THE EFFECTS OF AQUEOUS ANDROGRAPHIS PANICULATA EXTRACT ON LIPASE, GLUTATHIONE PEROXIDASE, AND GAMMA-GLUTAMYL TRANSFERASE IN WISTAR RATS.

CHIEDOZIE VICTOR ISAAC

MAT. NO: LSC 1605786

RESEARCH PROJECT SUBMITTED TO

THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

FACULTY OF LIFE SCIENCE

UNIVERSITRY OF BENIN

BENIN CITY

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THR AWARD

BACHELOR OF SCIENCE (B.Sc.) DEGREE IN

BIOCHEMISTRY

OCTOBER, 2023

CERTIFICATION

We the undersigned, certify that **IGHODARO BENJAMIN EFOSA** with matriculation number LSC1906519 carried out, compiled and reported this project work in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc, Hons) degree in Biochemistry, in the Department of Biochemistry.

_____ _____

DATE

PROF P.O UADIA

(PROJECT SUPERVISOR)

_____ _____

DR. S.I. OJEABURU DATE

(PROJECT CORDINATOR)

_____ _____

PROF. O.C. ONYENEKE DATE

(HEAD OF DEPARTMENT)

_____ _____

DATE

(EXTERNAL SUPERVISOR)

DEDICATION

This project is dedicated to God Almighty, for all his love, mercy, blessings, and for keeping me alive all through my years in the school. I also dedicate this work to my Parents for their love, financial support and words of encouragement.

ACKNOWLEDGEMENTS

My deepest gratitude goes to God for every help gratitude to me the led to the completion of this projection.

Next to him, are my parents, Mr and Mrs IGHODARO BENJAMIN, for the support which was given to me monetarily and through words that inspired the strength needed for this work.

I am sincerely grateful to my ever worthy project supervisor, Prof. P.O UADIA for every from of guidance that led to the successful completion of this work.

I would like to express my deepest appreciation to my project coordinator, Dr. SI Ojeaburu, my head of department, Prof. Oyeneke, and all my lectures, from whom attained knowledge was needed imbibed in me.

I would like to extend my gratitude to my parent for their their valuable words of encouragement.

Last but not the least, my thanks go to my siblings, friends and course mates and relatives who have always been helping and encouraging me.

Table of Contents

Type chapter title (level 1) **1**

Type chapter title (level 2) 2

Type chapter title (level 3) 3

Type chapter title (level 1) **4**

Type chapter title (level 2) 5

Type chapter title (level 3) 6

ABSTRACT

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 INTRODUCTION

A branching, annual plant with lance-shaped green leaves that grows to a height of 60 to 70 cm, Andrographis paniculata is an example of this (Mishra *et al,* 2007).(Kabeeruddin & Kitabul,1937) wrote in their journal that in Asian nations including India, Sri Lanka, Pakistan, Java, Malaysia, and Indonesia are among those where it thrives frugally. The name Kalmegh is the most popular one in India. One of the widely employed medicinal herbs in the country, and it may be found in the plains. Additionally, the plant is referred to as the "king of bitters"(Kabeeruddin & Kitabul,1937; Shahid, 2011). Since the entire plant, including the leaves, has a very bitter flavor. It has a long history of medicinal use In conventional Chinese, Indian, and Ayurvedic medicine, as well as Western medicine. (Pawar *et* al, 2016; Liu *et al,* 2016; Sivananthan & Elamaran, 2013).

A.paniculata Is frequently prescribed in Ayurveda to treat gonorrhea, malaria, dermatitis, intestinal worm infestation, jaundice, sore throat, fever, liver diseases, and jaundice (Pawar *et* al, 2016; Thokchom *et al,* 2018) . While treating diarrhea, laryngitis, gastrointestinal infections, and inflammation, the plant is also used in Chinese medicine to eliminate body heat from the body, including that caused by fevers and toxins (Hu *et al,* 2017; Yang *et* al, 2020) . A. paniculata serves as an immune system according to modern pharmacology anticancer, antibacterial, stimulant, cures myocardial ischemia, pharyngotonsillitis several more (Dai *et al,* 2019; Hidalgo *et al,* 2013) including anti-HIV, anti-inflammatory, etc. Additionally, recent research has found a treatment for SARS-CoV-2 using A. paniculata is possible (Banerjee *et al,*2021).

1.1.1. STATEMENT OF PROBLEM

The Aerial part of the plant is rich in a number of chemical constituents which has been in use throughout history as a medicinal plants for mankind.This study will helps to find out the

proximate analysis, anti-diabetic and phytochemical constituents of the leaves and extracts of the plant.

1.1.2. AIM AND OBJECTIVES OF THE STUDY

To determine the effect of Aqueous Extract of *Adrographic* Paniculata on the activity of Lipase, GGT, GPx on streptozotocin induced diabetic rat.

1.1.3. STATEMENT OF PROBLEM

The Aerial part of the plant is rich in a number of chemical constituents which has been in use throughout history as a medicinal plants for mankind.This study will helps to find out the proximate analysis, anti-diabetic and phytochemical constituents of the leaves and extracts of the plant.

1.1.4. AIM AND OBJECTIVES OF THE STUDY

To determine the effect of Aqueous Extract of *Adrographic* Paniculata on the activity of Lipase, GGT, GPx on streptozotocin induced diabetic rat.

