergo the dilatation (green arrow), with significant necrosis and inflammation of glomeruli (blue arrow). (40 X, H&E).

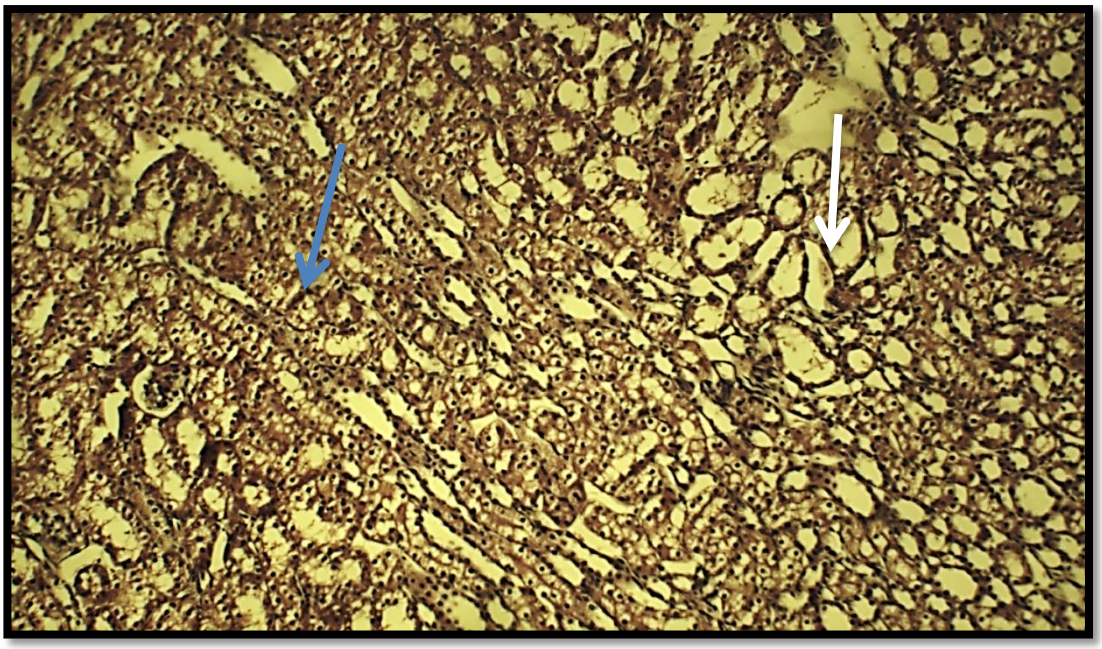


Figure (4-15) photomicrograph for rabbits kidney treated with (ginger and rosemary) infusion, showed significant necrotic renal tubule epithelia (blue arrow) in the left side, in the right side showed areas of nearly resembling normal tissue structure of renal tissue (white arrow). (40X, H&E).

Chapter Five: Discussion

5.1. Effect of Rosemary, Ginger infusion and their combination on Some Blood Parameters in Diabetic rabbits.

The count of RBC, PCV volume were showed a significantly decreased ($p \leq 0.05$) in a (GII) in comparison with (GI,GIII,GIV,GV). There is a significant increase in (GIII,GIV,GV) compared to (GII) and non significant change between them and control group as table (4-1) this agree with (ELkirdasy *et al*., 2015).

There are no significant differences observed Hb values in the control group and other treated groups.

Alloxane intravenous injection rapidly damages Langerhan islet, Destruction of pancreatic beta cells by alloxane may result from a reaction with glutathione or other sulphuric protein group that would inactivate the cell's essential enzyme or coenzyme. Alloxan injection may also lead to the creation of free radicals. That holds back DNA strands in beta cells. The activity of alloxane with inactive Ca+2 protein kinase and calmodulin-dependent protein has also been shown to be linked to insulin secretion (Bishop *et al*., 1985 and Shimran., 2003). Ginger may improve a number of alterations of hematologic parameters in diabetic bunnies.

That was a suggestion the development of anemia within DM is caused by enhanced enzyme-free glycosylation of RBC membranarian proteins, DM resulted increased production of lipid peroxides leading to RBC hemolysis. Hematological indexes are indicators and a reflection of the effects of food processing on animals in terms of the quality of the food ingested and the nutrients available to it satisfies physiological requirements. However, additional studies by measuring fragility in RBC and serum levels of folic acid, iron, cobalt, vitamin B12 and calcium necessary show the exact mechanism of action of ginger extracts on the growing number of RBC in diabetes rabbits. As a result, ginger extracts stimulate synthesis (erythropoiesis) and the concentration of erythrocytes up to RBC normalization in anemic diabetic rabbits. The corresponding statistical decrease in PCV in diabetic animals and its normalization with the processing of ginger extract suggests their role in erythropoiesis (Kennedy & Baynes., 1984).

Results from RBC, Hb and PCV suggest that ginger extracts have antioxidant properties and help stabilize the RBC membrane by bonding with proteins and

carbohydrates that are components of the RBC membrane and are thus able to prevent degradation of membrane and to counteract the anemic effect of alloxane (Ganong., 1991). No significant differences were found in Hb values in the control and non-control groups. No diabetes inducts or gives Z. Officinal resulted in a significant change in hemoglobin concentration (Olayaki *et al*., 2007). Hemoglobin values, a conjugate protein containing iron, that fulfills the physiologic function of oxygen transport and carbon dioxide, which did not show any major changes in diabetes or diabetes groups When Compared to the comparison group, it's probable the animals did not suffer any impairment in the respiratory capacity of any group, indicating that the blood oxygenation capacity of animals is not impacted by diabetes or treated diabetes, it could be said that the Ginger extract worked by lowering high glucose levels diabetes rabbits treated with ginger extract (Kennedy and Baynes.,1984).

The current results were showing a significant decrease ($P \leq 0.05$) of the WBCs count in group (GII) comparison with (GI, GIII, GIV, GV). A significantly increase ($P \leq 0.05$) of the WBCs in (GIII, GIV, GV) comparison to (GII), non significant change between them and a control group this finding agrees with (Olayaki *et al*., 2007).

The ginger increases the defense mechanism of the body against infections in diabetic rabbits (Olayaki *et al*., 2007) while rosemary enhance the immunity (Hamidpour *et al*., 2017).

DM is caused by enhanced enzyme-free glycosylation of RBC membranarian proteins, DM resulted increased production of lipid peroxides leading to delayed healing due decrease in production or efficiency of platelet (Olayaki *et al*., 2007).

Rosemary increases the numbers and the efficiency of the platelet (Arablou *et al*.,2014). Ginger because has powerful antioxidant and higher concentration of romance acid, improving the numbers and the effectiveness of the platelet (Ramadan *et al*., 2013)

5.2. Effect of rosemary, ginger infusion and their combination of Giucose and HB1AC Parameters in Diabetic rabbits.

A significant increase ($P \leq 0.05$) in glucose values was noticed for (GII) of in comparison with (GI, GIII, GIV, GV) agreed with (Sarhat *et al*., 2016) whereas, significantly decline ($P \leq 0.05$) in (GIII) agree with (Mabrouk., 2016), (GIV) agreement with (Sarhat et al., 2016) compared with (GI and GII) and no significant change between (GVand GI) this result agrees with (Mabrouk.,2016), as table (4-2).

