

UNIVERSITY OF GHANA

**COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY**

**ANTI-HYPERGLYCAEMIC AND ANTI-OXIDANT EFFECT OF
SYNEDRELLA NODIFLORA (L) GAERTN
IN STREPTOZOTOCIN-INDUCED DIABETES IN RATS.**

BY

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**A THESIS SUBMITTED TO THE UNIVERSITY OF GHANA,
LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE AWARD OF MASTER OF PHILOSOPHY
IN PHARMACOLOGY**

JULY, 2018

DECLARATION

DECLARATION BY THE CANDIDATE

I hereby declare that this is the product of my own research undertaken under supervision and has neither been presented either in whole or in part for another degree elsewhere. I am solely responsible for any residual flaws in the work.

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DECLARATION BY SUPERVISORS

We hereby declare that the principal work and presentation of the thesis was supervised by us in accordance with guidelines on supervision of thesis laid down by the University of Ghana.

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ACKNOWLEDGEMENTS

I will like to express my sincere gratitude to God and my Lord Jesus Christ for me seeing through this stage of my life till the end. What would I have done if YOU were not with me? I have never walked alone.

As for Pharm. Dr. Patrick Amoateng, my supervisor, my mentor, my friend and my big brother, I have nothing but praises for your support, expertise and counsel. You have put in so much and God bless you so much. You were undoubtedly pivotal in bringing me this far.

To my co-supervisor Dr. Kennedy Edem Kukuia thanks for being there, a warm smile and having a listening ear. God bless you sir.

To all the Lecturers in the department of Pharmacology and Toxicology, especially Dr Kwame BN Banga (H.O.D), Dr. Isaac J Asiedu-Gyekye, Dr. Seth K. Amponsah, Prof. Kwasi A. Bugyei and Prof. Alexander K Nyarko, God bless you all so much. You are deeply appreciated.

Also to my course mates, Mr. Joseph Torbi, Miss. Efua Koomson, Mr. Kwabena Oteng-Boahen, Mr. Richard N Lartey, Mr. Isaac Prah and Mr. Martin Akandawen etc, thank you all so much. I am really indebted to you all, God bless.

To my lovely wife Rosemond, love you so much. Thanks for your patience and support.

ABSTRACT

Background: *Synedrella nodiflora* is a common weed in Ghana that is boiled to treat epilepsy and threatened abortion. The ethanolic whole plant extract has been demonstrated to have anti-inflammatory, antioxidant, hypoglycemic, anticonvulsant, sedative, antidepressant and antipsychotic effects in murine models. The study presents the anti-oxidant and anti-hyperglycaemic effects of the ethanolic extract of the whole plant extract of *Synedrella nodiflora* (SNE) on a diabetic rat model.

Method: Diabetes was induced using an intraperitoneal injection of streptozotocin (STZ) (55 mg/kg). SNE (100, 300, 1000 mg/kg, *p.o*) and insulin (0.1, 0.3, 1.0 IU/kg, *s.c.*) were administered for 8 weeks. Bodyweight and fasting blood sugar were measured twice weekly. Serum biochemistry, haematological indices, organ histopathology, MDA and SOD assay were done.

Results: SNE induced a significant decrease ($P < 0.05$) in hyperglycaemia, with a corresponding increase in weight of the rats. The extract also reduced significantly the liver enzymes AST, ALT, ALP and increased albumin levels compared to the vehicle group ($P < 0.01$). SOD and MDA tests both showed significant increase ($P < 0.05$) and decrease ($P < 0.01$) respectively for SNE high dose of 1000 mg/kg, indicating antioxidant activity. Haematological indices showed a decrease in RBC and an increase in eosinophil count in the vehicle group ($P < 0.05$).

Conclusion: The ethanolic extract of the whole plant of *Synedrella nodiflora* possess anti-oxidant and anti-hyperglycaemic effects on rat model of diabetes.

TABLE OF CONTENTS

DECLARATION	i
ACKNOWLEDGEMENTS	ii
ABSTRACT	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	viii
LIST OF TABLES	viii
LIST OF ABBREVIATIONS	xi
CHAPTER ONE	1
1.0 GENERAL INTRODUCTION	1
1.1 BACKGROUND	1
1.2 RESEARCH QUESTIONS	3
1.3 PROBLEM STATEMENT	3
1.4 JUSTIFICATION OF THE STUDY	4
1.5 AIM OF RESEARCH	4
1.6 OBJECTIVES OF RESEARCH	5
CHAPTER TWO	6
2.0 LITERATURE REVIEW	6
2.1 INTRODUCTION	6
2.2 DIABETES	6
2.2.1 Types of diabetes.	6
2.2.2 Type1 diabetes (T1DM)	7
2.2.2.1 Pathophysiology of T1DM	7
2.2.3 Type 2 Diabetes Mellitus (T2DM)	7
2.2.3.1 Pathophysiology of T2DM	8
2.2.3.2 Insulin resistance in skeletal muscles	8
2.2.4 Gestational Diabetes Mellitus (GDM)	9
2.2.4.1 Pathophysiology of GDM	9

2.2.5 Other specific types (Monogenic diabetes).....	10
2.2.6 Symptoms of diabetes	10
2.2.6.1 Clinical features of type I diabetes	11
2.2.6.2 Clinical features of Type II diabetes	11
2.2.7 Pre-diabetes.....	11
2.2.8 Diagnosis.....	11
2.2.8.1 Glycated haemoglobin	12
2.2.8.2 Fasting Blood Sugar (FBS)/Random Blood Sugar (RBS)	12
2.2.8.3 Oral Glucose Tolerance Test (OGTT)	12
2.2.8.4 Anti-diabetic drugs.....	13
2.2.9 The Economic burden of Diabetes.....	17
2.2.10 Complications of diabetes.....	17
2.2.10.1 Macrovascular complications.....	17
2.2.10.2 Microvascular complications	18
2.2.10.2.1 Diabetic nephropathy.....	18
2.2.10.2.2 Diabetic retinopathy	18
2.2.10.2.3 Diabetic neuropathy.....	18
2.3 DIABETES: THE HERBAL APPROACH.....	19
2.3.1 Diabetes Mellitus and Medicinal plants.....	19
2.3.2 Diabetic complications and medicinal plants.	21
2.4 <i>SYNEDRELLA NODIFLORA</i> (L) GAERTN.....	22
2.4.1 Plants description	22
2.4.2 Folkloric uses	23
2.4.3 Phytochemical screening	24
2.4.3 Pharmacological tests.....	24
2.6 ANIMAL MODEL IN DIABETES	25
CHAPTER THREE	27
3.0 MATERIALS AND METHODS	27
3.1 STUDY DESIGN	27
3.2 PLANT EXTRACT PREPARATION	27
3.2.1 Plant acquisition.....	27

3.2.2 Preparation of the whole plant of <i>Synedrella nodiflora</i>	27
3.2.3 Phytochemical screening	28
3.2.3.1 Alkaloids (Dragendorff's Test)	28
3.2.3.2 Saponins (Frothing test)	28
3.2.3.3 Phytosterols (Lieberman-Burchard's test)	28
3.2.3.4 Terpenoids (Salkowski test)	29
3.2.3.5 Glycosides (General test)	29
3.2.3.6 Tannins (Ferric Chloride test)	29
3.2.3.7 Phenolic Compounds (Acetic acid test)	29
3.2.3.7 Flavonoids (Kumar test).....	30
3.2.3.9 Volatile Oils	30
3.3 EXPERIMENTAL ANIMALS AND HUSBANDRY	30
3.4 INDUCTION OF DIABETES.....	31
3.4.1 Preparation of citrate buffer	31
3.4.2 Process of diabetes induction with STZ.....	31
3.4.3 Oral glucose tolerance test	32
3.4.4 Grouping	32
3.4.5 Preparation of drugs.....	32
3.4.6 Drug administration	33
3.4.7 Fasting blood sugar (FBS) determination	33
3.4.8 Determination of percentage change in bodyweight and relative organ weight.....	33
3.5 PREPARATION OF SD RATS FOR BLOOD TESTS AND HISTOPATHOLOGY ...	34
3.5.1 Sacrificing of rats.....	34
3.5.2 Preparation of organs	34
3.5.3 Preparation of blood.....	35
3.5.4 Preparation of serum	35
3.5.5 Kidney and liver Function tests	35
3.5.6 Haematological analysis	35
3.6 HISTOPATHOLOGY	36
3.6.1 Processing of Organs into slides.....	36
3.6.2 Examination of slides.....	36

3.7 ANTIOXIDANT ACTIVITY	36
3.7.1 Determination of Superoxide Dismutase (SOD) Activity	37
3.7.2 Procedure for SOD activity determination	37
3.7.3 Malondialdehyde (TBARS) determination	38
3.7.4 Procedure for MDA determination	38
3.8 ANALYSIS OF RESULTS	39
CHAPTER FOUR.....	39
4.0 RESULTS.....	40
4.1 PHYTOCHEMICAL SCREENING.....	40
4.2 INDUCTION OF DIABETES.....	40
4.3 ANTIHYPERGLYCAEMIC ACTIVITY OF SNE	42
4.4 WEIGHT PARAMETERS	46
4.4.1 Percentage change in bodyweight.....	46
4.4.2 Relative Organ Weight	48
4.5 BLOOD BIOCHEMISTRY	50
4.6 HAEMATOLOGY	52
4.7 ANTIOXIDANT ACTIVITY.....	54
4.7.1 SOD and MDA assay.....	54
4.8 HISTOPATHOLOGY	56
4.8.1 Liver	57
4.8.2 Kidney.....	58
4.8.3 Lungs.....	59
4.7.4 Brain (cerebral cortex)	60
4.7.5 Spleen.....	61
CHAPTER FIVE	62
5.0 DISCUSSION	62
5.1 DISCUSSION.....	62
5.2 CONCLUSION	69
5.3 RECOMMENDATIONS.....	70
REFERENCES	70