1.1.5. SPECIFIC OBJECTIVES OF THE STUDY

To determine the effect of Aqueous Extract of *Adrographic* Paniculata on

(1) The activity of lipase in diabetic rats
(2) The activity of GPx in diabetic rats
(3) The activity of GGT in diabetic rats

1.2 LITERATURE REVIEW

1.2.1 SCIENTIFIC CLASSIFICATION OF PLANT SPECIES

Domain……Eukaryota

Kingdom……..Plantae

Phylum…….Tracheophyta

Subphylum……Angiospermae

Class……..Magnoliopsida (Dicotyledoneae)

Order…….Tubittorae

Family……Acanthaceae

Genus…….Andrographis

Specie……. Paniculata Nees

1.2.2 NOMENCLATURE

Preferred scientific name: *Andrographis Paniculata*

Common name: King of Bitter, -The Creat (Abhishek *et al.,* 2010).

Nigerian names: Yoruba- Meje , Hausa- Garadugu

1.2.3 DESCRIPTION

It is a 0.5-1.0 m tall, erect, annual herb with several branches and a tap root. The leaves are glabrous, lanceolate, 3–7 cm 1-2.3 cm in size, green, with an acuminate apex and a tapering base.

Flowers are tiny and solitary; the hairy corolla is either white or pale pink in color. Fruit is a capsule with many seeds that is linear, oblong, and sharp at both ends. While it is commonly farmed in China, Thailand, the East and West Indies, and Mauritius, it grows in South Eastern Asia, specifically in India, Sri Lanka, Pakistan, and Indonesia. In general, it can be found in all types of vegetated regions, including pine, evergreen, and deciduous forest areas, as well as along roads and in settlements. (Abhishek *et* al., 2010).

1.2.4 HABITAT

There are many different environments where it can be found, including plains, hill slopes, wastelands, farms, dry or wet regions, sea coasts, and even road sides (Abhishek *et* al., 2010).

1.2.5 ECOLOGICAL CONDITION

It may be grown from seeds on any type of soil with ease (Abhishek et al., 2010).

1.2.6 CULTIVATION

It is grown as a crop during the rainy season (Kharif) in India. For the plant to thrive, the environment must be hot, humid, and sunny. Kalmegh can be grown on a variety of soil types, including lateritic and loam soils with a moderate fertility. Additionally, it can be grown on shaded wastelands. Following the end of the monsoon, the plant begins to flower as it develops luxuriantly with the approach of the next rainstorm. Broken seeds are used for reproduction in nature. Layering can also be used for vegetative propagation in some unique situations because each node can produce enough roots. Small and dormant for 5–6 months, seeds are tiny. Three beds of 10 x 2 m each need to be tilled, ground up, and leveled in order to raise crops on a hectare of land.For developing healthy seedlings, it is advisable to utilize organic manure liberally in the nursery. On each bed surface, 250–300g of seeds are disseminated, and a thin layer of a soil and compost combination should be applied to cover them. Till seedlings sprout (in 6-7 days), the bed should be thoroughly covered with the right kind of mulch and periodically watered by a fountain. To prevent seedling elongation, mulch must be removed as soon as

germination (70–80%). To protect them from the heat, seedlings should ideally be nurtured in a shaded area. When seedlings are one month old, they are transplanted into well-prepared and laid-out beds with a row spacing of 45-60 cm and a plant spacing of 30-45 cm. After transplantation, beds need to be watered to maximize the herb output. It can be cultivated on infertile to moderately fertile soil, however increasing the herb yield by applying 80 kg of nitrogen and 40 kg of phosphorus per hectare can help. Two nitrogen doses can be administered: one as a basal dose and the other after 30-45 days of transplanting. For growing nurseries, an additional 3-6 tonnes of well-rotten farmyard manure are needed. In 90 to 100 days, the maximum amount of herb biomass can be harvested before the leaves begin to shed. Throughout the winter, the crop is dormant. The amount of the active ingredient andrographolide in leaves is high around the period of flower commencement. Since the entire plant contains active ingredients, the harvested material is dried in the shade before being ground up. In the monsoon season, a well-maintained crop yields 3.5 to 4 tonnes/ha of herb (Chauhan and Gyanendra, 2003; Maheshwar *et* al, 2002; Seema *et* al., 2003).

Fig 1: The leaves of *Andrographis Paniculata*

Source: Akilandeswari *et al.*, 2019

1.2.7 BIOACTIVE PHYTOCONTITUENTS

Flavonoids, lactones, and diterpenes are all present in A. paniculata. Flavonoids have been separated from the leaves and are primarily found in the roots of plants. There are alkanes, ketones, and aldehydes in the aerial sections. Although it was first believed that the lactone andrographolide was the source of the leaves' bitterness, later research revealed that the leaves really contained two bitter compounds, andrographolide and a chemical known as kalmegin four lactoneschuanxinlian. In China, the aerial portions were used to isolate the compounds A (deoxyandrographolide), B (andrographolide), C (neoandrographolide), and D (14-deoxy-11,12dihydroandrographolides). Six diterpenoids of the ent-labdane type, including two diterpenes, glucosides, and four diterpene dimmers (bis-andrographolides A, B, C, and D), have been isolated from aerial parts. Diterpene glucoside (deoxyandrographolide 19 beta-d-glucoside) has been found in the leaves (Akilandeswari et al; 2019).