A substantial increase ($P \leq 0.05$) in HB1AC values for the (GII) comparison to (GI,GIII,GIV,GV), whereas treated groups, significantly decline ($P \leq 0.05$) in (GIII) this result corresponds to (Mang *et al*., 2006 and Khan *et al*., 2014). (GIV) the result is agreed with (Mozaffari-khosravi *et al*., 2014 and Khandouzia *et al*., 2015).

A possible mechanism for the hypoglycemic effect of Rosmarin, it was suggested that the amount of insulin be raised (Vanithadevi & Anuradha., 2008).

Furthermore, an A recent study has found that rosemary leads to cell regeneration β cells, indicating that rosemary reduces glycemia by stimulating insulin secretion from the remaining or renewed beta cells (Alnahdi., 2012). In addition, reduction of fasting glucose can result from rosemary, which may inhibit intestinal uptake of glucose by inhibiting the intestinal a-amylase enzyme (McCue and Shetty., 2004) or an enzyme called a-glucosidase (Koga *et al*., 2006). As well, the remarkable antidiabetic impacts of rosemarinus officnalis may be the consequence of its powerful antioxidants properties. It also has the ability to produce hypoglycemic activity an independent mechanism for insulin secretion, e.g. suppressing protein glycation and inhibiting endogenous generation of glucose (Bakirel *et al*., 2007). Treatment of alloxane-induced diabetic rats with leaves of rosemary extract significantly decreased glycemia . This has induced the liver to return to normal homeostasis during experimental diabetes (International Journal of Pharma Tech Research., 2014).

The anti-hyperglycemic activity of rosemary leaf extract can be due to a stimulative effect on insulin secretion or improvement of the effect of insulin (Alnahdi, 2012). Rosemary can have an additional pancreatic action mechanism or rosmarinic acid that improves pancreatic function and therefore insulin secretion (Tavafi *et al*., 2011). Moreover, the remarkable antidiabetogen properties of rosemary

leaf extract could be because of its powerful antioxidant properties. It could also produce its hypoglycemic action by a mechanism independent of insulin secretion such as inhibition of protein glycation and inhibition of endogenous glucose generation (Bakirel *et al*., 2008). Hypoglycemic effect of ginger by enhancing glucose absorption in muscle cells without use insulin regulating gene expression of insulin receptor-inhibiting and stimulative proteins (Arablou *et al*., 2014).

Gingerol and shogaol present in ginger supplements that affect various levels of insulin signalling waterfall or insulin postreceptor complex by increasing the expression of glucose transporters and other molecular modulators of insulin activity that increase glucose uptake by muscle cells. (Arablou *et al*., 2014 and Hyun *et al*.,2014). Another mechanism of action of ginger that could explain this action could be caused by inhibition of oxidative stress. Ginger and shogaol are active components of ginger and may have contributed to the hypoglycemic effect found during this study The findings of this blood glucose study are similar to those of (Nafiseh *et al*., 2015). Ginger therapy in the diabetic group revealed a significant reduction in serum glucose levels and it conforms to (Abd Elwahab and Ali., 2015). Which studied the effect of ginger on renal tissues in diabetes-induced streptozotocin rats showed that serotonin receptors can be implicated in the hypoglycemic effect of ginger Serotonin receptors attenuate insulin release and ginger may counteract this suppressor effect (Sultana *et al*., 2014 and Salim., 2014).

Moreover, explained the hypoglycemic effect of ginger by inhibiting the glycolytic activity of the brush edge enzymes of the small intestine by polyphenolic compounds of ginger (alkaloids, tannins and flavinoids). Flavinoids like anti-oxidants can overcome the harmful effects of oxidative stress. Which causes a change in the function of pancreatic beta cells due to, thus diminishing the hyperglycemic effect of diabetes (Eleazu *et al*., 2013). Describe another ginger mechanism for overcoming the diabetic effect of STZ, like ginger causes inhibition of the main enzymes that control carbohydrate metabolism and increased insulin liberation/sensitivity improve the absorption of glucose into peripheral adipose and skeletal tissues. Ginger also reduces lipids, which also enhances the insulin-resistant state (Yiming *et al*., 2012).

In alloxane- of induced diabetic rabbits, blood glucose levels rose due to the permanent destroying pancreatic beta cells, leading reduction in blood insuline levels (Hala *et al*., 2006).

5.3. Effect of rosemary, ginger infusion and their combination on Albumin and on liver function Parameters in Diabetic rabbits.

5.3.1. Albumin:

A significant decrease (P≤ 0.05) in albumin were noticed (GII) with whom this work agrees (Goodman and Gilman., 1985) in comparison with (GI, GIII, GIV, GV) whereas, significantly increase (P≤ 0.05) in (GIII) This work coincides with (Selmi *et al*., 2017), (GIV) accordance with (Sedlak and Lindsay., 1968).

It is known that structural changes occur in the liver as a result of the absence of insulin in diabetes . The functions of the liver may also be affected by the changes in the levels of insulin. Hyperemia in sinusoids and central veins show that the liver is having difficulty in its function and is toxically damaged. Administered ginger shows that it ameliorates liver damage These findings are also supported by histological findings(El-Kott et al., 2010).

Rosemary given indicates that it enhances liver damage ,has antioxidant properties protect the cell damage the histopathological findings support these findnges(Selmi *et al*., 2017).

5.3.2. Concentration of Aspartate Amino Transferase (AST):

A significantly (P≤ 0.05) in the AST concentration was observed in the group of (GII) (Goodman Gilman., 1985 and Davi., 2005). Compared to control and other, (GI,GIII,GIV,GV) whereas (GIV) agree with (Sedlak and Lindsay 1968) and no signficinant change between (GI and GV) as in table (4-2).

The functions of liver may also be affected by the changes in the levels of insulin. It is known that structural changes occur in the liver as a result of the absence of insulin in diabetes (El-Kott et al., 2010).

An increase in the enzyme activities may be mainly due to leakage of these enzymes from the liver cells into the blood stream which gives an indication of the hepatotoxic effect of STZ induction (Ramadan et al., 2013).

R. Officinalis has been reported to possess antioxidant activity; furthermore, the extract exhibited significant radical-scavenging activity probably due to the higher concentration of caffeic acid derivatives, especially rosmarinic acid (Ramadan et al., 2013).

Administered ginger shows that it ameliorates liver damage (El-Kott et al., 2010). These findings are also supported by histological findings.

5.3.3. Alanine Transferase concentration (ALT):

It is from table (4-3). The output was a substantial increase ($P \leq 0.05$) in the concentration of alloxan- induced diabetes group with whom this work concurs (Goodman and Gilman., 1985; Davi., 2005), in relation to control groups and other treatment groups. Whereas, significant decrease ($P \leq 0.05$) among alloxane - induced diabetes treated with aqueous rosemary extract agree (Selmi *et al*., 2017). The group treated with Aqé ginger extract agreed with (Sedlak and Lindsay., 1968) and the group combination of two plant extracts (rosemary + ginger) resulting in the increase of the aforementioned parameter means attaining that of the control group.