LIST OF FIGURES

Figure 1: Picture of <i>Synedrella nodiflora</i> showing leaves and flowers	24
Figure 2: The effect of STZ injection on SD rats in diabetes induction.....	42
Figure 3: The effect of the administration of SNE and insulin in diabetic rats over 8 weeks..	44
Figure 4: Effect of diabetes on bodyweights of diabetic rats receiving vehicle and SNE.....	47
Figure 5: The effect of SNE on SOD activity (A) and MDA levels (B) in STZ-induced diabetic male SD rats after 8week of administration.....	55
Figure 6: Photomicrographs of liver isolated from rats after 8 weeks of continuous administration.....	57
Figure 7: Photomicrographs of kidneys isolated from rats after 8 weeks of continuous administration	58
Figure 8: Photomicrographs of lung isolated from rats after 8-week continuous administration..	59
Figure 9: Photomicrographs of cerebral cortex of brains isolated from rats after 8-week continuous administration	60
Figure 10: Photomicrographs of spleens isolated from rats after 8-week continuous administration	61

LIST OF TABLES

Table 1: Diabetes drugs, mechanisms of action and side effects.....	14
Table 2: Plants used in diabetes, mechanisms of action and model.....	20
Table 3: Pure compound for treating diabetic complications and their mechanisms of action.....	21
Table 4: Animal models for diabetes and their limitations.....	26
Table 5: Phytochemical screening results	40
Table 6: ED ₅₀ of insulin and SNE.....	44
Table 7: Effect of diabetes on relative organ weights of diabetic rats receiving vehicle and SNE (100-1000 mg/kg, <i>p.o</i>) for 8 weeks.....	48
Table 8: The effect of SNE (100–1000 mg/kg, <i>p.o</i>) on blood biochemistries in STZ–induced diabetic SD rats after 8-week administration	50
Table 9: The effect of SNE (100–1000 mg/kg, <i>p.o</i>) on haematological indices in STZ -induced diabetic SD rats after 8week administration compared to control and vehicle.....	52

LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AGE	Advanced Glycation Endproducts
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
Bas	Basophils
DAG	Diacylglycerol
DN	Diabetic Neuropathy
Eos	Eosinophils
FBS	Fasting Blood Sugar
FPG	Fasting Plasma Glucose
HCT	Haematocrit
HDL	High Density Lipoprotein
HGB	Haemoglobin
LDL	Low Density Lipoprotein
Lymp.	Lymphocytes
MDA	Malondialdehyde
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
NO-cGMP	Nitric Oxide- Cyclic Guanine Monophosphate
PLT	Platelets

PKC	Protein Kinase C
RAGE	Receptor for Advanced Glycation Endproducts
RBC	Red Blood Cells
SD	Sprague Dawley
SOD	Superoxide Dismutase
SNE	Synedrella Nodiflora Extract
STZ	Streptozotocin
TBARS	Thiobarbituric Acid Reactive Substances
Tot Chol.	Total cholesterol
Tot. Pro	Total Protein
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
WBC	White blood Cells

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 BACKGROUND

Diabetes is a well-known chronic disorder characterized by many metabolic changes in the body as a result of insufficiency in secretion of insulin and peripheral resistance to insulin (Georga *et al.*, 2018). The worldwide prevalence of diabetes is on the rise due to obesity, poor feeding habits and sedentary lifestyle (Klonoff, 2009). There were about 518,400 known cases of diabetes in Ghana as at 2017 (International Diabetes Federation, 2017). Diabetes presents with long term complications that lead to damage of the retina, kidney, and liver. It can also lead to cardiovascular diseases (Dokken, 2008). Recent prospective studies have provided unequivocal evidence on the crucial role of prolonged hyperglycaemia in the development of chronic diabetic complications (Matuszewski *et al.*, 2013).

Hyperglycaemia causes tissue damage through multiple mechanisms including increased flux of glucose and other sugars through the polyol pathway, increased intracellular formation of advanced glycation end products (AGEs), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C isoforms, and the over activity of the hexosamine pathway (Vinik *et al.*, 2013). A lot of researches have shown that oxidative stress and inflammation in diabetes play key roles in inducing many secondary chronic complications (Cefalu, 2009; Kawano, 2014; Wellen and Hotamisligil, 2005).

In view of the many metabolic changes that occur in diabetes, many drugs are employed to tackle the cause, complications and symptoms at the same time. Treatment of diabetes is

challenging as the pathological mechanisms of diabetes and its complications are multidirectional and complicated (Zychowska *et al.*, 2013).

Diabetes is treated with drugs of many classes including, biguanides, sulfonylureas and alpha-glucosidase inhibitors. These drugs may be used individually or in combination depending on the patients' response. This raises concerns about drug-drug interactions, compliance and cost of treatment. There are also concerns of long term overstimulation of the beta pancreatic cells by sulfonylureas which may lead to secretory failure (Ramedì and Nichols, 2008). Side effects like lactic acidosis and weight gain have been well established for biguanides and sulfonylureas, respectively (BNF 68, 2014). All these create clinical challenges in the management of diabetes. Cost of managing diabetes has placed enormous economic burden on patients, family members and healthcare delivery as a whole (Zhang and Gregg, 2017).

Plants have been an alternate source of medicines for many over the years. Plants may have a significant role to play in diabetes (Matough *et al.*, 2012; Tiwari *et al.*, 2002). Apart from their ease of accessibility, plants are known to possess anti-oxidative, anti-inflammatory, analgesic and anti-hyperglycaemic activity (Arumugam *et al.*, 2013). All of these properties will tackle key components of diabetes and possibly its complications (Bhagour *et al.*, 2016; Tiwari *et al.*, 2018).

Synedrella nodiflora is a plant that originated from the Americas, but is locally abundant around streams and rivers in West Africa. Traditionally it is used in the treatment of hiccup, epilepsy and threatened abortion by boiling and drinking of the whole plant (Mshana *et al.*, 2000). It has been demonstrated to possess anticonvulsant, analgesic and anti-inflammatory properties (Amoateng *et al.*, 2011; Haque *et al.*, 2012). It has also been proven to have low toxicity even at high doses in rats (Adjei *et al.*, 2014; Amoateng *et al.*, 2016).

Of much importance to this study is its anti-hyperglycaemic, anti-oxidant properties in addition to its anti-inflammatory and analgesic properties already established (Amoateng *et al.*, 2015; Haque *et al.*, 2012; Woode *et al.*, 2009). Oxidative stress, inflammation, and hyperglycaemia are the main factors that influence the progression of diabetes. *Synedrella nodiflora* may be a very useful plant in diabetes if it can significantly reduce these key factors that play major roles in diabetes in a rat model.

1.2 RESEARCH QUESTIONS

- Can the extract significantly reduce hyperglycaemia in the diabetic rat model?
- Will the anti-oxidative properties result in reduction of oxidative stress markers *in vivo*?
- Does the extract have any organo-protective effects in diabetic rats?
- What other effects will the extract have on blood biochemistry and haematology in diabetes?

1.3 PROBLEM STATEMENT

Diabetes causes a lot of complications. Diabetes can affect the heart, kidney, liver and eye. It also inculcates hyperglycaemia, inflammation and oxidative stress simultaneously to achieve its adverse outcomes (Giacco and Brownlee, 2010). These complications can be very distressing to patients. Diabetic neuropathy for example is characterized by pain, hypersensitivity, numbness of the limbs which can result in possible foot amputations. The total cost of treatment of diabetes and all its complications as well as symptoms continue to present a burdensome socio-economic challenge to patients, relatives, clinicians and healthcare systems (Zhang and Gregg, 2017).

1.4 JUSTIFICATION OF THE STUDY

Synedrella nodiflora is very common and easily accessible in Ghana. It has been proven to have low toxicity in chronic and acute administration of high doses in rats (Adjei *et al.*, 2014a; Amoateng *et al.*, 2014b; Amoateng *et al.*, 2016). The plant also possesses anti-inflammatory and *in vitro* antioxidant properties (Amoateng *et al.*, 2011; Haque *et al.*, 2012). Also the methanolic extract of the plant has shown hypoglycemic activity in alloxan-induced Long-Evans diabetic rats, given by intra-peritoneal injection for 3 days (Zahan *et al.*, 2012). This study however seeks to further study the effects of an ethanolic extract of the whole plant in streptozotocin-induced diabetic rats. This will provide further pharmacological information regarding the anti-diabetic potential of the plant towards drug discovery. The hypoglycaemic and *in vitro* antioxidant properties of the ethanolic extract from the plant provides the basis for further research into the possible anti-hyperglycaemic and *in vivo* anti-oxidant activity. *In vitro* antioxidant activity may not always translate into activity *in vivo*. An *in vivo* antioxidant activity could be beneficial since oxidative stress plays vital role in worsening of diabetes and its complication. An agent that could possibly reduce sugar levels, provide antioxidant protection, reduce inflammation and also reduce pain will be an excellent alternative to current drugs that do not have all these properties combined. It is also a cheaper alternative to current treatments.

1.5 AIM OF RESEARCH

This research aims at investigating the anti-oxidant and anti-hyperglycaemic effect of the ethanolic extract of *Synedrella nodiflora* (L.) Gaertn in diabetic rat models.

1.6 OBJECTIVES OF RESEARCH

- Induce diabetes in Sprague-Dawley rats with streptozotocin (STZ) and evaluate the anti-hyperglycaemic properties of the extract in comparison with a standard drug by comparing fasting blood sugar (FBS) levels.
- Evaluate the effect the extract on oxidative stress by measuring SOD and MDA levels.
- Assess the effect of the extract on blood biochemistry and heamatology in diabetes.
- Assess the histopathological effect of the extract on major organs in diabetic rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

2.2 DIABETES

Diabetes mellitus is a group of complex metabolic disorder characterized by deficiency in insulin secretion, impaired insulin action, or a combination of both leading to hyperglycaemia (Crawford, 2017). Diabetes is a chronic illness requiring continuous medical care with multifactorial risk-reduction strategies targeting glycemic control. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source (Le Roith and Zick, 2001). Chronic hyperglycaemia is associated with micro vascular and macro vascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, heart disease, and stroke (Goldenberg and Punthakee 2013). Its long term complications include damage to the heart, retina, kidney and the peripheral nerves. Damage to the nerves lead to diabetic neuropathy (DN). It is reported that dyslipidemia coupled with lipid peroxidation in diabetes induce many secondary chronic complications including atherosclerosis and neural disorders (Upadhyay *et al.*, 2018).