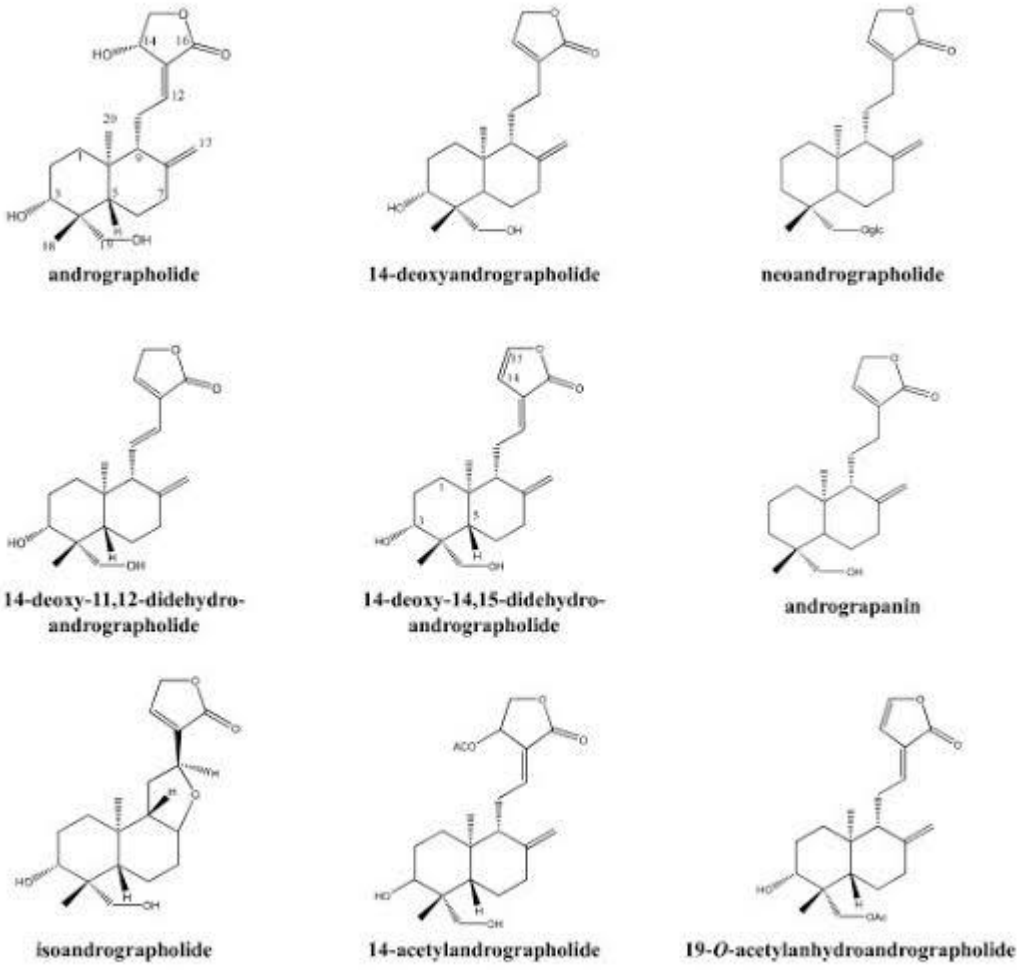

FIG 2: IMAGE OF THE STRUCTURE OF BIOACTIVE PHYTOCONSTITUENTS OF *ANDROGRAPHIS PANICULATA*

SOURCE: GOGGLE

1.2.7.1 TRADITIONAL USE

- A.Paniculata is a traditional medicine that is said to regulate body temperature, improve the body's natural detoxification processes, and protect against common colds, upper respiratory tract infections, sinusitis, and fever (Gabrielian *et* al., 2002).
- A. Paniculata is used as a cure for poison caused by snake and insect (Samy *et* al., 2008).

- The plant may have therapeutic properties that could help treat liver disorders and alleviate the symptoms of common colds and coughs (Geethangili *et al.,* 2008).
- Typical applications Since ancient times, lower urinary tract infections, herpes, fever, sore throats, and respiratory illnesses have all been successfully treated with the traditional medicinal plant Andrographis paniculata (Jarukamjorn and Nemoto, 2008)
- A. paniculata is sometimes prescribed to treat skin conditions such as leprosy, gonorrhea, scabies, and boils. Its 'blood purifying' properties are also believed to be effective in reducing fever symptoms.

1.2.7.2 Contemporary Use

- Pharmacological and clinical research has indicated that A. paniculata may have beneficial effects in the treatment of diseases such as cancer and HIV (Shahid, 2011).

1.2.7.3 Antidiabetic activity

The study evaluated the effect of A. paniculata in rats with streptozotocin-induced diabetes. The results showed that A. paniculata could lower blood glucose levels and improve the health of the rats' beta cells. The study also found that a specific compound in A. paniculata, andrographolide, could improve the function of pancreatic beta cells and increase insulin production. This suggests that A. paniculata may be useful in the treatment of diabetes (Nugroho *et* al., 2013). (Zhang et al., 2012) investigated the effects of andrographolide on type I diabetes in mice. The mice were given water or different doses of andrographolide for four weeks. The mice that received andrographolide had improved glucose tolerance and their pancreatic islets showed reduced damage. There was a decrease in the production of Th1 and Th17 cytokines, such as IFN-γ and IL-17. The production of the Th2 cytokines IL-10 and TGF-β was increased. (Chaursk et al., 2012) studied the anti-diabetic activity of the chloroform fraction of the ethanol extract of A. paniculata in type 2 diabetic mice. The results showed that the fraction significantly improved fasting blood glucose, oral glucose tolerance, serum insulin levels, and tissue glucose content. In addition, the activity of the enzymes glucose-6-phosphatase and hexokinase was evaluated in body tissues.