Attributable this disruption of enzymatic activity of lipid peroxidation due to diabetes. This leads to lowering free radicals within the cell membrane of hepatic cells (Sedlak and Lindsay., 1968).

An increase in the enzyme activities may be mainly due to leakage of these enzymes from the liver cells into the blood stream which gives an indication on the hepatotoxic effect of STZ induction(Ramadan et al.,2013).

Rosemary has an antioxidant effect; In addition, the extract displayed significant radical scanning activity, probably as a result of the higher concentration of caffeic acid derivatives, particularly rosmarinic acid (Ramadan et al.,2013).

Administered ginger shows that it ameliorates liver damage (El-Kott et al., 2010). These findings are also supported by histological findings.

5.4. Effect of rosemary, ginger infusion and their combination on lipid profile Parameters in Diabetic rabbits.

In the following table (4-4). Compared to control groups and other treatment groups varies, Significant reduction (P≤ 0.05) of alloxane - induced diabetes treated with an aqueous rosemary extract agreement with (Cani *et al*., 2018 and Hadree et al., 2019), ginger treated group Aqé extract Accord with (Elshater *et al*., 2009) and the alloxan - induced diabetes group treated with two plant extracts (rosemary + ginger) resulting in increased means of the above mentioned parameters to attain that of the comparison group.

The triglyceride concentration was substantially higher (P ≤ 0.05) in the alloxan-induced diabetes in relation to the reference group and other treatment groups wherase, significant decline (P≤ 0.05) in alloxane - induced diabetes treated with aqueous rosemary extract is consistent with (Cani *et al*.,2018 and Hadree *et al*.,2019), group treated with the aqueous extract of ginger accord with (Akhani *et al*.,2004 and Bhandari *et al*.,2005) and alloxan-induction diabetes group treated with the extract of two herbs (rosemary + ginger) leading to an increase in the means for the above parameters to reach the control group.

Significant reduction in HDL (P≤ 0.05) in alloxane - induced diabetes is consistent (Goodman and Gilman 1985) wherase, significant increase (P≤ 0.05) in alloxan diabetes managed with rosemary extract (Cani *et al*.,2018 and Hadree**,***et al*.,2019), Group treated by ginger extract agree with (Elshater *et al*., 2009) and the alloxan-induced diabetes group treated with Two plant extracts (rosemary + ginger) causing the average increase of the parameters mentioned above to reach the control group like table (4-4).

The alloxan injection has caused an increase in serum cholesterol; the marked hyperlipidemia that characterizes the diabetic condition can thus result from non-inhibited lipolytic hormone actions on fat deposition because of the lack of insulins (Almdal *et al*., 1988). The observed reduction in lipid levels following rosemary intake has been suggested in other studies to be caused as a reduced dietary fat intake sustained by one, enhanced fecal fat excretion (Ibarra *et al*., 2011). The possible explanation came from improved metabolic glucose, like that direct protein

metabolism by anabolizing instead of catabolic processes, whereby proteins such as apolipoprotein A1 (Apo-A1) are synthesized, which makes up 70 per cent of the HDL-C structure, resulting increased its concentration (Al-Jamal & Alqadi., 2011). Rosemary leaf extract had hypolipidemic potential because of the gradual metabolic control of rosemary leaf extract about the mechanisms involved with lipid removal from the somatic. The decline may be due to the antioxidant effect of rosemary components in the form of rosmarinic acid, which modified the oxidation fatty acid content in the liver and lowers triglyceride biosynthesis in rats (Iweala and Oludare., 2011).

The reduction of TC and LDL-C through rosemary leaf extract may be caused by inhibiting pancreatic lipase and hormone-sensitive lipase by a variety of constituents in particular rosmarinic acid extract together with with more phenolic phenomena (Al Sheyab *et al*., 2012). Observed a rise in HDL-C in rats afterwards extracted from rosemary leaves (Alaa and Brahamaflesh., 2010). Related regeneration of pancreatic cells and improvement of insulin secretion by surviving cells can result in inhibition of lipid peroxidation (Alnahdi., 2012).

Ginger stimulates converting cholesterol into biliary acids, an important means of eliminating cholesterol in the body and reducing cholesterol levels (Rafiq., 2013). This reduction in most lipid parameters, attributes to the severe lipolysis caused by both extracts to overcome the effect of insulin deficiency (Elkirdasy et al., 2015).

5.5. Effect of rosemary, ginger infusion and their combination on serum Antioxidant activity in diabetic rabbits.

5.5.1. Estimate of glutathione concentration (GSH):

Significantly reduced ($P \leq 0.05$) the concentration of GSH (GII) agreement with (Sarhat *et al*., 2016) compared to the (GI,GIII,GIV,GV) groups, whereas , showed a significant rise ($P \leq 0.05$) (GIII) arrangement with (Harb *et al*., 2018 and Hadree *et al*., 2019), (GIV) in accordance with (Sarhat *et al*., 2016) non significant change with (GI and GV) as table (4-5).

Reported that the water extract from the rosemary plant contains effective chemical compounds such as (carmosic acid, rosmarinic acid) with anti-oxidant activity

(Maggi-Capeyron *et al*., 2002). Its ability to remove free radicals, prevent oxidative stress and decrease the oxidation of fats (Harach *et al*., 2010). Rosemary has the ability to stabilize free radicals with electrons. Rosemary's high recoverability, primarily for free radicals, is considered as an anti-oxidant action mechanism (Moreno *et al*., 2006).

Ginger has been reported to reduce lipid peroxidation by affecting the enzyme levels (Ahmed *et al*., 2000). Ginger has also been shown to reduce cellular oxidation, regenerative super-oxide anion and hydroxyl radicals. Hydrolyzed phenolic fractions without ginger and phenolic fractions with ginger have free radical scanning activity. Decreased tissue levels of GSH increase cell damage caused by oxidizing stress (Siddaraju *et al*., 2007).

5.5.2. Serum malondialdehhyde (MDA) estimation:

According to the outcome of MDA which show a significant increase ($p \leq 0.05$) in (GII) agreement with (Sarhat *et al*., 2016) concurring diabetes compared to the (GI,GIII,GIV,GV) while , demonstrated a significant decline ($P \leq 0.05$) of diabetes (GIII) agreement with (Harb., 2018 and Hadree *et al*., 2019), (GIV) consistent with (Sarhat *et al*., 2016) and non significant change between (GI and GV) table (4-5).

Reported that the water extract from the rosemary plant contains effective chemical compounds such as (carmosic acid, rosmarinic acid) with anti-oxidant activity (Maggi-Capeyron *et al*., 2002). Its ability to remove free radicals, prevent oxidative stress and decrease the oxidation of fats (Harach *et al*., 2010). Rosemary has the ability to stabilize free radicals with electrons. Rosemary's high recoverability, primarily for free radicals, is considered as an anti-oxidant action mechanism (Moreno *et al*., 2006).