2.2.1 Types of diabetes.

The common types of diabetes include; Type1 Diabetes Mellitus (T1DM), Type 2 Diabetes Mellitus (T2DM), Gestational Diabetes Mellitus and Other Specific Types also known as Monogenic Diabetes.

2.2.2 Type1 diabetes (T1DM)

Type1 diabetes (T1DM) results from pancreatic beta cell destruction. Most childhood diabetes are of type 1. This type of diabetes is due to an autoimmune process for which the etiology of the beta cell destruction is unknown. T1DM is a chronic disorder of glucose homeostasis, leading to a absolute deficiency in insulin resulting in hyperglycemia.

2.2.2.1 Pathophysiology of T1DM

T1DM begins with a predisposition at birth with the inheritance of genetic risk factors. Development of autoimmune type 1 diabetes is typically divided into stages, starting with genetic susceptibility and ending with complete loss of the β -cells of the pancreatic islets (Georga *et al.*, 2018). The β -cells are destroyed by autoantibodies which are produced by the presentation of autoantigens to autoreactive lymphocytes (Brinkman, 2017). Environmental factors such as infections may trigger autoimmune destruction of the beta pancreatic cells by inciting cross-reactivity against antigens on the beta-cells that bear a similar molecular structure (Rewers and Ludvigsson, 2016). The process involving immune activation to clinically relevant islet cell destruction may take years but is marked early by the presence of serum autoantibodies.

These may include: Glutamic Acid Decarboxylase Autoantibodies (GADA), Tyrosine phosphatases IA-2 and IA-2b, Zinc transporter (ZnT8), Insulin Autoantibodies (IAA) (Chahine, 2017).

2.2.3 Type 2 Diabetes Mellitus (T2DM)

Type 2 diabetes mellitus (T2DM) has become an epidemic burden in worldwide. Diabetes mellitus is a common metabolic disorder associated with chronic complications such as macro-

micro vascular complications. The onset of T2DM is often silent and many years may pass before diagnosis (Barrett, 2017).

2.2.3.1 Pathophysiology of T2DM

In type 2 diabetes, the two main pathological defects are impaired insulin secretion through dysfunction of pancreatic β -cell and impaired insulin action through insulin resistance. T2DM leads to a decrease in pancreatic beta-cell function over a period of time. This may occur concurrently with insulin resistance and hyperglycemia (Khanam *et al.*, 2017).

The pathophysiology of type 2 diabetes includes aberrant secretion of glucagon, resulting in elevated glucagon concentrations in both the fasting state and after a meal (Girard, 2017). Postprandial glucagon suppression may also be flawed. Some T2DM patients can also have an increase in glucagon secretion in response to a meal. It is also known that such patient's alpha pancreatic cells maybe resistant to the glucagon suppressive effects on glucose and insulin. The resulting hyperglucagonemia may increase hepatic glucose output, all leading to persistent hyperglycemia (Haedersdal *et al.*, 2018).

2.2.3.2 Insulin resistance in skeletal muscles

Insulin resistance in skeletal muscle manifests itself primarily as a reduction in insulin stimulated glycogen synthesis due to reduced glucose transport. Ectopic lipid accumulation in skeletal muscles and liver plays an important role in inducing insulin resistance (Baynest, 2015). Multiple defects in insulin signaling are responsible for impaired glucose metabolism in insulin resistance. Increased lipids promote insulin resistance through the activation of signaling

pathways like protein kinase C (PKC), ceramide, and other lipid molecules and the accumulation of lipids in target tissues can induce insulin resistance (Gustafson *et al.*, 2015). Lipid accumulation within myocytes and hepatocytes is strongly associated with insulin resistance in type 2 diabetic patients, in non-diabetic offspring of type 2 diabetic subjects, in subjects with impaired glucose tolerance, and in obese subjects (Sesti, 2006).

Furthermore, excess adipose tissue secretes the metabolites leptin, adiponectin, and tumor necrosis factor-alpha and these can decrease cell sensitivity to insulin (Caselli, 2014).

2.2.4 Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus identifies with women who develop diabetes mellitus during pregnancy. Women who develop Type 1 diabetes mellitus during pregnancy and women with undiagnosed asymptomatic Type 2 diabetes mellitus that is discovered during pregnancy are classified as GDM. In most women who develop GDM, the disorder may start in the second or third trimester of pregnancy (Piper *et al.*, 2017).

2.2.4.1 Pathophysiology of GDM

In pregnancy, women develop some insulin resistance, which most likely evolved as a means to facilitate energy delivery to the fetus. Insulin resistance occurs as a response to placental hormones released during pregnancy. Several pregnancy hormones are thought to disrupt the usual action of insulin as it binds to its receptor, most probably by interfering with cell signalling pathways, leading to increase in insulin resistance (Mack and Tomich, 2017). Insulin resistance

may progress to GDM by revealing autoimmune-mediated or genetic abnormalities in pancreatic beta-cell function and/or worsening of chronic insulin resistance in pregnant women (Evensen, 2012).

2.2.5 Other specific types (Monogenic diabetes)

Certain types of diabetes mellitus of various known etiologies are grouped together to form the classification called “Other Specific Types” (Punthakee *et al.*, 2018). Individuals in this group include persons with genetic defects of their beta-cell function (this type of diabetes was formerly called MODY or maturity-onset diabetes in youth), persons with diseases affecting their exocrine pancreas, such as pancreatitis or cystic fibrosis, persons with dysfunction associated with other endocrinopathies (e.g. acromegaly), and persons with pancreatic dysfunction from drug use, chemicals or infections. This group comprise less than 10% of DM cases (Goldenberg and Punthakee, 2013).

2.2.6 Symptoms of diabetes

The symptoms of both T1DM and T2DM are similar. They may however develop more rapidly and clearly in type 1 diabetes. Some of the symptoms include weight loss, polyurea, polydipsia, polyphagia, constipation fatigue, cramps, blurred vision, and candidiasis. Diabetic patients may become susceptible to microvascular complications (neuropathy, retinopathy and nephropathy) and macrovascular disease (coronary artery, heart, and peripheral vascular diseases) after longstanding hyperglycemia (Baynest, 2015).

2.2.6.1 Clinical features of type I diabetes

In addition to the above symptoms, T1DM also present with diabetic ketoacidosis (DKA), accompanied with vomiting, abdominal pain, and lethargy. It can also present as an incidental finding performing a urine or blood test for other reasons. DKA can be life-threatening when it presents as an acute complication of T1DM (Brinkman, 2017).

2.2.6.2 Clinical features of Type II diabetes

Most cases are diagnosed because of complications or accidentally. There is also a high risk of large vessel atherosclerosis commonly associated with hypertension, hyperlipidemia and obesity. Most patients with type 2 diabetes die from cardiovascular complications and end stage renal disease (Zappas and Granger, 2016).

2.2.7 Pre-diabetes

Individuals with impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) have intermediate conditions in the transition between normality and diabetes. People with IGT or IFG are at high risk of progressing to type 2 diabetes.

2.2.8 Diagnosis

A positive test result for any of the below tests isn't conclusive. It should be repeated to exclude laboratory error, unless history and clinical signs do not leave any doubt about the diagnosis.

Preferably, the same test method should be used. In case of conflicting results from two different tests, the test being positive for diabetes should be repeated.

2.2.8.1 Glycated haemoglobin

The HbA1C test is recommended for diagnosis of diabetes based on a threshold of $\geq 6.5\%$. The HbA1C test may be influenced by ethnicity, haemoglobinopathies, acute/chronic blood loss, anemia and becomes invalid in conditions with abnormal red cell turnover (Nitin, 2010). This test is relatively expensive and not yet readily available.

2.2.8.2 Fasting Blood Sugar (FBS)/Random Blood Sugar (RBS)

Fasting blood sugar is the level of sugar in the blood after an 8 hour period fasting. This test is usually done in the early morning when the patient has eaten nothing. The normal range for a non-diabetic should be between 4-6 mmol/l. Chronic Fasting blood sugar (FBS) levels greater than 7 mmol/l (126 mg/dl) could be indicative of diabetes. Random blood sugar is also the sugar level of the blood 2 hours after eating a meal. With the classic symptoms of hyperglycaemia or a hyperglycaemic crisis, a random plasma glucose ≥ 11.1 mmol/l (200 mg/dl) may be diagnostic of diabetes.

2.2.8.3 Oral Glucose Tolerance Test (OGTT)

OGTT is also used clinically to diagnose impaired glucose tolerance and as a standardized test of carbohydrate metabolism and insulin secretion. It requires an oral administration of glucose and an observation plasma level of glucose and insulin levels over time, usually 2 hours. A

prolonged elevation even after 2 hours of administration in both plasma glucose and insulin will imply an impaired glucose tolerance and insulin resistance. An OGTT of ≥ 11.1 mmol/l (200 mg/dl) is suggestive of diabetes. The test should be performed according to the WHO instructions with an equivalent of 75 g anhydrous glucose dissolved in water.

2.2.8.4 Anti-diabetic drugs

Over the years many anti-diabetic drugs have been developed to lower blood sugar. The drugs are varied and act via various mechanisms. Despite the great strides made in anti-diabetic drug development, contemporary orthodox drugs are still present with various challenges. These include side effects, compliance issues and cost. The table below provides examples of orthodox drugs, brand names, their mechanism of action and side effects.

Table 1: Diabetes drugs, mechanisms of action and side effects.

Drug classes	Examples of drugs	Mechanism of action	Side effects
Sulphonylureas	Gliclazide (Diamicon®) Glimepiride (Amaryl®) Glyburide (DiaBeta®)	Stimulate the pancreas by binding to specific site of on the β -cell K_{ATP} channel complex and inhibiting its activity.	Hypoglycemia Weight gain
Thiazolidinediones	Pioglitazone (Actos®) Rosiglitazone (Avandia®)	Act as ligands for peroxisome proliferating activation factor γ (PPAR γ) Regulates of genes that control glucose and lipid metabolism. Leads to increased glucose uptake in liver increased sensitivity to insulin.	Foot swelling due to water retention, weight gain. Pioglitazone : increased risk of bladder cancer Rosiglitazone : increased risk of non-fatal heart attack

Meglitinides	Nateglinide (Starlix®)	Stimulate insulin release by closing cell K_{ATP} channel complex and inhibiting its activity. This causes calcium influx into the β -cell and lead to depolarization and release of insulin.	Hypoglycaemia
Biguanides	Metformine (Glucophage®)	Increase the activity of AMP-dependent kinases causing increased glucose uptake, decrease in gluconeogenesis and lipogenesis, increased glucose sensitivity and decreased peripheral resistance	Diarrhoea, metallic aftertaste , lactic acidosis
Alpha-glucosidases inhibitors	Acarbose (Glucobay®)	Inhibits enzymes in the intestine that metabolize carbohydrates. Slows down absorption of carbohydrates.	Bloating and flatulence.
Dipeptidyl-peptidase-4 (DPP-4) inhibitors	Linagliptine (Trajenta®) Saxagliptine (OnglyzaMC)	Increase insulin secretion from β -cells and decrease glucagon secretion from α -cells by increasing glucagon-like peptide-1.	Pharyngitis, headache.