1.2.8 GAMMA-GLUTAMYL TRANSFERASE

Gamma-glutamyl transferase (GGT) is an enzyme that helps break down glutathione. GGT is found in many tissues, but most of it in the body is produced by the liver. GGT in the blood is mostly carried by lipoproteins and albumin. Several factors affect the level of GGT in the blood, including alcohol intake, body fat content, plasma lipids and glucose levels, and certain medications (Grundy, 2007).

1.2.9 GLUTATHIONE PEROXIDASE (GPx)

YGlutathione peroxidase (GPx) is a family of enzymes that use reduced glutathione to convert hydrogen peroxide or organic hydroperoxides to water or alcohols. Some GPxs contain selenium and are encoded by opal TGA codons. Four major selenium-dependent GPx isozymes have been identified in mammals: classical GPx, gastrointestinal GPx, plasma GPx, and phospholipid GPx (Mathis *et al.,* 2008).

1.2.10 LIPASE

Lipases are esterases that break down and form water-insoluble acyl compounds, such as mono-, di-, and triacylglycerols. Lipases can be found in a wide range of organisms and are unique in their substrate selectivity. While the definition of lipases is still debated, they are typically defined as enzymes that can act on long-chain fatty acid esters (more than C6) (Jun-Young Park & Kyung-Min Park, 2022).

CHARTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1 1 EQUIPMENT

- Nose mask
- Mechanical Blender (Cixi Honest, China).
- Bench-top centrifuge (centrifuge 80–20, Techmel & Techmel, USA).
- Spectrophotometer (Bruker Physik, Germany).
- Weighing Balance (Gallenkamp, England).
- Micropipettes [200ųl and 1000ųl (Sigma, USA)]
- Gavage (Bajaj Instruments, India).
- Cages
- Dissecting kit (Gold Cross, England).
- Ceramic plates
- Muslin Cloth
- Beakers (Pyrex, Nigeria).
- Rotatory Evaporator (US Lab, USA)
- Conical Flasks (Technico, India).
- Cotton wool
- Glucometer and strips (Yasee GLM–79, China).
- Syringes (2ml and 5ml [SeaskyMedical, China]).
- Hand gloves

2.1.2 CHEMICALS AND REAGENTS

All the chemicals used in this experiment were of the highest possible purity. They included the following chemical:

- Sucrose (Chempur, Germany).

- Picric Acid (Dow, USA).
- Malondialdehyde Assay Kit (Randox Lab Ltd, U.K).
- Glutathione Peroxidase Assay Kit (Randox Lab Ltd, U.K).
- Reduced Glutathione Assay Kit (Randox Lab Ltd, U.K).
- Streptozotocin (Sigma, London).
- Chloroform (Sigma–JHD, Germany)
- Sodium Citrate (May and Baker, England)
- Sodium Chloride (Chempur, Germany).

2.1.3 EXPERIMENTAL ANIMALS

Eighteen (18) Male wistar rats were obtained, weighed, housed in clean cages where they were fed standard diet and water. They were procured from the animal house from the department of Anatomy, University of Benin, Nigeria. On transferred to the Animal house of the University of Benin.

2.1.4 ANIMAL FEED

Grower mash was purchased from Uselu Market in Benin City, Nigeria. The grower mash was part of the Chikun Grower brand, which is a type of pelleted feed commonly used for chickens and other poultry in Nigeria.They were fasted overnight before sacrificed.

2.1.5 ACCLIMATIZATION TREATMENT

The cages and the entire animal house were disinfected prior to the commencement of the study. The animals were given two weeks to acclimatized in the animal house before the commencement of the study.

2.1.6 PLANT SAMPLE AND IDENTIFICATION

The A. paniculata plants used in this study were sourced from the Uselu Market in Benin City, Nigeria, and were then identified as true specimens by prof. Aigbikhan Emmanuel Izaka with voucher number UBH-A599 from the Department of Plant Biology and Biotechnology at the University of Benin, Benin City and subsequently deposited in the Herbarium for future reference.

2.2 METHODS

2.2.1 THE AQUEOUS EXTRACT OF *A. PANICULATA* WAS PREPARED BY THE FOLLOWING METHOD

270 grams sample of dried and grounded 'King of bitters' was weighed using a weighing balance. A sample was poured into a conical flask containing 1350 ml of distilled water. The mixture was stirred and brought to boil. After 10 minutes of boiling, the mixture was allowed to cool. Filtration was carefully carried out using a muslin cloth. The filtrate was poured into an air-tight flask and stored.

2.2.2 ACCLIMATIZATION OF ANIMALS

The experiment began with the purchase of 18 male albino rats from the animal house at the University of Benin. The rats were of varying weights, ranging from 111 to 178 grams, and were divided into 3 groups. Each group was placed in a different galvanized cage and allowed to acclimatize for 7 days, during which time they were given grower pellets and water and libitum. After this acclimatization period, the baseline blood glucose levels of the rats were measured to provide a control value for comparison with subsequent measurements.

2.2.3 INDUCING DIABETES USING STREPTOZOTOCIN

Sodium citrate buffer (0.1M, pH 4.5) was used to dissolve freshly prepared streptozotocin, with a concentration of 300mg per 12ml of buffer solution. The rats were then injected with this solution, using a dose of 50ml/kg body weight.

To observe the effects of streptozotocin, the rats were divided into 3 groups, each with a different number of rats.

1. Aqueous Control (AC, 5 rats) – Rats were injected with the buffer only and weren't treated with the aqueous extract.

2. Aqueous Untreated (AU, 7 rats) – Rats were injected with STZ only and weren't treated with the aqueous extract.
3. Aqueous Treated (AT, 6 rats) – Rats were injected with STZ and treated with the aqueous extract.