Ginger has been reported to reduce lipid peroxidation by affecting the enzyme levels (Ahmed *et al*., 2000). Ginger has also been shown to reduce cellular oxidation, regenerative super-oxide anion and hydroxyl radicals. Hydrolyzed phenolic fractions without ginger and phenolic fractions with ginger have free radical scanning activity. (Siddaraju *et al*., 2007). Who reported that on a combination of medicinal plants provides better glycemic control in a short time with a small dose than just one medicinal plant. This helps to avoid adverse effects from a large dose of each one

Which means their biological effects should be different and therefore they should have different biological effects (Ahmad *et al*., 2020).

5.6. Histopathological changes:

5.6.1. Pancreas:

The pancreas for the check rabbit showed normal structures of pancreatic acinar tissue in figure (4-1) . Microscopic examination of the pancreas of alloxan induced diabetes rabbit showed sever congestion and necrotic changes of pancreatic acini, marked damage of Langerhans islet cells.

pancreas of alloxan in diabetic rabbits showed sever congestion and necrotic changes of pancreatic acini , marked damage of Langerhans islet cells figure(4-2). Necrosis and atrophy of the β-cells and Congestion in the pancreatic blood vessels, these results conform to the results of other studies (Qadori., 2011 and Khattab *et al*., 2013).

In figure (4-3) rabbit pancreas treated with rosemary aqueous extraction showed degenerative changes (vacuolation) in acinar epithelia, degeneration (cloudy swelling) of Islet cells. Rosemary is a good source of antioxidant and therefore may be capable of preventing tissue damage it can protect pancreas tissues from lipid peroxidation on diabetic rats(El-Naggar *et al*., 2016).

In figure (4-4) induced diabetes rabbit pancreas treated with ginger infusion showed slight congestion, degeneration of the acinar epithelium and abnormal pancreatic septa with normal intertubular duct wall.

Ginger is a good source of antioxidant and therefore may be capable of preventing tissue damage it can protect pancreas tissues from lipid peroxidation on diabetic rats(Al-Qudah et al.,2016)

In figure(4-5) induced-diabetic rabbit pancreas treated with a combination of both herbal extracts reveled normal pancreatic acinar tissue , normal interlobular duct and ductile epithelium with normal collagen surrounding the duct and close to normal connective tissue septa due to the synergism between ginger and rosemary as the histopthological findings.

5.6.2. Liver:

The hepatic tissues of comparison groups (Figure 4-6) , showed a natural histological architecture.

Figure (4-7) liver of rabbits in alloxan –induced diabetes group, there are severed portal vein congestion with sever inflammatory cell infiltration in portal area demonstrates that there is severe portal vein congestion with severe inflammation and areas of fibrosis in group-induced alloxan diabetes. Whereas structural changes occur in the liver due to lack of insulin in diabetes (Koyuturk *et al*., 2005). The functions of the liver may also be affected by the changes in the levels of insulin. It is known that structural changes occur in the liver as a result of the absence of insulin in diabetes (El-Kott et al., 2010).

liver in previously alloxan-induced diabetes animal and treated with rosemary aqueous extract revealed, significant inflammatory response characterized by heavy infiltration of mononuclear cells, areas of fibrosis on hepatic tissue and marked tissue depletion represented by spaces between hepatic cord in figure (4-8). Was reported that review of liver sections in mice Treatment by Rosemary showed an improvement in hepatic architecture as well as histological aspects of hepatocytes were virtually identical from the control mice (El-Naggar *et al*., 2016). Who indicated as a histopathological exam has shown the extracts of rosemary may improve pathology abnormalities and decrease inflammatory cell immigration to liver tissues (Xiang *et al*., 2013).

diabetic animal liver treated with ginger infusion , showed severe portal vein congestion, mild hepatocyte necrosis with infiltration of inflammatory cells (4-9). The liver of the ginger-treated diabetic rats shows well-preserved glamorous and tubule tissue. Normal histological appearance was associated with minor vacuolization in some cells (El-kott *et al*., 2010).

In figure (4-10) rabbit liver treated with two herbal infusion (ginger and rosemary) showed improvement in the hepatic tissue structure (right side), slight necrosis and inflammation, whereas the left side revealed hepatic depletion and sever fibrotic changes due to the synergism between ginger and rosemary as the histopthological findings.

5.6.3. Kidney:

The histological section of the kidney is shown in Figure (4-11) of the control group, normally revealed renal histology. The findings indicate a major effect of diabetes on the rat kidney. The main effect, the factor of diabetes, was associated with high blood sugar and was responsible for the dilation of the proximal and distal tubules in the cortex. The side effect, known as the individual response factor, was related to inflammatory processes (Leegwates and Kuper 1984). The excellent recovery of kidney function expected with the treatment of rosemary can be explained by the regeneration capacity of kidney tubules (Thakran *et al*., 2004).

(4-12) kidney in alloxan diabetic rabbits diabetes animal, showed renal tubules dilatation with hyperplasia of epithelial lining with diffuse inflammatory infiltration in renal tissue and glomerular tissue .The primary effect, the diabetes factor, was associated with hyperglycemia and was responsible for dilatation of proximal and distal tubules in the cortex. The secondary effect, named the individual response factor, was associated with inflammatory processes . Diuresis is a common feature associated with diabetes, which may be the reason for structural changes observed with glomeruli . The excellent recovery of renal function expected with treatment of rosemary can be explained by the regenerative capability of the renal tubules (Elkirdasy et al., 2015)

In figure (4-13) Kidney of a diabetic rabbit treated with rosemary infusion the upper portion showed significant dilatation and inflammation of renal tubules, necrotic renal epithelial lining, below shows nearly slight changes with necrosis and severe inflammation in renal tubules .Treatment of rosemary can be explained by the regenerative capability of the renal tubules. The role of Rosemary in reversing the diabetic state at the cellular level besides the metabolic normalization, further proves its potential as an antidiabetic assert (Elkirdasy et al., 2015).

In figure (4-14) Diabetic animal kidney treated with ginger infusion showed necrotic epithelial tissue of renal tubules and the tubules undergo the dilatation, with significant necrosis and inflammation of glomeruli. Oral therapy of the rat's aqueous ginger extract normalized the histological structure of the kidney (Al-Qudah *et al*.,

2018). Ginger ethanolic extract by normalizing renal histology and improving renal function (Hamed and El-Rigal., 2012).

In figure(4-15) for rabbit kidney treated with (ginger and rosemary)infusion , showed significant hyperplastic, necrotic renal tubule epithelia in the left side, in the right side showed areas of nearly resembling normal tissue structure of renal tissue due to the synergism between ginger and rosemary as the histopthological findings.

Chapter Six: Conclusions and Recommendations

6.1. Conclusions:

1. Administration of the alloxan in rabbits causes decrease in hematological parameters,albumin,GSH, increase in glucose,HB1AC,hepatic enzyme ,lipid profile and MDA.