Glucagon-like peptide-1 (GLP-1) agonist	Dulaglutide (Trulicity®)	Suppress hepatic glucose production	Nausea, diarrhea, vomiting.
	Liraglutide (Victoza®)	Increase insulin secretion from β -cells and decrease glucagon secretion from α -cells.	
	Exenatide (Byetta®)		
Sodium glucose cotransporter 2 (SGLT2) inhibitors	Dapagliflozine (Forxiga®)	Lowers renal threshold for glucose, and reduce renal reabsorption of filtered glucose from tubular lumen leading to increased urinary glucose excretion.	Vulvovaginitis, balanitis urinary tract infections, hypoglycemia.
Insulins	Short acting- insulin lispro(Humalog®),	Bind to plasma membrane receptor and initiate a cascade of signaling events that results in the utilization of glucose and glycogen synthesis.	
	insulin aspart (Novolog®).		
	Intermediate acting - NPH(Humulin N®).		
	Long acting – insulin glargine(lantus®).		

2.2.9 The Economic burden of Diabetes.

Diabetes and its complications impose direct or indirect economic stress on individuals, families, health care systems and countries (Aronson *et al.*, 2018). People living with diabetes and their families are impacted negatively as the cost of treatment is usually high and can affect family income and may also affect the work of the diabetic individual. Many strategies have been employed to prevent the increase in type 2 diabetes and to reduce its negative economic impact. These include cost reductions in healthy foods, taxation or reformulation of unhealthy foods, promoting physical activity, and nutritional education and informative food labelling. Also health care policies that allow for early detection of diabetes, ease and accessibility to affordable diabetes medication are also essential (Zhang and Gregg, 2017).

2.2.10 Complications of diabetes

Complications of diabetes can be classified into microvascular and macrovascular complications.

2.2.10.1 Macrovascular complications

Diabetes can affect the brain in various ways including the risk of stroke, cerebrovascular diseases like cognitive impairment. There is also an increased risk of coronary heart disease. Peripheral vascular disease may occur as blood vessels begin to narrow. This can also culminate in reduced blood flow and oxygen to the periphery. Lack of oxygen and blood flow can cause nerves to malfunction and increase numbness in the limbs. Lack of sensitivity predisposes patients to injury. Lack of blood /oxygen supply cause wounds to heal slowly. Gangrene may set in and amputation if the limb maybe required (Melendez-Ramirez *et al.*, 2010).

2.2.10.2 Microvascular complications

Microvascular complications include diabetic nephropathy, diabetic retinopathy and diabetic neuropathy.

2.2.10.2.1 Diabetic nephropathy

Specific renal functional and morphological changes lead to diabetic nephropathy and are as a result of hyperglycemia. The high sugar content causes changes in the structure of the basement membrane of the glomerulus. The altered structure causes hyperfiltration, albuminuria, renal hypertrophy etc. Glomerular hypertension in diabetes, renal insufficiency and the defective kidney may eventually lead to kidney failure (Surya *et al.*, 2014).

2.2.10.2.2 Diabetic retinopathy

Hyperglycemia is a leading cause of blindness in diabetic patients. Hyperglycemia and blood pressure can damage capillaries in the eye leading to retinopathy, cataracts and glaucoma (Khalil, 2017).

2.2.10.2.3 Diabetic neuropathy

Diabetic neuropathy are a heterogeneous group of nerve disorders that results from perpetual hyperglycemia associated with diabetes. It is one of the major complications of diabetes believed to affect which approximately half of all diabetes patients (Tiwari *et al.*, 2018). DN can affect various nerves over the body. Diabetic neuropathy treatment is cumbersome. This is because there are no specific medication, and the pathomechanism of the development of diabetic neuropathy and its associated pain is multidirectional and complicated. It also leads to morbidity, mortality and diabetic amputations (Vinik *et al.*, 2013).

2.3 DIABETES: THE HERBAL APPROACH

There are varied approaches in modern medicine formulation to ameliorate the harmful effects of diabetes and its subsequent complications. The use of herbal formulations as alternative is gaining more attention because they are thought to have fewer side effects and cost less (Parikh *et al.*, 2014). Also, some of these plants may possess antioxidant, anti-inflammatory, analgesic and anti- hyperglycaemic properties all in one plant giving them an advantage over most orthodox drugs. A lot of current research is focusing on herbal drug preparations and plants as potential substitutes or alternatives in the treatment of diabetes mellitus and their complications. Moreover the cost of treatment of diabetes is still a burden to many around the world (Zhang and Gregg, 2017).

2.3.1 Diabetes Mellitus and Medicinal plants

Herbal medicines have been used for management of diabetes for decades. Ethnobotanical studies have reported about 800 plants that are claimed to possess antidiabetic properties around the world (Alarcon-Aguilara *et al.*, 1998). Many plants have been successfully tested for their antidiabetic/antihyperglycaemic effects in STZ-induced and alloxan- induced diabetic animal models (Arulselvan *et al.*, 2014; Arumugam *et al.*, 2013). Plants act by various mechanism to exert their anti-hyperglycemic activity. A few anti-diabetic plants and their possible mechanisms of action are listed in the table below.

Table 2: Plants used in diabetes, mechanisms of action and model

Plant name/part	Mechanism of Action	Model	Reference
<i>Artemisia afra</i> (Leaves)	Regeneration of pancreatic beta cells, Enhances glucose utilization.	STZ	(Sunmonu and Afolayan, 2013)
<i>Dioscorea spp.</i> (Whole plant)	Improves glucose utilization in skeletal muscle	STZ	(Niu <i>et al.</i> , 2010)
<i>Kigelia pinnata</i> (Flower)	Improve lipid profile, body weight. Stimulates GLUT4 expression	STZ	(Kumar <i>et al.</i> , 2012)
<i>Psidium guajava</i> (Leaves)	Inhibits activity of α -glucosidase Stimulates glucose metabolic enzymes	Alloxan	(Huang <i>et al.</i> , 2011)
<i>Sapindus trifoliatus</i> (Fruits)	Has insulin-like activity. Increase glucose usage peripherally.	Alloxan	(Sahoo <i>et al.</i> , 2010)
<i>Terminalia bellerica</i> (Fruits)	Augments secretion of insulin by modulation of cAMP and calcium	STZ	(Latha and Daisy, 2013)
<i>Scoparia dulcis</i> (Whole plant)	Antioxidant, stimulates insulin secretion, secretagogue activity	STZ	(Latha <i>et al.</i> , 2009)

(Arulselvan *et al.*, 2014; Bhagour *et al.*, 2016)

2.3.2 Diabetic complications and medicinal plants.

Generally plants that have anti-hyperglycaemic properties may improve diabetic complications. However plants that have analgesic, anti-inflammatory and anti-oxidant properties in addition are preferred. Other plants have also shown different pathways to inhibit diabetic neuropathy. A few pure herbal compounds have been isolated, and have been shown to improve diabetic complication like neuropathy and retinopathy. These compounds are shown in table below:

Table 3: Pure compound for treating diabetic complications and their mechanisms of action.

Compound	Mechanism of Action	Reference
Quercetin	Central analgesic activity	(Ferreira <i>et al.</i> , 2013)
Rutin	Metal chelating property Anti-oxidant, anti-inflammatory anti- hyperglycaemic.	(Oh, 2016) (Tian <i>et al.</i> , 2016)
Baicalein	Anti-oxidant, anti-inflammatory, Inhibits sorbitol pathway.	(Stavniichuk <i>et al.</i> , 2011)
Naringenin	Anti-oxidant	(Al-Rejaie <i>et al.</i> , 2015)
Hesperidin	Anti-oxidant, anti-inflammatory, analgesic	(Visnagri <i>et al.</i> , 2014)
Diosmine	Anti-oxidant, anti-inflammatory	(Jain <i>et al.</i> , 2014; Wojnar <i>et al.</i> , 2017)

2.4 SYNEDRELLA NODIFLORA (L) GAERTN

Synedrella nodiflora (L.) Gaertn (asteraceae) also known as node weed is a herb native to tropical America. It is however found throughout the West-African sub region, especially in marshy or cool and moist places. It is the plant of interest in this study.

2.4.1 Plants description

Synedrella nodiflora is an erect branched seasonal shrub usually 30-80 cm tall and it belongs to the Asteraceae family (USDA 2010). The plant has a shallow root system that is strongly branched. The stems are sometimes woody in nature and may branch directly from the base of the plant. The plant normally has long internodes with a hairy swollen node. The plant can grow roots on the lower parts of the stem in moist environment. The leaves appear in opposite pairs, around 4-9 cm long, elliptic to ovate in shape, commonly displaying three veins and margins that are finely toothed. Its petioles are short and finely hairy, and joined by a ridge across the stem.

It flowers in small bunches of about 2 to 8, at the node and tips throughout the upper parts of the plant. The inflorescence normally presents with yellow coloured petals. It produces dimorphic dark brown seeds. Ray floret seeds are flat, oblong, 3-5 mm long, teeth pointing upward along the paler marginal wing. Disc floret seeds are thicker, elongate, 3-4 mm long, with 2-4 stiff bristles at the apex. Both seeds produce the same plant (Adjibode *et al.*, 2015).