After the injections, the rats were given access to 10% sucrose water for the next 24 hours. The rats' blood glucose levels were measured before the treatment and before sacrifice.

2.2.4 TREATMENT OF ANIMALS

5g of A. paniculata extract was dissolved in a 100ml beaker of distilled water. The rats in the AT group were given 300ml/kg of the aqueous extract orally via gavage every day for 21 days.

2.2.5 ANIMAL SACRIFICE AND SAMPLE COLLECTION

The animals were treated humanely according to the guidelines set out in Lyden (2016). After the final extract treatment, the animals were sacrificed 24 hours later. Each rat was anesthetized with chloroform, and the thoracic and abdominal regions were opened with a dissection kit. Blood samples were taken by cardiac puncture, while the entire pancreas was excised and stored in a sample bag on ice.

2.2.5.1 PREPARATION OF SERA SAMPLES

The blood samples were centrifuged at 3000rpm for 15 minutes to separate the serum. The serum was then collected using Pasteur pipettes and stored at -20C until required for biochemical analysis.

2.2.5.2 PREPARATION OF TISSUE HOMOGENATE SUPERNATANT

The pancreas was first weighed to determine its mass, and then homogenized in saline to create a suspension of pancreatic tissue. The suspension was then centrifuged at 3000rpm for 15 minutes, after which the clear supernatant containing cellular components was collected and stored in plain bottles at -20°C until further analysis.

2.2.6 THE LEVEL OF FASTING BLOOD GLUCOSE WAS DETERMINED USING THE FOLLOWING PROTOCOL

PRINCIPLE

The glucometer's manual described the method of measuring fasting blood glucose levels as per the protocol of Barham and Trinder (1972). The glucose meter's enzyme-based reaction works by combining the glucose in the patient's blood sample with enzymes in a disposable test strip or reaction cuvette. This reaction produces a color change proportional to the concentration of glucose in the solution, which is measured by the meter and displayed on the screen.

PROCEDURE

After fasting overnight, a drop of blood was collected from the tip of each rat's tail using a needle and a glucometer was used to measure the blood glucose level of each rat. The blood glucose levels were recorded before and after the administration of STZ, and at the time of sacrifice. The rats were considered diabetic if their blood glucose levels were above 200mg/dl.

2.2.7 DETERMINATION OF BODY WEIGHT

The body weight of each rat was determined by placing the rat on a weighing balance, following the instructions in the balance's manual. This method was adapted from the work of Tonyushkina and Nichols (1983).

PRINCIPLE

An object placed on a digital balance displaces the strain gauge, which measures the change and converts it into an electrical signal that's passed through a digital converter. The digital converter displays the object's weight on the balance's screen.

PROCEDURE

The initial weight and final weight of each animal were recorded using a weighing balance. The initial weight was measured the day the animals were purchased, and the final weight was measured a day before they were sacrificed.

2.2.8 MORTALITY

Aqueous Control – None of the 5 rats in this group died before the day of sacrifice.

Aqueous Untreated – Four (4) out of the seven (7) rats died before the day of sacrifice.

Aqueous Treated – Two (2) out of the seven (6) rats died before the day of sacrifice.

2.2.9 BIOCHEMICAL ASSAYS

2.2.8.1 QUALITATIVE DETERMINATION OF LIPASE FROM SERUM OR PLASMA

TEST PRINCIPLE

Lipases are glycoproteins with a molecular weight of 47000 daltons. They are triglyceride hydrolases that catalyzed the cleavage of triglycerides to diglycerides with subsequent formation of monoglycerides and fatty acids. The determination of lipase is central for differential diagnosis of diseases of the pancreas. The method described here is based on the cleavage of a a specific chromogenic lipase substrate 1, 2, o-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester emulsified with bile acids. The combination of bile acid and colipase is used in this assay to detect the lipase activity. In the assay, the rate of formation of methylresorufin is measured photometrically and is directly proportional to the activity of lipase.

MANUAL PROCEEDURE

Wavelength	Temperature	Cuvette	Measurement
580nm (578nm)	37°c	1cm light path	Against Distilled water

Pipette into tubes as follows (37^0 C)		
	Blank	**Calibrator**
R1 Buffer	1000µl	1000µl
R2 Buffer	500µl	500µl
Distilled water	30µl	_____
Calibrator/sample	_____	30µl

Mix and incubate at assay temperature for 1 minute, Measure initial absorbance of the sample, start the stopwatch at the same time.

Measure absorbance at 1.2 minutes. Calculate ΔAbs/minute.

Calculation:

ΔAbs/min of Calibrator =(ΔAbs Calibrator - ΔAbs Blank)

ΔAbs/min of Sample = (ΔAbs Sample - ΔAbs Blank)

Lipase activity = ΔAbs / min of Sample × Calibrator value

∴ ΔAbs/ min of Calibrator

QUALITY CONTROL

It is recommended that a laboratory uses normal and abnormal reference Control sera to verify the performance of the procedure, both performance of the reagent and any instrumentation employed in the determination. Results obtained should fall within the specified ranges.

2.2.8.2 DETERMINATION OF GLUTATHIONE PEROXIDASE (GPX) ACTIVITY

Glutathione peroxidase was estimated by the method described by Nyman (1959).

PRINCIPLE

This is based on the oxidation of pyrogallol to purpuragallin by peroxidase, resulting to a light brown colouration, which was read at 430nm.