2. Administration of rosemary , ginger infusion and their combination in alloxan diabetic rabbits causes an increase in hematological parameters, albumin, GSH, Decreased glucose, HB1AC, hepatic enzymes, lipid profile and MDA.

3.Histological changes in pancreas, liver and kidney in diabetic rabbits showed congestion, inflammation, necrotic changes while in diabetic rabbits treated with rosemary and ginger infusion showed improved pathological abnormalities and reduce the immigration of inflammatory cells. In diabetic rabbits treated with two herbal(rosemary and ginger) infusion showed normalized the histological structures and improvement of these organs.

6.2. Recommendations:

1.The study should be done for 2 months for full recovery.

2. Study the Rosemary, Ginger infusion and their combination on the nervous system.

3. Study the Rosemary, Ginger infusion and their combination on the wound healing

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Appendices

Appendices

Appendix (I):

Complete Blood Count:

Procedure:

The sample is collected drawing the blood into a tube containing an anticoagulant typically (EDTA) to stop it from clotting The testing is typically performed by an automated analyzer Analysis begins when a well mixed blood sample is placed on a rack in the analyzer. The instrument utilizes flow cells, photometers and apertures analyze different elements in the blood. On board the analyzer, the sample is diluted and aspirated into at least two different channels, one of which is used to count red blood cells and platelets, the other to count white blood cells. Additional channels may be used for differential white blood cell counts and specialized measurements of platelets.

Calculation:

Blood cell counts occurs by flow cytometry, in which a very small amount of the specimen is aspirated, diluted and passed through an aperture and a flow cell. Sensors count and identify the number of cells passing through the aperture using two main principles: electrical impedance and light scattering Impedance-based cell counting operates on the Coulter principle, which measures the drop in current as cells pass through an aperture to count cells and calculate their sizes. Because red blood cells, white blood cells and platelets have different average sizes, this technique allows the three types of cells to be differentiated. Light scatteringtechniques direct a laser at individual cells and determine cellular size and complexity by measuring the amount of light scattered at different angles. Forward scatter, which refers to light scattered between 0 and 10 degrees of the beam's axis, correlates with cellular size, while side scatter (light scattered at a 90-degree angle) correlates with cellular complexity. White blood cells, red blood cells and platelets, as well as individual types of white blood cells, can be distinguished based on light scattering characteristics.

Appendices

Appendix(II):

Estimation of Glucose:

Principle:

D-Glucose + H2O + O2 _____ > Gluconic acid + H2O2.

POD

2H2O2 + 4-Aminoantipyrine + p-Hydroxybenzoic acid

_____> colored quinonic derivative + 4 H2O.

REAGENTS:

Kit size	**4 x 125 ml**	**8x125 ml**	**5x5 ml**
Cat. No.	**ADX101**	**ADX102**	**ADX103**
Kit contents			
1) Glucose Reagent	**4x125 ml**	**8x125 ml**	**----**
2) Glucose Standard (100 mg/dl)	**1x5 ml**	**1x5 ml**	**5x5ml**

REAGENT COMPOSITION:

1) Glucose Reagent Concentrations in the reagent solution are:
   Phosphate buffer pH 7.0 120.00 mmol/L
   4-Aminoantipyrine 0.80 mmol/L
   Phenol 4.50 mmol/L
   Glucose Oxidase < 16.00 KU/L
   Peroxidase > 1.25 KU/L
   Preservatives and stabilizers.
2) Glucose Standard Glucose Concentration – 100 mg/dL (5.55 mmol/L).

Appendices

TEST PROCEDURE:

Wavelength 505 nm

Temperature 37º C

Prewarm the Reagent to the reaction temperature.

	Blank (ml)	Standard (ml)	Sample (ml)
Glucose reagent	1.000	1.000	1.000
Glucose standard	--	--	0.010
Sample	--	0.010	--

Mix well and incubate for 10 min. at 37º C Or 20 -25 min, At 15–25 ºC. After incubation, zero spectrophotometer with the reagent blank. Read and record the incubated Standard and samples. Final Color stability: A minimum of 1 hour, when protected from direct sunlight.

Sample O.D.

Calculation: Sample OD\standard OD x 100 = mg glucose / dl Standard.

Units (mg/dl) x 0.0555 = mmol/L.

Appendix(III):

Estimation of HB1 AC.

PRINCIPLE:

The percentage HbA1c whole blood can be directly determined by utilizing the interaction of antigen and antibody. Total hemoglobin and HbA1c have the same unspecific absorption rate of Latex particles. When we add mouse antihuman HbA1c monoclonal antibody (containing Multiple subunits), “Latex--HbA1c--mouse antihuman HbA1c monoclonal antibody--HbA1c--Latex” complex is formed. The amount of this complex is proportional to the amount of HbA1c absorbed onto the surface of latex particles. The amount of agglutination is measured absorbance. The HbA1c value is obtained from a calibration.

Appendices

REAGENTS COMPOSITION:

Reagent 1 (R1) Latex Reagent: Latex 0.13%, Buffer, stabilizer Sodium azide (0.95 g/L) Reagent 2

(R2) HbA1c Antibody: Buffer, Mouse anti-human HbA1c monoclonal antibody: 0.05 mg/mL, goat anti-mouse IgG polyclonal antibody 0.08 mg/DL., Stabilizers Reagent 3

(R3) Hemolysing reagent: Water and stabilizers.

TEST PROCEDURE FOR PREPARATION OF CALIBRATION CURVE.

Wavelength: 660 nm

Temperature: 37 °C

Prewarm the Reagent to the reaction temperature.

Reagent	**Cal -1**	**Cal -2**	**Cal -3**	**Cal -4**	**Cal -5**
HbA1c Reagent-1 (R1) (ml)	**0.300**	**0.300**	**0.300**	**0.300**	**0.300**
Calibrator(ml)	**0.800**	**0.800**	**0.800**	**0.800**	**0.800**
Mix well and incubate for 5 min at 37 ◦C					
HB1ac Reagent (R2)(ml).	**0.100**	**0.100**	**0.100**	**0.100**	

Mix well, incubate 5 min. At 37 °C and read the absorbance of blank, calibrates at 660 nm.

Appendices

Calculations:

Δ O.D of Calibrator = O.D Calibrator-O .D Blank. Plot the Δ O.D of each calibrator versus assigned concentration (HbA1c%) on a non-linear graph.

TEST PROCEDURE FOR PREPARATION OF SPECIMEN:

Wavelength : 660 nm

Temperature: 37 °C

Prewarm the Reagent to the reaction temperature.

	Blank (ml)	Sample(ml)
HbA1c Reagent-1 (R1)	**0.300**	**0.300**
Sample	**--**	**0.008**
Distilled Water	**0.008**	**--**
Calibrator	**--**	**--**
Mix well and incubate for 5 min at 37 ◦C		
HbA1c Reagent-2 (R2)	**0.100**	**0.100**

Mix well, incubate 5 min, at 37 ◦C and read the absorbance of blank, samples at 660 nm.