2.4.2 Folkloric uses

In Ghana, it is known in the Akan dialect as ‘*tutumrika ko hwé épo*’ (run to watch the sea). The aqueous extract of the full plant is used orally to treat epilepsy. The leaves are also used to treat hiccup and also to treat threatened abortion (Mshana *et al.*, 2000). The leaves of *Synedrella nodiflora* leaves are usually eaten as food by horses, rabbits, guinea pigs and pigs (Hidayat, 2001). Some humans are known to eat it without any toxicity reported so far (Li *et al.*, 2014). In Ghana farmers sometimes use the plant leaves as post-harvest protectants (Cobbinah *et al.*, 1999). In Nigeria, some local tribes use the whole plant to treat cardiac problems and to stop wounds from bleeding (Idu and Onyibe, 2007). In countries like Malaysia and Indonesia, the plant is used as a poultice for sores, headaches, ear aches, stomach aches and to treat rheumatism (Burkill, 1985; Sumi *et al.*, 2011).



Figure 1: Picture of *Synedrella nodiflora* showing leaves and flowers (CABI, 2018).

2.4.3 Phytochemical screening

Phytochemical screening has been done using solvents like chloroform, methanol, butanol, ethanol, hexane and water. The plant possesses many phytochemical constituents including flavonoids, phenolics, steroids, triterpenoids, saponins, alkaloids, phytosterol glycosides, tannins, saponins, reducing sugars and phytosterols (Adjibode *et al.*, 2015). It also had a high total phenolic content (Dutta *et al.*, 2012).

2.5.3 Pharmacological tests

The plant has shown anti-oxidant / anti-lipid peroxidative properties in vitro (Amoateng *et al.*, 2011; Onocha *et al.*, 2016). The constituents may also be responsible for other proven activities of *Synedrella nodiflora* extracts including anti-convulsant activity (Amoateng *et al.*, 2011), anti-

inflammatory activity (Haque *et al.*, 2012), analgesic (Amoateng *et al.*, 2017), antimicrobial (Bhogaonkar *et al.*, 2011) and hypoglycemic effects (Adjibode *et al.*, 2015). Its ethanolic extract also show no toxicity on biochemical, haematological and histopathological parameters in sub-acute, acute and long term administration in male Sprague – Dawley rats (Adjei *et al.*, 2014; Amoateng *et al.*, 2014; Amoateng *et al.*, 2016). Finally the ethanolic extract has also exhibited analgesic effect in paclitaxel and vincristine induced neuropathy (Amoateng *et al.*, 2015; Amoateng *et al.*, 2017). These properties have opened the door for much research to be done on this plant for its potential medicinal benefits in managing diabetes for humans.

2.5 ANIMAL MODEL IN DIABETES

Animal models have been used extensively in diabetes to study the pathophysiological changes that take place in the condition and to evaluate the effect of drugs/potential drugs on the disease. Diabetes can be induced in animal models by chemical, viral, genetic or by spontaneous autoimmune mechanisms. Certain models may also present limitations that may not be found in other models. The STZ- induced diabetic model is one of the most widely used and reliable methods. Below is a table of a few examples of animal models in diabetes, induction mechanism, main features and possible limitations.

Table 4: Models for diabetes and their limitations

Induction mechanism	Model	Limitations
Chemical	Streptozotocin Alloxan	May affect other internal organs
Spontaneous	NOD mice	Gender differences.
Autoimmune	BB rats	Unpredictable
Genetic	AKITA mice	Not validated by antineuropathic drugs
Obese model (Polygenic)	OLETF rats	Exhibit renal complications
Viral	Cocksackie B virus Kilham rat virus	Outcome is dependent on replication levels of the virus as well as timing of the infection
Induced obesity	High fat feeding	Genetic difference is response to high fat diet

(King, 2012)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN

The study was an experimental study design.

3.2 PLANT EXTRACT PREPARATION

3.2.1 Plant acquisition

The whole plant samples (stems, leaves and roots) of *Synedrella nodiflora* were harvested in University of Ghana at the Botanical Gardens (Latitude, 5.6599, longitude, -0.1840) in the month of October, 2017. Harvesting was done in the rainy season of the year when the plant was in abundance, in the cool of the morning. The plant was authenticated in the Ghana Herbarium of Department of Botany in the University of Ghana, Legon. A voucher specimen labeled (PA01/UGSOP/GH17) was kept.

3.2.2 Preparation of the whole plant of *Synedrella nodiflora*

The collected plant material was air dried for 7 days and powdered using a hammer mill (Liming®, China). A 3 kg quantity of the sample was weighed, extracted with 70 % (v/v) ethanol by cold maceration for 3 days and concentrated in a rotary evaporator (Rotavapor R-210, Buchi, Switzerland) at 60 °C. It was finally oven dried (Gallenkamp®, UK) at 50 °C to a constant weight. A dark greenish slurry residue of 31.3 g was obtained and refrigerated until reconstituted into a solution for administration. This extract was labelled as SNE and stored in a refrigerator for later use . A yield of 10.4 % w/w was obtained.

3.2.3 Phytochemical screening

Screening of SNE for the presence of alkaloids, tannins, saponins, triterpenoids, flavonoids, volatile oils, phenolic and glycoside was done according to various tests specified in literature (Evans and Evans, 2009; Sofowora, 1982).

3.2.3.1 Alkaloids (Dragendorff's Test)

A 0.5 g quantity of SNE was extracted with 20 ml of ammoniacal alcohol and filtered. The residue obtained after evaporating the filtrate was shaken with 1 % H_2SO_4 and filtered. The filtrate was made alkaline with dilute ammonia solution and shaken with chloroform; the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 1 % H_2SO_4 and one drop of Dragendorff's reagent (Sigma Aldrich Co. Ltd. Irvine, UK) was added. An orange-red precipitate shows alkaloids are present.

3.2.3.2 Saponins (Frothing test)

A 1 g quantity of SNE was dissolved in distilled water and filtered. The filtrate was given a vigorous shake and left to stand for 5 minutes. The presence of persistent froth on standing indicated the presence of saponins in this extract.

3.2.3.3 Phytosterols (Lieberman-Burchard's test)

Using 5 ml chloroform, 0.5 g of SNE was extracted with and filtered into a test tube. Several drops of acetic anhydride (Sigma Aldrich Co. Ltd. Irvine, UK) were added and mixed carefully. Two (2) drops of concentrated H_2SO_4 (BDH Laboratories, England) was added to test tube gently through the side. Formation of violet to blue coloured ring at the junction of two liquids showed the presence of steroid moiety.

3.2.3.4 Terpenoids (Salkowski test)

With 5 ml chloroform, 0.5 g of SNE was extracted and filtered into a test tube. The careful addition of concentrated H_2SO_4 (BDH Laboratories, England) to test tube wall produced a violet colouration showing the presence of terpenoids.

3.2.3.5 Glycosides (General test)

A 0.5 g quantity SNE was extracted by warming with 5 ml of dilute H_2SO_4 on a water bath for 2 minutes and filtered. The filtrate was turned alkaline by adding 2-5 drops of 20 % NaOH (BDH Laboratories, England). 1 ml of Fehling's solution A and B (Sigma Aldrich Co. Ltd. Irvine, UK) was added to the filtrate and dried on a water bath for 2 minutes. The presence of a brick-red precipitate indicated the presence of glycosides.

3.2.3.6 Tannins (Ferric Chloride test)

A 0.5 g quantity of SNE was boiled with distilled water for 5 minutes. The boiled extract was cooled, filtered and made up to 25 ml. To 1 ml of the extract, 10 ml of distilled water was added followed by 2-10 drops of 1 % Ferric chloride solution (Sigma Aldrich Co. Ltd. Irvine, UK). A blue-black or blue-green colouration showed a positive test for tannins.

3.2.3.7 Phenolic Compounds (Acetic acid test)

A 0.5 g quantity of SNE was boiled with distilled water for 5 minutes. The boiled extract was cooled, filtered and made up to 25 ml. To 1 ml of the extract, 10 ml of distilled water was added followed by 3-4 drops of acetic acid (Sigma Aldrich Co. Ltd. Irvine, UK). The solution turned red indicating the presence of phenolic compounds.

3.2.3.7 Flavonoids (Kumar test)

A 0.5 g quantity of SNE was dissolved in water and filtered; to this 2 ml of 10 % NaOH (BDH Laboratories, England) solution was added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute HCl indicated the presence of flavonoids.

3.2.3.9 Volatile Oils

To a 0.3 g quantity of the extract a 5 drops of alcoholic solution of Sudan III was added and mixed thoroughly. No reddish coloured globules were obtained, indicating the absence of volatile oils.

3.3 EXPERIMENTAL ANIMALS AND HUSBANDRY

Male Sprague-Dawley rats (160-200 g) 8 weeks old were obtained from the Centre for Plant Medicine Research, Mampong, Ghana, and transported to Animal House Section of the Noguchi Memorial Institute. The animals were allowed to acclimatize and adapt to the new environment a week before the study commenced. They were put in cages of five rats each in stainless steel cages (34 cm × 47 cm × 18 cm) with wood shavings as bedding. The room for the cages was adequately ventilated at normal room temperature (22–26 °C). The relative humidity of 60–70 % with a natural light-dark cycle was maintained during the period. Good sanitation practices were observed with regular cleaning of cages and change of bedding. The rats were fed with rat feed (Agricare Ltd, Kwadaso, Kumasi, Ghana) and given clean water *ad libitum*. The processes and procedures employed during the experiment stayed in agreement with the methods approved by the Scientific and Technical Committee (STC) of the Noguchi Memorial Institute for Medical Research (N.M.I.M.R) [reference number STC-6 (International Diabetes Federation) 2012–13] and also by the Noguchi Institutional Animal Care and Use Committee (N.I.A.C.U.C), College of Health Sciences, University of Ghana with protocol number NIACUC-2017-01-1E. The

procedures for the research work was permitted by the Department of Pharmacology, University of Ghana.

3.4 INDUCTION OF DIABETES MELLITUS

3.4.1 Preparation of citrate buffer

To prepare the buffer solution, 1.76 g of sodium citrate (Mwt- 294, Zigma-Aldrich, St Loius, USA) was dissolved in 60 ml of distilled water to obtain a 0.1M solution. 1.05grams of citric acid monohydrate powder (Mwt-210, Zigma-Aldrich, St Loius, USA) was also dissolve in 50 mls of water to obtain 0.1M solution. 44.5mls of 0.1M citric acid was combined with 55.5mls sodium citrate to obtain 100 ml of citrate buffer pH of 4.5. The amount of streptozotocin needed was calculated in relation to the total weight of all the rats, weighed and dissolved in the citrate buffer.