PROCEDURE

To 0.2ml of serum, 2.5ml of phosphate buffer (pH 7.4), 2.5ml of H2O2 and1.5ml of pyrogallol were added. The reaction was allowed to stand for 30min at room temperature. The colour developed was read at 430nm.

Calculation:

Enzyme activity =$\frac{\text{OD/min} \times \text{vt (U/mg protein)}}{E \times Vs \times Y}$

Where:

OD = absorbance

Vt = total volume of reaction mixture

E = Molar extinction co-efficient

Vs = volume of sample

Y = mg of protein used

2.2.8.3 QUALITATIVE DETERMINATION OF Y-GLUTAMYL TRANSFERASE IN SERUM AND PLASMA.

TEST PRINCIPLE

The substrate L-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted to 5-amino-2-nitrobezoate by Y-GT measured at 405nm. The increase in absorbance is proportional to y-GT activity.

L-y-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{\text{Y-Gt}}$

L-Y-glutamylgkycylglcine + 5-amino- 2- nitrobenzoate.

MANUAL PROCEDURE:

Wavelength	**Temperature**	**Cuvette**	**Measurement**
Hg 405 nm (400-420 nm)	25, 30 and 37^{o}c	1 cm light path	Against Air or Distilled water

PROCEDURE 1: SAMPLE START

Pipette into a cuvette as follows:

	Blank	**Sample**
Sample	-	1000µl
Working Reagent	1000µl	1000µl

Mix well and allow to stand for 1 min at 25°C (30 or 37°C) Measure the absorbance of the sample per minutes for 3 minutes. (ΔAbs/min)

Procedure 2: Substrate Start

Pipette into cuvette as follows:		
	Blank	**Sample**
R1 Buffer/Glylglycine	1000µl	1000µl
Sample		100µl
Mix well and allow to stand for 3 minutes at 37°C. Add:		
R2 Substrate	200 µl	200µl
Mix well and allow to stand for 2 minutes at 37°C, Measure the absorbance of the sample per minute for 4 minutes. (ΔAbs/ min)		

Calculation:

To calculate the GGT activity, use the following formula:

U)L = 1158 × Δ A 405 nm/min for procedure 1. (Sample start)

U/L = 1368 × Δ A 405 nm/min for procedure 2. (Substrate start)

CHAPTER 3

RESULTS

3.1 WEIGHT OF RATS

Table 3.1 gives a detailed look at how the weights of animals changed during a diabetic research study. “Basal weight” is the starting weight when we got the animals. “Weight at Day 0” is their weight when we induced diabetes using STZ “Day 7” is their weight one week after treating them with an extract from *Andrographis paniculata.* “Day 14” is their weight when we analyzed them after two weeks.

TABLE 3.1

Group 1 (Normal Control)	**Basal weight (g)**	**Wt. of animals after acclimatization- Day 0 (g)**	**Wt. of animals on the 8th day of treatment- Day 7 (g)**	**Wt. of animals on the 14th day of treatment- Day 14 (g)**
R1	**148.01**	**190.51**	**134.28**	**256.78**
R2	**144.36**	**182.42**	**222.80**	**245.06**
R3	**146.10**	**181.03**	**216.45**	**244.02**
R4	**143.77**	**169.05**	**190.43**	**208.05**
R5	**146.44**	**181.24**	**212.65**	**229.71**

Group 2 (Diabetic untreated)	**Basal weight (g)**	**Wt. of animals after acclimatization- Day 0 (g)**	**Wt. of animals on the 8th day of treatment- Day 7 (g)**	**Wt. of animals on the 14th day of treatment- Day 14 (g)**
R1	**171.46**	**195.69**	**144.42**	**144.76**
R2	**172.43**	**197.96**	**159.74**	**153.04**
R3	**160.37**	**179.51**	**220.35**	**239.27**

Group 3 (Diabetic treated with aqeous extract)	**Basal weight (g)**	**Wt. of animals after acclimatization- Day 0 (g)**	**Wt. of animals on the 8th day of treatment- Day 7 (g)**	**Wt. of animals on the 14th day of treatment- Day 14 (g)**
R1	**150.45**	**174.46**	**137.42**	**126.55**
R2	**156.13**	**184.75**	**172.83**	**152.63**
R3	**151.99**	**186.69**	**146.02**	**132.67**
R4	**157.82**	**161.86**	**132.95**	**124.02**

3.2 CALCULATION FOR % INCRASE OR DECREASE IN WEIGHT

Initial Weight – Weight at Day 0

Final Weight –Weight at Day 14

$$\frac{final\ weight - initial\ weight}{initial\ weight} \text{ x } \frac{100}{1}$$

TABLE 3.2

Group 1 (Normal Contol)	% Increase % Decrease in weight
R1	36.33 %
R2	45.31%
R3	40.39%
R4	26.91%
R5	37.39%
Group 1	% Increase % Decrease in weight

(Diabetic Untreated)	
R1	-23.72%
R2	-10.70%
R3	-13.49%
R4	10.65%
Group 3 **(Diabetic treated with aqueos extract)**	% Increase % Decrease in weight
R1	-19.17%
R2	-17.45%
R3	-19.49%

3.3 WEIGHT OF ORGANS

Tables 3.3

Group 1 (Normal Control)	Wt. of liver (g)	Wt. of pancreas (g)
R1	7.420	0.640
R2	5.020	0.575
R3	7.630	0.569
R4	8.839	0.720
R5	5.820	0.640
Group 2 (Diabetic Untreated)	Wt. of liver (g)	Wt. of pancreas (g)
R1		0.230
R2		0.515
R3		0.260
R4		0.560
Group 3 (Diabetic treated with aqueos extract	Wt. of liver (g)	Wt. of pancreas (g)
R1	5.475	0.200
R2	5.230	0.200
R3	4.554	0.300

3.4 MORTALITY

Table 3.4

Group 1 (Normal control)	**Nil**
Group 2 (Diabetic untreated)	**3**
Group 3 (Diabetic treated with aqueous extract)	**3**

3.5

Chapter 3

RESULTS

Table 1: Effect of Aqueous Extract of *Andrographis Paniculata* on the activity of Lipase in diabetic animals.