Calculations:

Δ O.D of Sample = O.D Sample - O.D Blank HbA1c % of the sample is calculated by interpolation of the OD of sample on the calibration curve.

The TRUEchemie HbA1c assay is traceable to the International Federation of Clinical Chemistry (IFCC) reference standard. The default result unit for the assay is % HbA1c. For alternative units, manual calculations can be used according to below equations: NGSP % HbA1c to IFCC mmol/Mol: [%HbA1c x 10.93]-23.50 IFCC mmol/Mol to NGSP %HbA1c: mmol/Mol x 0.09148]+2.152 HbA1c results are calculated from a spline data reduction method to generate a calibration curve.

Appendices

Appendix(VI):

Albumin

Assay principle:-

Measurement of albumin is based on its binding to the indicator dye bromocresol green (BCG) at pH 4.3 to form a blue – green colored complex. The intensity of the blue – green colored is directly proportional to the concentration of Albumin in the sample its determined by monitoring the increase in absorbance at 623 nm, or 578 nm.

Albumin + BCG PH 4.3→Albumin_BCG complex

*Reagents Standard albumin

4.0 g/dl.

*Reagents (R)

Succinate Buffer 200 mmol / l

Bromocresol green 0.4 mmol / l

Sodium azide 4.0 mmol /1

Procedure:

	Blank	**Standard**	**Specimen**
Reagent (R)	**2.5 ml**	**2.5 ml**	**2.5 ml**
Standard	**---**	**10 µl**	**---**
Specimen	**---**	**---**	**10 µl**

Mix, incubate for approximately 5 minutes at 20-25 Cº. The measure absorbance of specimen (A specimen) and standard (A standard) against reagent blank within 60 minutes.

Calculation:

Albumin concentration (g/DL) = $A\ specimen \backslash A\ standerd$ x 4

Appendices

Appendix (IX):

Lipid Profile:

Estimation of serum cholesterol concentration (mg/dl):

Principle:

Easter of cholesterol+H2O *Chol. estease* Cholesterol + Fatty acids Cholesterol +O2 *Chol. oxidase* Cholest-4-en-one+H2O2 H2O+4-Aminophenazone + phenol *peroxidase* Quinonimine Reagent:

Reagent:

Reagent **(1)** Buffer solution: pipes PH 6.9 mmol/L, phenol 26 mmol/L

Reagent **(2)** vial of enzyme: cholesterol oxidase 300 U/L, peroxidase 1250 U/L, cholesterol esterase 300 U/L, 4-aminophenazone 0.4 mmol/L Reagent.

(3): cholesterol standard 200 mg/dl 1. Manual procedure: Cholesterol concentration in serum samples was measured according to the following:

a. Reagent and serum samples were brought to room temperature

b. Serum sample, blank and standard was treated as follows:

c. Tube contents were mixed and left to stand for 5 minutes at 37 C before reading

d. The absorbance of the standard was measured and a sample was read via spectrophotometer at wavelength 505 nm against the blank.

Tubes	Blank	Standard	Sample
Cholesterol standard (s)	-	10 ml	-
Sample	-	-	10 ml
Working Reagent	1ml	1ml	1ml

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Calculation:

Result were calculated according to the following equation:

Total Cholesterol concentration = (O.D sample)/ (O.D/ standard)× nn =200 mg/dl.

Estimation of serum triglyceride concentration (mg/dl):

Principle:

Triglyceride $lipoprotein\ lipase$ Glycerol + fatty acid Glycerol + ATP $Glycerol\ kinase, Mg{+}{+}$ Glycerol-3-phosphate+ADP Glycerol-3-P+O2 $3{-}G{-}P{-}oxidase$ Dihydroxyacetone ne-p+H2O H2O2+4-Aminophenazone+p+Chlorophenol$peroxidase$ Quinonimine+ H2

Reagent:

Reagent (1) buffer solution: pipes buffer PH, 7.2, 50 mm0l/L, p- chlorophenol 2 mmol/L Reagent

(2) Enzyme: lipoprotein lipase 150 000 U/I, glycerol kinase 800 U/U/I, glycerol-3-phosphate oxidase 4000 U/I, peroxidase 440 U/I, 4-aminophenazone 0.7 mmol/L, ATP 0.3 mmol/L.

Reagent (3) triglyceride Standard (S): Glycerol 200mg/dl. O.

Procedure:

Triglyceride concentration in serum samples was measured according to the following:

a. Wave length/filter. 505nm (Hg546nm)/green

b. Temperature 37 C/R. T

c. Light path 1 cm

Pipette into clean dry test tubes labelled as Blank (B), standard (S), and Test(T). Mix well and incubated at 37 C for 5 min or at R. T (25 C) for 15min. Measure the absorbance of the standard.

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Calculation:

The results were calculated according to the following equation:

Triglyceride concentration mg/dl = (O.D sample) / (O.D standard) × n = 200 mg/dl.

Additive sequence	Blank	Standard	Test
Working reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Triglyceride	- Appendix	0.01	-
standard Sample	-	-	0.01

Estimation of serum HDL-Cholesterol concentration (mg/dl):

Principle:

Cholesterol esters + H2O *Chol.esterase* Cholesterol + fatty acid Cholesterol +½O2 + H2O *Chol.oxidase* Cholestenone + H2O2 2 H2O2 + 4-Aminoantipyrine + DCFS *peroxidase* Quinoneimine + 4H2O

Reagent:

Reagent (1) Good's buffer (pH 6.6)100 mmol/l, cholesterol esterase 1400 U/l, cholesterol oxidase 800 U/l, catalase 600 kU/l, N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) 0.6 mmol/l.

Reagent (2) Good's buffer (pH 7.0) 100 mmol/l peroxidase 3 kU/l 4–aminoantipyrine (4-AA) 4 mmol/L.

Tubes	Blank	Standard	Sample
Distilled water	50 ml	-	-
Cholesterol standard (S)	-	50ml	-
Sample supernatant	-	-	50 ml
Reagent	1.0ml	0ml	1ml

Appendices

Procedure:

HDL-Cholesterol concentration in serum samples was measured according to the following steps:

serum sample 40 – 60 mg/dl 1.04 1.55mmol/l, wavelength 600 nm, temperature 37°C CORMAY HDL DIRECT is intended for automated analysers.

a. Reagent (A, B) and serum sample were brought to room temperature. b. Serum sample, blank and standard was treated as follows:

c. 0.2 ml of sample was mixed with 0.5 ml of reagent (A) in centrifuge tube and let stand for 10 minutes at room temperature.

d. Centrifuged at a minimum of 4000 r.p.m. For 10 minutes.

e. The temperature was collected carefully.

f. Sample supernatant, blank, standard and reagent (B) was treated as follows:

g. Tubes contents were mixed thorough and incubated for 10 minutes at 37 C.

h. The absorbance (A) of the standard was measured and a sample was read via spectrophotometer wavelength 500 nm against the blank.

Calculation:

The results were calculated according to the following equation: HDL-cholesterol concentration in the sample (mg/dl) = (Absorbance of the sample/Absorbance of standard) × concentration of standard × sample dilution factor (1.7).