3.4.2 Process of diabetes induction with STZ

Induction of diabetes was done 1 week after arrival of rats (after acclimatization). Animals to be induced were fasted for 16 hours and subsequently weighed and given a single intra-peritoneal injection of STZ (55 mg/kg) (streptozotocin, Zigma-Aldrich, St Loius, USA) already dissolved in a freshly prepared citrate buffer of pH 4.5. Rats in control group were given only citrate buffer of equal volume. The rats were allowed access to feed and water *ad libitum* after the injection.

Three (3) days after the injection, FBS (fasting blood sugar) was measured for all the animals using a blood glucose monitor (One Touch Select Plus®, Lifescan, Gubelstrasse, Switzerland).

FBS was measured at 7 am in the morning, one hour before feeding. Animals with values greater than 11 mmol/L were considered to be diabetic.

3.4.3 Oral glucose tolerance test

All STZ injected Sprague-Dawley rats were fasted overnight and orally administered with bolus glucose load of 2 g/kg at 08:00 AM. Blood samples were periodically measured from the tail vein at 0, 30, 60, 90, and 120 min after administration of the glucose. The levels of blood glucose were evaluated by using a glucose monitor (One Touch Select Plus®, Lifescan, Gubelstrasse, Switzerland). All SD rats with a blood glucose level above 11 mmol/L after 2 hours were considered to be diabetic. This test is a confirmatory test for diabetes (Ernsberger and Koletsky, 2012).

3.4.4 Grouping

The Sprague Dawley rats were divided into 8 groups of 5 each. The first group comprised of rats that received no STZ. The rats injected with STZ, with FBS and OGTT >11 mmol/L were divided into 7 groups containing 5 animals each. The vehicle (citrate buffer) group was not treated with any drug. Subsequently there were three groups for insulin (0.1 IU/kg, 0.3 IU/kg and 1 IU/kg bodyweight) and SNE (100 mg/kg, 300 mg/kg and 1000 mg/kg bodyweight).

3.4.5 Preparation of drugs

Insulin human (rDNA) (100 IU/ml solution, Actrapid®, novo nordisk, Denmark) was administered as reference drug was serially diluted with Normal saline (500 mls, Sanbao Pharmaceuticals Ltd, Tema, Ghana) to obtain the minute quantities needed. This solution was

freshly prepared daily before administration subcutaneously. SNE was mixed with distilled water and administered orally by gavage.

3.4.6 Drug administration

The control and diabetic control group received no treatment except food and water. Insulin group received a subcutaneous injection of Insulin human (rDNA) twice daily. SNE group received a daily gavage at 7:30 am, 30 minutes before feeding. Drug administration was done every day for 8 weeks before animals were sacrificed.

3.4.7 Fasting blood sugar (FBS) determination

FBS of animals were measured twice weekly and the weekly average calculated throughout the eight (8) weeks. Fasting blood sugar was taken at 7am every morning one hour before feeding using the OneTouch Select plus glucometer.

3.4.8 Determination of percentage change in bodyweight and relative organ weight.

The first and final weights of the rats were recorded on the day of induction of diabetes and on the last day of the experiment when the rats were sacrificed. The percentage changes of body weight of each group were calculated using the formula below:

$$\text{Percentage change in body weight} = \frac{(\text{final weight} - \text{initial weight})}{\text{initial weight}} \times 100$$

The harvested liver, spleen, kidney, brain and heart were washed with normal saline, dried with tissue paper, and weighed. The weight of the excised organs with respect to the body weight of the rat at the end of the experiment was calculated from the formula below:

$$\text{Relative organ weight} = \frac{\text{absolute organ weight (g)}}{\text{rat body weight on sacrifice day (g)}} \times 100$$

3.5 PREPARATION OF SD RATS FOR BLOOD TESTS AND HISTOPATHOLOGY

3.5.1 Sacrificing of rats.

On the last day of the 8th week all animals were weighed. They were then chloroformed and sacrificed after 8 weeks. Blood was collected from the jugular vein to into serum separating gel tubes (BD Vacutainer® blood collection Tube Product, USA) and EDTA tubes (Mediplus vacutainer K3, Sunphoria Co. Ltd., Taiwan) for serum preparation and haematological analysis respectively.

3.5.2 Preparation of organs

Rat organs (liver, kidney, lungs, brain and spleen) were harvested, cleaned and dried with tissue. The organs were then weighed. All organs were examined by trained veterinary doctors for any pathological lesions and placed in labeled containers containing 10 % neutral buffered formalin. The organs were then transported to a pathology laboratory for processing and examination.

3.5.3 Preparation of blood

About 2 mls of blood was collected into EDTA tubes and carefully turned back and forth by rubbing them in the palms to allow even mixing of the blood with EDTA to prevent clotting. After 2 minutes of swiveling, the samples were transported on ice in thermos flask for haematological analyses.

3.5.4 Preparation of serum

To obtain the serum 3-4 ml of blood were allowed to clot at room temperature (27 °C) in the gel tubes after they were collected. The tubes were then centrifuged (Heraeus Labofuge 300, UK) at 3000 rpm for 20 minutes. A clear supernatant obtained was collected by pipetting into plain tubes. These tubes were labeled accordingly and were cold-chain transported immediately to the lab for biochemical analysis.

3.5.5 Kidney and liver Function tests

From the serum prepared using the above procedure, the values for the level liver transaminases [aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (ALP)], total protein (TP) and albumin (ALB), kidney urea (Ur) and creatinine (Cr) were determined. Lipid profile (HDL, LDL, total cholesterol) and were made using an automated clinical chemistry analyzer (ABX Pentra C200, Horiba Medical, USA).

3.5.6 Haematological analysis

An automated haematology analyser (Sysmex XP -300 TM Automated Analyser, USA) was employed in the assessment of the following haematological parameters from blood collected

above. The parameters included white blood cells (WBC), neutrophils (NEU), lymphocyte (LYM), red blood cell (RBC), haemoglobin (HGB), mean corpuscular volume (MCV), haematocrit (HCT), mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration (MCHC), basophils and eosinophils, platelet (PLT).

3.6 HISTOPATHOLOGY

3.6.1 Processing of Organs into slides

Portions of the organs were selected into labeled tissue processing cassettes and processed into paraffin blocks. Each was passed through ascending grades of alcohol (70%, 80%, 90% and absolute) and further two changes of absolute alcohol for dehydration, cleared in three changes of xylene and finally infiltrated and embedded in paraffin wax. Five micron sections were cut from each block, mounted on microscope slides and stained by the haematoxylin and eosin method.

3.6.2 Examination of slides

The sections of the liver, spleen, lung, brain and kidney samples of the sacrificed animals were examined by a qualified histopathologist who was blind to the experimental profiles under a light microscope after they have been stained with eosin and haematoxylin.

3.7 ANTIOXIDANT ACTIVITY

3.7.1 Determination of Superoxide Dismutase (SOD) Activity

Superoxide anion (O_2^-) is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage through oxidative stress. SOD reacts with superoxide anion to form hydrogen peroxide and oxygen in the body. This is a natural protective action against oxidative stress in the body. Hence the presence of Superoxide anion in a sample could be indicative of SOD depletion and high oxidative stress. The anion's presence can be detected by using the SOD Assay Kit because it reacts with highly water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) that produces a water-soluble formazan dye. Measurement of the IC_{50} (50% inhibition activity of SOD or SOD-like materials) can be determined by a colorimetric method.

3.7.2 Procedure for SOD activity determination

19160 SOD determination kit (Sigma-Aldrich, 3050 Spruce Street, St. Louis MO 63103, USA) was used for SOD assay. WST (water soluble tetrazolium salt) working solution was prepared by diluting 1 ml of WST solution with 19 ml of buffer solution. The enzyme working solution was centrifuged for 5 seconds and mixed by pipetting. It was then mixed with 2.5 ml of the dilution buffer. Each sample was assigned four blank wells. The wells were labelled blank 1, blank 2 and blank 3 respectively. Twenty microliters (20 μ) of sample solution was added to each sample and blank 2 well. 20 μ l of ddH₂O (double distilled water) was also added to each blank 1 and blank 3 well. 200 μ l of WST Working Solution was subsequently added to each well and mixed. Then 20 μ l of Dilution Buffer was added to each blank 2 and blank 3 wells. Finally 20 μ l of enzyme

working solution was added to each sample and blank 1 well and mixed thoroughly. These wells were incubated in the plate at 37 °C for 20 min. The absorbance at 450 nm was read using a microplate reader. SOD activity (inhibition rate %) was calculated using the following equation:

$$\text{SOD activity (inhibition rate \%)} = \frac{(\text{blank1} - \text{blank3}) - (\text{sample} - \text{blank 2})}{(\text{blank 1} - \text{blank3})} \times 100$$

This method is similarly employed by (Chiari-Andréo *et al.*, 2017; Ukeda *et al.*, 1997).

3.7.3 Malondialdehyde (TBARS) determination

Biological cells when posed to high levels of oxidative stress accumulate mixture of aldehydes which are derived from the decomposition polyunsaturated fatty acids. Aldehydes can be cytotoxic. Malondialdehyde (MDA) and MDA-like products are the most common lipid peroxidation products formed. The analysis of MDA by the classical TBA (2-thiobarbituric acid) test is still a simple but dependable method for determining oxidative stress.

3.7.4 Procedure for MDA determination

Rat serum sample of 0.5 ml was mixed with 0.5 ml of ice-cold 10% Trichloacetic acid and incubated for 30 minutes to deproteinize it. Then 1.0 ml of 0.67% Thio-Barbituric Acid was subsequently added to the mixture mixed properly and heated at 100 °C for 30 minutes. The mixture was then allowed to cool. 4.0 ml of n-butanol was added to the mixture and vortexed for 30 seconds. It was then centrifuged at 500 g for 10 minutes. The supernatant obtained was drawn into 96- well plates using a micropipette. Each sample was duplicated. The absorbance was then measured at 535 nm using the spectrophotometer (GloMax Explore GM35000,

Promega Corporation, Madison, Wisconsin, USA). The results were expressed in $\mu\text{mol/L}$ using the extinction coefficient of $1.56 \times 10^5 \text{ Lmmol}^{-1}\text{cm}$ (Kamal et al., 1989).