Lipase				
	Abs 1 Min	Abs 2 Min	ΔAbs Min	Activity of Lipase

STD	1.935	1.952	0.017	
1	1.866	1.908	0.042	252
1	1.888	1.938	0.05	300
1	1.866	1.908	0.042	252
2	1.875	1.902	0.027	162
2	1.896	1.918	0.022	132
2	1.955	1.968	0.013	78
3	1.913	1.9.29	0.016	96
3	1.913	1.929	0.016	96
3	2.087	2.105	0.018	108

Group	Lipase Activity
Normal Control	268+- 3.464
Diabetic control	124+- 3.
Diabetic Treated	100+- 2

For both standard and samples, subtract abs 1 min from abs 2 min. then, use the formula in the procedure leaflet to calculate the activity of lipase. The activity of standard lipase enzyme (calibrator) is 102 U/L

Calculation

ΔAbs/min of Calibrator = (ΔAbs Calibrator - ΔAbs Blank)

ΔAbs/min of Sample= (ΔAbs Sample -ΔAbs Blank)

Lipase activity = $\frac{\Delta Abs/min\ of\ Sample}{\Delta Abs/min\ of\ Calibrator}$

Table 2: Effect of Aqueous Extract of Andrographis Paniculata on the activity of GGT in diabetic animals.

GGT AQUEOUS EXTRACT

SAMPLE	Abs 0 min	Abs 1 min	ΔAbs min	U/l
1	0.371	0.372	1× 10^-3	1.158
1	0.34	0.341	1× 10^-3	1.158
1	0.371	0.377	3×10^-3	3.474
2	0.6	0.687	0.087	100.746
2	0.432	0.439	7×10^-3	8.106
2	0.444	0.45	6×10^-3	6.948
3	0.75	0.754	4×10^-3	4.632
3	0.563	0.569	6×10^-3	6.948
3	0.657	0.662	5×10^-3	5.79

Group	GGT AQUEOUS ACTIVITY
Normal Control	1.93+- 0.876
Diabetic control	38. 6+- 3.167
Diabetic Treated	5.79+- 0.132

For GGT, substrate abs 0 min from abs 1 min and multiply the difference by 1158 as contained in the proceedure leaflet for GGT.

Calculation:

To calculate the GGT activity, use the following formula:

U/L= 1158 × ΔA 405nm/ min for procedure 1. (Sample start)

U/L= 1368 × ΔA 405 nm/min for proceedure 2. (Substrate start).

Table 3: Effect of Aqueous Extract of *Andrographis Paniculata* on the activity of GPx in diabetic animals.

GPx DIABETES AQ

Sample	Abs 1 min	GPx enzyme activity
1	1.392	36.7488
1	1.26	33.264
1	1.35	35.64
1	1.641	43.3224
1	1.097	28.9608
2	1.3974	36.89136
2	1.096	28.9344
2	1.554	41.0256
3	0.783	20.6712
3	1.14	30. 096
3	1.043	27.5352
3	0.982	25.9248

Group	GPx enzyme activity
Normal Control	35.5872+- 1.591
Diabetic control	35.61712+- 3.046
Diabetic Treated	26.0568+- 1.574

GPx enzyme activity (v/ml) = OD/min × Vt / E× Vs

OD= Absorbance

Vt = 6.6 ; E = 2.5; Vs= 0.1

CHAPTER 4

DISCUSSION AND CONCLUSION

REFERENCE

Abhishek N et al.(2010). Biological activities of Kalmegh (Andrographis paniculata Nees.) and its active principles. A review.1(2):125-135.

Akilandeswari G, Vijaya AA, Sampathkumar P, Vinayaga PM, Preethi B. (2019). A prospective Review on phyto-pharmacological Aspect of *Andrographis* Paniculata. 10(1):15-19.

Banerjee, S.; Kar, A.; Mukherjee, P.K.; Haldar, P.K.; Sharma, N.; Katiyar, C.K (2021). Immunoprotective potential of Ayurvedic herb Kalmegh (Andrographis paniculata) against respiratory viral infections—LC–MS/MS and network pharmacology analysis.phytochem. Anal.32, 629–639.

Barham, D. and Trinder, P., (1972). "An improved colour reagent for the determination of blood glucose by the oxidase system".Analyst . 97: 142 – 145.

Chauhan SK and Gyanendra T (2003). Effect of sources and levels of nitrogen on partitioning of andrographolide in Kalmegh (Andrographis paniculata Nees.), Ind J Plant Physiol. 8, 60-62.