Appendix(VII):

Estimation of Serum Malondialdehyde (MDA):

Malondialdehyhe was estimated by Thiobarbituric acid (TBA) assay method of Buege & Aust, 1978 on a spectrophotometer .

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Principle:

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm.

Stock TCA – TBA – HCl Reagent:

It was prepared by dissolving 15% W/V trichloroacetic acid and 0.375% W/V thiobarbituric acid and 0.25N HCl to make 100 ml (2.1 ml of concentrated HCl in 100 ml). This solution was mildly heated to assist in the dissolution of TBA. Dissolved 15 gm TCA and 0.375 mg thiobarbituric acid in 0.25 N HCl and volume was made up to 100 ml with 0.25 N HCl.

Procedure:

To 0.4 ml of serum, 0.6 ml TCA-TBA-HCl reagents were added. It was mixed well and kept in boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added to eliminate centrifugation. This absorbance of pink color was measured at 535 nm against blank, which contained distilled water in place of serum. In blank 0.4 ml distilled water and 0.6 ml TCATBA-HCl reagent was mixed and boiled. Blank was always taken.

Calculation:

extinction coefficient of MDA at 535 nm is = 1.56×105

MDA concentration = χ / 0.0624 nmol / ml.

Appendix(VIII):

Glutathione (GSH):

Working Assay Mixture preparation

Immediately prior to use, prepare the working Assay mixture as shown in the table.

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Table 1 preparation is suitable for 48 reactions (100ml/well).

Reagents	**Volume**
Glutathione Assay buffer [1]	**5 ml**
Glutathione reductase	**8.7 µl**
NADPH Solution	**10 µl**

Note NADPH is light sensitive, therefore, make the assay buffer in a brown vial or cover the vial with aluminum foil.

Ellman's Working Solution preparation:

Immediately prior to use, add 65 µl Ellman's Reagent stock solution to 2.5 ml of 1X Glutathione Assay Buffer to make a working solution. You require 50 µl working solutions/ well.

NOTE: Ellman's Reagent is light sensitive, therefore, make the working solution in a brown vial or cover the vial with aluminum foil.

Assay protocol:

1.Make dilutions of GSSG in microcentrifuge vials using 400 µM GSSG stock to

achieve final concentration of 1 µM, 0.8 µM, 0.6 µM, 0.4 µM, 0.2 µM and 0.1 µM

in 1X Glutathione Assay Buffer. Prepare 1 µM stock of Oxidized Glutathione Standard by adding 2.5 µl of 400 µM

Oxidized Glutathione Standard solution to 1 ml of 1 X Glutathione Assay Buffer and mix well.

Tube	Oxidized glutathione Standard [1 µm]	1x Glutathione Assay Buffer	Final concentration of Oxidized glutathione
A	-	200 µl	0
B	20 µl	180 µl	0.1

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C	40 µl	160 µl	0.2
D	80 µl	120 µl	0.4
E	120 µl	80 µl	0.6
F	160 µl	40 µl	0.8
G	200 µl	-	1

NOTE: The detection limit of this assay falls in 0.1-2.5 µM for GSSG and 0.2-5 µM for GSH, so different standard curves can be used.

NOTE: The standards should have the same concentration of Deproteination Reagent as the samples to ensure accurate estimations.

2. Aliquot 50 µl GSSG standards into the wells, performing in at least duplicate. (See format below).

3. Dilute the 5% Deproteination Solution in the samples to 1:10 with 1X Glutathione Assay Buffer.

NOTE: Lower than 0.5% Deproteination Reagent in sample is acceptable for the assay, however >0.5% Deproteination Reagent is not recommended as it may interfere with the assay. The standards should have the same concentration of Deproteination Reagent as the samples to ensure accurate estimations.

4. Aliquot 50 µl samples into the wells, performing in at least duplicate. (See format below).

5. Add 100 µl of freshly prepared working assay mixture per well.

6. Incubate the plate at room temperature for 5 minutes.

7. Rapidly add 50 µl of freshly prepared Ellman's Reagent working stock solution per well and mix several times by pipetting up and down.

8. Cover the plate with aluminum foil or incubate plate in dark on shaker until absorbance is checked. For kinetic method absorbance at 0 minute is also recorded.

9. Glutathione concentration can be determined by endpoint method or by kinetic method.

Appendices

End point method: Read the plate at 405-415 nm, 25 minutes after addition of Ellman's Reagent.

Kinetic method: Read the plate at 405-415 nm at 5 minutes' interval after addition of substrate for 30 minutes.

Appendix (IV):

Serum Aspartate aminotransferase Activity (AST) concentration:

Serum Aspartate aminotransferase activity (AST) is determined by using a special kit (SPECTRUM AST – kit, Egypt- IFUFCC22), by using a device (Spectrophotometer Sesil, England).

Principle:

Calorimetric determination of AST activity is obtained according to the following reactions:

AST: Aspartate + α keto glutarate ⟷ Oxaloacetate + glutamate

The reaction:

The oxaloacetate formed is measured in its derivative form, 2,4- DI nitrophenylhydrazone.

Reagents:

Reagent 1 AST	Phosphate buffer pH7.5 L-Aspartate 2- Oxoglutarate Sodium Hydroxide Sodium Azide	100 mmol / l 100 mmol / l 5 mmol / l 140 mmol/l 12 mmol/l

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Reagent2 Color Reagent	2.4dinitroph enylhydrazine HCL	2 mmol /l 8.4 %

Procedure:

Wavelength: 546 nm (530–550 nmZero adjustment: reagent blank: Pipette into test tubes:

Reagent	**Reagent blank**	**Sample**
Reagent (Buffer)	0.5ml	0.5ml
Sample	-	100µl
Distilled water	100µl	-

Mix and incubate for exactly 30 minutes at 370.

Reagent 2	0.5ml	0.5 ml

Mix and incubate for exactly 20 minutes at 20-250C.

Sodium hydroxide	5ml	5ml

Mix and measure absorbance of specimen against reagent blank at 546nm after 5 minutes.

Absorbance	Value of AST U/L	Absorbance	Value of AST U/L
0.020	7	0.100	36
0.030	10	0.110	41

0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.0170	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

Linearity:

The assay is linear up to 89 U/L. If the absorbance exceeds 0.170 at 546 nm, the sample should be diluted 1+9 using sodium chloride and repeat the assay (result x10).

Appendix(V):

Serum Alanine aminotransferase Activity (ALT)

Determination:

Serum Alanine aminotransferase activity ALT is determined by using a special kit (SPECTRUM ALT – kit, Egypt- IFUFCC25), by using a device (Spectrophotometer Sesil, England).

Principle:

Calorimetric determination of ALT activity is obtained according to the following reactions:

ALT: Alanine + α keto glutarate $\rightleftharpoons$ pyruvate + glutamate.

The pyruvate formed is measured in its derivative form, 2,4dinitrophenylhydrazone.