3.8 ANALYSIS OF RESULTS

GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and ED_{50} determination. $P < 0.05$ was considered statistically significant in all analysis. The graphs were plotted with Sigma Plot for the Window version 11.0 (Systat Software Inc., Germany)

CHAPTER FOUR

4.0 RESULTS

4.1 PHYTOCHEMICAL SCREENING

**Table 2: Results of Phytochemical screening of the ethanolic extract of *Synedrella nodiflora*.
+ indicates presence and - indicates absence of constituent.**

CONSTITUENT	RESULT
Alkaloids	+
Saponins	+
Taninns	+
Phytosterols	+
Phenolics	+
Volatile oils	-
Terpinoids	+
Flavonoids	+
Glycosides	+

Absent:- Present:+

4.2 INDUCTION OF DIABETES

The Fasting blood sugar of both control and treatment group were measured before and 72 hours after the injection of STZ (55 mg/kg bodyweight). There was a significant increase in fasting blood sugar levels for the STZ-treated group ($P < 0.0001$, $F_{3,68} = 22.36$, Fig. 2A) in comparison to the control group. Subsequently the vehicle group (untreated) maintained a high fasting blood sugar over the 8 week period significantly higher than the control group ($P < 0.0001$, $F_{4,25} = 27.56$, Fig. 2B).

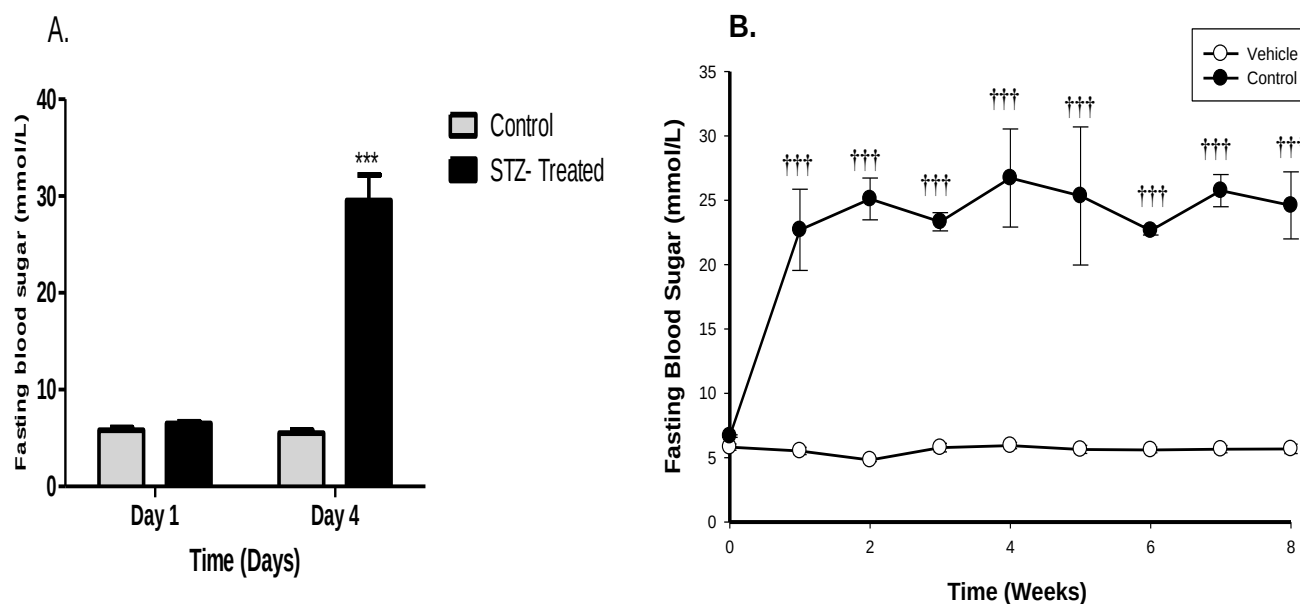


Figure 2: The effect of STZ injection of SD rats in diabetes induction. The FBS levels of control and STZ-treated rats (55 mg/kg, i.p) before and 72 hrs after treatment (A). The FBS levels of vehicle group and control group from week 1 to week 8 of experiment (B). Values are means $\pm$ s.e.m. (n=5). * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$; compared to control group (one-way anova followed by bonferroni's *post hoc* test) for A. † $P \leq 0.05$; †† $P \leq 0.01$; ††† $P \leq 0.001$; compared to control group (two-way ANOVA followed by Newman-keuls *post hoc* test) for B.

4.3 ANTIHYPERGLYCAEMIC ACTIVITY OF SNE

The administration of SNE 1000 mg/kg gave a significant reduction in fasting blood sugar levels on the 4th week ($P < 0.05$, $F_{4,38} = 21.14$, Fig.3C). Other SNE doses gave no significant reduction over the 8 weeks ($P > 0.05$). The total fasting blood sugar activity (calculated as AUCs) showed that the doses of SNE gave no significant reduction ($P < 0.05$, $F_{4,32} = 32.82$, Fig.3B) in insulin over 8 weeks. The administration of insulin (0.1-1.0 IU/kg) showed a significant reduction in the FBS for only 1IU/kg dose of insulin on the 3rd and the 8th week ($P < 0.05$, $F_{3,19} = 27.56$, Fig.3A). All other doses of insulin did not give any significant reduction in fasting blood sugar compared to the control ($P > 0.05$). The total fasting blood sugar activity (calculated as AUCs) showed that the highest of insulin (1 IU/kg) gave a significant reduction ($P < 0.05$, $F_{3,68} = 20.82$, Fig.3B) in insulin over 8 weeks. An ED_{50} of 187.9 mg/kg and 0.156 IU/kg were obtained for the SNE extract and insulin respectively.

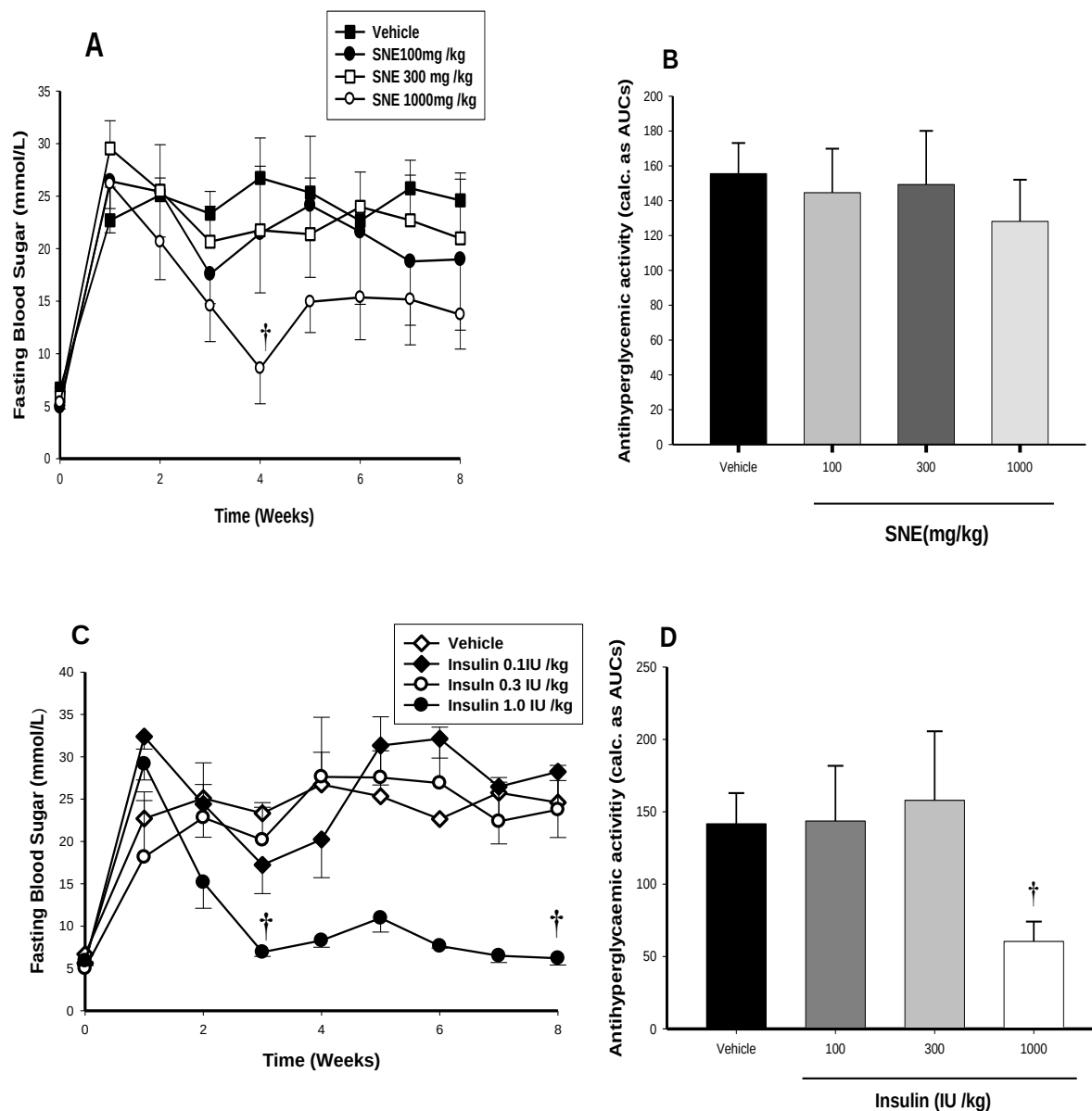
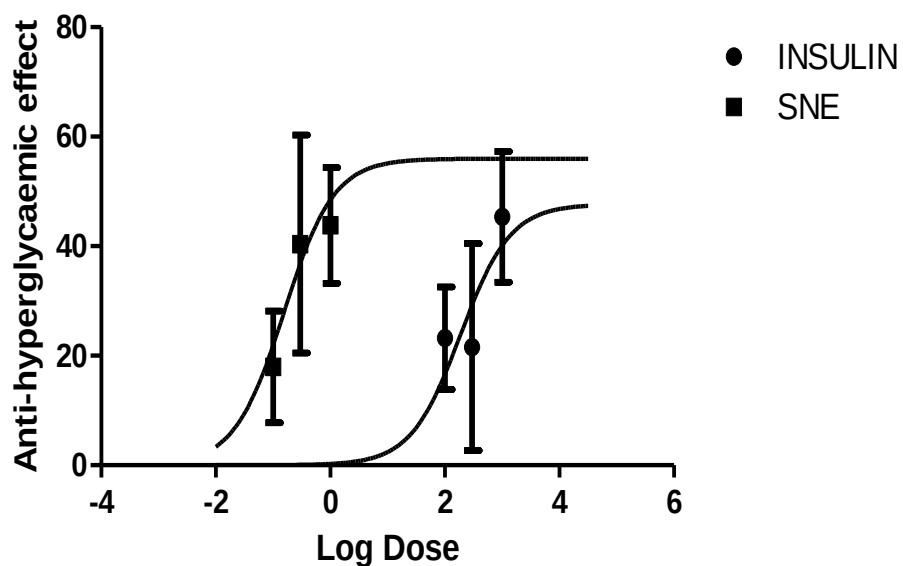