Chaurasia A, Kharya MD, Sharma B, Roy P (2012). Glucose metabolism and diabetogenic gene expression analysis of chloroform fraction of Andrographis Paniculata (Nees) whole herb in diabetic albino mice. Indian J Med Res.135(5):636-41

Dai, Y.; Chen, S.R.; Chai, L.; Zhao, J.; Wang, Y.; Wang, Y (2019). Overview of pharmacological activities of Andrographis paniculata and its major compound andrographolide. Crit. Rev. Food Sci. Nutr. 59, S17–S29

Gabrielian ES, Shukarian AK, Goukasova GI, et al (2002). A double blind,Placebo-controlled study of *Andrographis paniculata* fixed combination Kan Jang in the treatment of acute Upper respiratory tract infections Including sinusitis. Phytomedicine.9:589-597

Gabrielian ES, Shukarian AK, Goukasova GI, Chandanian GL, Panossian AG (2002) A double blind, placebo-controlled study of Andrographis paniculata fixed combination Kan Jang in the treatment of acute upper respiratory tract Infections including sinusitis. Phytomed 9: 589-597

Geethangili M, Rao YK, Fang SH, Tzeng YM (2008) Cytotoxic constituents from Andrographis paniculata induce cell cycle arrest in jurkat cells. Phytother Res 22: 1336-1341.

Grundy, S.M.(2007). 'Gamma-glutamyl transferase, Arteriosclerosis, Thrombosis, and vascular Biology, 27(1), pp. 4-7.

Hidalgo, M.A.; Hancke, J.L.; Bertoglio, J.C.; Burgos, R.A. Andrographolide a new potential drug for the long term treatment of rheumatoid arthritis disease. In Innovative Rheumatology; InTech: London, UK, 2013.

Jarukamjorn K, Nemoto N (2008) Pharmacological aspects of Andrographis Paniculata on Health and its Major Diterpenoid Constituent Andrographolide. Jounal of Health Science 54(4):370-381

Jun-Young Park, Kyung-Min Park (2022), "Lipase and Its Unique Selectivity: A Mini-Review", Journal of Chemistry, vol. 2022. Page 11.

Hu, X.Y.; Wu, R.H.; Logue, M.; Blondel, C.; Lai, L.Y.W.; Stuart, B.; Flower, A.; Fei, Y.T.; Moore, M.; Shepherd, J.; et al. Andrographis Paniculata (ChuAn Xin Lián) for symptomatic relief of acute respiratory tract infections in adults and children: A systematic review and meta-analysis. PLoS ONE 2017, 12, e0181780.

Kabeeruddin M (1937) Kitabul Advia. Vol 2. Delhi, India: Aligarh Barqi Press: 148.

Liu, X.Y.; Niu, X.; Feng, Q.J.; Yang, X.Z.; Wang, D.W.; Zhao, T.; Li, L.; Du, H (2016). A new biocompatible microemulsion increases extraction yield and bioavailability of Andrographis paniculata. Chin. J. Nat. Med.14, 683–691

Maheshwari SK, Sharma RK, Mishra PK and Gangrade SK (2002). Response of kalmegh (Andrographis paniculata) to dates of planting and harvesting in a shallow black soil, JMAPS. 24, 969-971.

Margis, R. et al. (2008) 'Glutathione peroxidase family – an evolutionary overview', FEBS Journal, 275(15), pp. 3959–3970.

Mishra SK, Sangwan NS, Sangwan RS (2007). Andrographis Paniculata (Kalmegh): A review, Pharmacognosy Reviews. 1:283-298.

Nugroho AE, rais IR, Setiwan I, Pratrwi PY, Hadibalata T, Tegar M, Pramono S (2013). Pancreatic effects of andrographolide isolated from Andrographis paniculata (Burm f.) Nees. Indian J Exp Biol.51(12):1101-8

Nyman, N. (1959). Determination of glutathione peroxidase in tissue. Analytical Biochemistry . 28: 481.

Pawar, A.; Rajalakshmi, S.; Mehta, P.; Shaikh, K.; Bothiraja, C (2016). Strategies for formulation development of andrographolide. RSC Adv. 73, 69282–69300.

Samy RP, Thwin MM, Gopalakrishnakone P, Ignacimuthu S (2008) Ethnobotanical survey of folk plants for the treatment of snakebites in Southern Part of Tamilnadu, India.J Ethnopharmacol 115: 302-312

Seema N, Mohod NB, Wankhade SG and Paturde JT (2003).Effect of Planting and harvesting dates on yield and quality of kalmegh (Andrographis paniculata), JMAPS. 25, 981-983.

Shahid A, (2011). Andrographis paniculata: A review of Pharmacological activities and clinical effects, Alternative Medicine Review. 16:66-77.

Sivananthan M, Elamaran M (2013) Medicinal and Pharmacological properties of Andrographis paniculata. International Journal of Biomolecules and Biomedicine 3: 1-12

Thokchom, A.; Talukdar, A.; Chingakham, B.; Ningthoujam, S.; Choudhury, M (2018). Traditional usage of ethnomedicinal plants in treating liver disorders at Manipur, North East India. Eur. J. Med. Plants. 23, 1–10

Tonyushkina, K. and Nichols, J.H. (1983). "Weight Balance: A Review of Technical Challenges to Obtaining Accurate Results". Journal of Diabetes, Science and Technology. 3(4): 971–980.

Yang, Y.; Cao, T.; Guo, Q (2020). Characterization of a traditional Chinese medicine plant the chloroplast genome of Andrographis Paniculata. Mitochondrial DNA Part B Res.5, 1949–1951.

Zhang C, Gui L, Xu Y, Wu T, Liu D (2012). Preventive effects of andrographolide on the Development of diabetes in autoimmune diabetic NOD mice by inducing Immune tolerance. J Complement Integr Med.10:19.