Reagents:

Reagent 1 ALT	Phosphate buffer pH7.5 D-Alanine 2-Oxoglutarate Sodium Azide	100 mmol /l 200 mmol /l 6 mmol /l 12 mmol/l
Reagent 2 Color	2.4 dinitrophe	2 mmol /l

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Reagent	nylhydrazine	

Procedure:

Wave length: 546 nm (530 – 550 nm) adjustment: reagent blank: Pipette into test tubes:

Reagent	**Reagent blank**	**Sample**
Reagent (Buffer)	0.5ml	0.5 ml
Sample	-	100µl
Distilled water	100µl	-

Mix and incubate for exactly 30 minutes at 370C.

Reagent 2	0.5 ml	0.5 ml

Mix and incubate for exactly 20 minutes at 20-250C.

Sodium hydroxide	5 ml	5 ml

Mix and measure absorbance of specimen against reagent blank at 546nm after 5 minutes.

Calculation:

Obtain the ALT activity from the following table.

Absorbance	Value of ALT U/L	Absorbance	Value of ALT U/L
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77

0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

Linearity:

The assay is linear up to 94 U/L. If the absorbance exceeds 0.5 at 546nm, the sample should be diluted 1+9 using sodium chloride and repeat the assay (result x 10).

Appendix (X):

Histological Technique(E & H) stain:

The Pancreas,liver and kidney of each animal were quickly removed and rapidly weighed then prepared for histological study according to Mescher method,(2010) with aid of the light microscope as the following steps:

* Fixation:

The specimen fixated in the formalin 10 % for 24 – 48 hours.

* Washing and dehydration :

After fixation the specimens washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later. By dehydration the water had been completely extracted from fragments by bathing them successively in a graded series of of ethanol and water (70 %, 80 %, 90 %, and 100 % ethanol).

* Clearing:

Bathing the dehydrated fragments in solvent (xylene) for 30–60 minutes, this step was repeated 3 times. As the tissues clearing, they generally became transparent.

Appendices

* Infiltration and embedding :

Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52 C. The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin.

* Sectioning:

After holdes from the oven, the specimen lets at room temperature to be solid and removed from their containers in order to sectioning they were put in the rotarymicrotome and were sliced by the microtome, a steel blade sections 5 micrometers thick. The sections were floated on water bath (50 – 55 o C) , then transferred into glass slides coated with Mayers albumin as adhesive substance and left to dry.

* Staining:

The histological sections of the studied organs were stained with Hematoxylin - Eosin stain.

الـــــــــــــــــــخلاصة :

كان الغرض من هذه الدراسة هو تقييم التأثير العلاجي نقيع إكليل الجبل والزنجبيل ومزيج من (إكليل الجبل والزنجبيل) في الأرانب المصابة بلسكري. خمسون أرنباً مختلطاً صحية عشرة ارانب سيطرة واربعون ارنب استحداث الاصابة بالسكري ثم وزعت عشوائياً وربيت على مدى 4 أسابيع. المجموعة الأولى مجموعة السيطرة السالبة (الماء المقطر) بدون مكملات على الماء المقطر .المجموعة الثانية هو مجموعة السيطرة الموجبة في وريد الاذن بأعطاء الألوكسان بجرعة واحدة (100 مجم / كجم . المجموعة الثالثة اعطاء جرعة من نقيع إكليل الجبل عن طريق الفم (200 مجم / كجم من وزن الجسم) للأرانب المصابة السكري.المجموعة الرابعة يعطى نقيع الزنجبيل عن طريق الفم بجرعة (250 مجم / كجم من وزن الجسم) في الأرانب المصابة بداء السكري. المجموعة الخامسة مزيج من (نقيع إكليل الجبل والزنجبيل) في ألأرنب المصابة السكري واستمرت الدراسة لمدة 4 أسابيع .

وفي نهاية الدراسة تم جمع الدم لتقييم نسبة السكر في الدم ، HB1AC ، مؤشرات الدم (RBC، PCV، HB ، الصفائح الدموية و WBC) ، مصل لتحليل إنزيم الكبد (AST و ALT) ، ملف الدهون (الكوليسترول ، الدهون الثلاثية و HDL) ومعايير مضادات الأكسدة (GSH و MDA). تم عزل البنكرياس والكبد والكلى كجزء من الدراسة النسيجية. أظهرت النتائج زيادة معنوية($p \leq 0.05$) في الجلوكوز ، HB1AC ، إنزيمات الكبد (AST & ALT) ، الألبومين ، محتوى الدهون (الكوليسترول والدهون الثلاثية) ومضادات الأكسدة (MDAs) انخفاضًا معنويًا في ($p \leq 0.05$) (HDL & GSH) .في المجوعة الثانية ظهرت زيادة معنوية (p t ($0.05 \geq$في السكر, فحص السكر التراكمي , أنزيمات الكبد (AST&ALT) ,الألبومين ,الدهون (cholesterol andTG triglycerides)ومضادات الأكسدة,MDAs) وأنخفاض معنوي($p \leq 0.05$) في HDL&GSH) أضهرت زيادة معنوية ($p \leq 0.05$) زيادة في المؤشرات الدموية والألبومين وGSH وأنخفاض معنوي فيt في السكر, فحص السكر التراكمي , أنزيمات الكبد (AST&ALT) ,الألبومين ,الدهون (cholesterol andTG)ومضادات الأكسدة (MDAs) في المجموعة الثالثة والمجموعة الرابعة والمجموعة الخامسة وأضهرت نتائج مقاربة لمجموعة السيطرة(المجوعة الخامسة). لا يوجد اختلاف معنوي في (HB) بين المجموعة السيطرة والمجموعات المعالجة الأخرى.

أظهرت التغيرات النسيجية في البنكرياس والكبد والكلى في مرض السكري الناجم عن الألوكسان احتقانًا والتهابًا وتغيرات نخرية بينما في مرض السكري الناجم عن الألوكسان المعالج بنقيع بإكليل الجبل و الزنجبيل أظهر تحسنًا في التشوهات المرضية وتقليل هجرة الخلايا الالتهابية. أظهر مزيج نقيع أكليل الجبل والزنجبيل اظهر تحسنا في التركيب النسيجي و تحسنا لهذه الأعضاء.

الاستنتاج من التجربة الأرانب المصابة بالسكري انخفاض بالدم والالبومين GSH وزيادة في الكلكوزوفحص السكر التراكمي وانزيمات الكبد والدهون وMDA. الأرانب المصابة بالسكري والمعالجة بنقيع اكليل الجبل والزنجبيل ومزيج كلاهما زيادة بالدم والالبومين GSHوقلة في الكلكوزوفحص السكر التراكمي وانزيمات الكبد والدهون وMDA

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب البيطري

فرع الفسلجة والكيمياء الحياتية والأدوية

تأثير نقيع أكليل الجبل والزنجبيل في بعض المعايير الدمية والكيميوحيوية في الأرانب المصابة بالسكري.

رسالة مقدمة الى مجلس كلية الطب البيطري /جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في علوم الطب البيطري / الفسلجة.

كتبت بواسطة

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