Figure 3: The effect of the administration of SNE (100 mg/kg, 300 mg/kg and 1000 mg/kg) and insulin (0.1 IU/kg, 0.3 IU/kg and 1 IU/kg) in diabetic rats over 8 weeks. The graphs on the left (A and C) show the time course of effects over the 8-week period and the right (B and D) show the total anti-hyperglycaemic activity calculated from the AUCs for the test duration. Values are means $\pm$ SEM (n=5). †P $\leq$ 0.05, ††P $\leq$ 0.01, †††P $\leq$ 0.001 compared with vehicle-treated group (two-way analysis of variance followed by Bonferroni's post hoc test). One way analysis of variance was employed for AUCs.

Table 6: ED₅₀ of insulin and SNE.

Drug	ED ₅₀
Insulin (human (rDNA))	0.156 IU/ kg
SNE (<i>Synedrella nodiflora</i> ethanolic Extract)	187.9 mg/kg



Log dose-response curves showing the effect of SNE (100 - 1000 mg/kg) and insulin (0.1 IU-1.0 IU/kg) on hyperglycaemia in STZ-induced diabetic rats. Each point is the mean $\pm$ S.E.M. of 5 animals.

4.4 WEIGHT PARAMETERS

4.4.1 Percentage change in bodyweight

The percentage change in bodyweight was calculated by finding the difference between final and the initial weight and expressing it as a percentage of the initial weight. Induction of diabetes caused a significant weight loss ($P < 0.001$, $F_{3,68} = 13.86$, Fig 4.) in the vehicle and SNE doses (100 and 300 mg/kg) compared to the control after 8 weeks of treatment. There was a less significant loss in weight for SNE 1000 mg/kg compared to the control ($P < 0.01$, $F_{4,54} = 15.66$, Fig 4.) after 8 weeks.

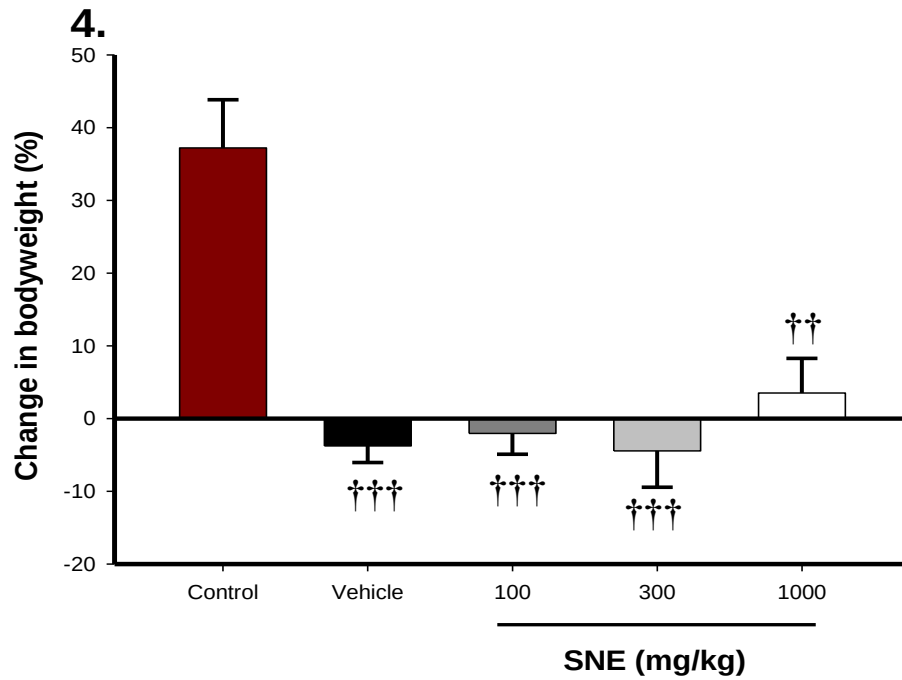


Figure 4. Effect of diabetes on bodyweights of diabetic rats receiving vehicle and SNE (100-1000 mg/kg, *p.o.*). Values are means $\pm$ SEM ($n=5$). $^{\dagger}P \leq 0.05$, $^{\dagger\dagger}P \leq 0.01$, $^{\dagger\dagger\dagger}P \leq 0.001$ compared with control group (one-way analysis of variance followed by Newman–Keul’s post hoc test)

4.4.2 Relative Organ Weight

For relative organ weights, a significant increase was seen in only liver weights for the vehicle group ($P < 0.05$, $F_{4,25} = 14.32$, Table 7). All other groups had no significant difference in relative organ weights for all organs ($P > 0.05$, Table 7).

Table 7: Effect of diabetes on relative organ weights of diabetic rats receiving vehicle and SNE (100-1000 mg/kg) for 8 weeks.

Organs	Relative organ weight (%)				
	Vehicle	SNE 100 mg	SNE 300 mg	SNE 1000 mg	P- VALUE
Liver	6.00 ±0.58[†]	4.17±0.20	4.45±0.38	4.28 ±0.31	0.02
Kidney	0.52±0.02	0.42±0.02	0.52±0.03	0.48±0.03	0.35
Lungs	1.33±0.38	1.03±0.03	0.92±0.11	0.88±0.08	0.12
Brain	0.97±0.03	0.95±0.05	0.90±0.06	1.00±0.06	0.23
Spleen	0.23±0.03	0.23±0.03	0.23±0.03	0.26±0.02	0.28

Values are means ± SEM (n=5). For P-value, [†]P ≤ 0.05, ^{††}P ≤ 0.01, ^{†††}P ≤ 0.001 compared with vehicle-treated group (B) (one-way analysis of variance followed by Bonferroni's post hoc test)

4.5 BLOOD BIOCHEMISTRY

Blood biochemistry after 8 weeks of treatment showed that there was a significant decrease in creatinine level for all diabetic groups i.e. ($P < 0.01$, $F_{4,13}=9.497$, Table 8) for vehicle and 300 mg/kg SNE and ($P < 0.05$, $F_{3,17}=3.233$, Table 8) for 100 and 1000 mg/kg SNE group. Albumin levels also decreased significantly for the vehicle group ($P < 0.01$, $F_{4,25}=4.343$, Table 8). There were also significant increases in AST ($P < 0.01$, $F_{3,18}=3.429$, Table 11), ALT and ALP ($P < 0.05$, $F_{4,22}=8.215$, Table 8) for the vehicle group in comparison with the control. The SNE groups (100-1000 mg/kg) gave no significant change ($P > 0.05$, Table 8) in AST, ALT, ALP and albumin in comparison with the control. There were no significant changes in lipid profile, urea, total protein and globulin ($P > 0.05$, Table 8)

Table 8: The effect of SNE (100–1000 mg/kg, p.o) on blood biochemistries in STZ –induced diabetic SD rats after 8-week administration .

Parameter	Control	Vehicle	SNE 100 mg	SNE 300 mg	SNE 1000 mg	P value
Urea (mmol/L)	9.07±0.54	14.62±2.18	10.59±0.24	14.65±.36	15.68±2.62	0.06
Creat(mmol/L)	75.08±9.81	34.80±2.22**	45.43±4.45*	38.43±4.01**	48.60±5.02*	0.02
Tot. Chol.	2.10±0.28	2.17±0.21	2.21±0.22	2.71±0.16	2.25±0.30	0.53
Triglycerides	2.45±0.45	2.55±0.60	1.700±0.25	1.42±0.14	1.50±0.08	0.08
HDL(mmol/L)	0.81±0.13	1.06±0.31	0.89±0.17	1.19±0.11	0.95±0.15	0.52
LDL(mmol/L)	0.68±0.13	0.48±0.04	0.47±0.04	0.52±0.05	0.57±0.06	0.43
Tot. Pro. (g/L)	85.58±2.52	76.60±4.16	89.93±5.66	85.65±1.76	83.62±1.76	0.13
Albumin(g/L)	39.56±1.07	29.07±1.51**	37.60±2.73	36.30±1.70	35.54±1.05	0.01
Globulin (g/L)	46.04±2.10	47.53±4.33	52.33±3.36	49.35±1.82	48.36±1.67	0.53
ALT(U/L)	106.70±26.00	613.70±190.60*	140.70±15.66	192.40±40.65	219.40±58.92	0.04
AST(U/L)	191.30±106.20	1336.00±395.40**	199.10±20.79	193.00±27.40	318.70±56.70	0.01
ALP(U/L)	297.70±41.79	892.00±149.00*	558.00±51.80	651.90±197.70	449.70±249.90	0.02

Data are mean ± SEM (n = 5). *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001 compared to control group (one-way ANOVA followed by a Bonferroni's post hoc test)

4.6 HAEMATOLOGY

The vehicle group showed a significant increase in eosinophil count ($P < 0.01$, $F_{4,20} = 17.15$, Table 9) after 8 weeks. All the SNE groups showed no significant change in eosinophil count compared to the control ($P > 0.05$, Table 9). The vehicle group also gave a significant decrease in RBCs ($P < 0.001$, $F_{3,20} = 11.11$, Table 9) and haemoglobin level ($P < 0.01$, $F_{4,20} = 13.133$, Table 9). There was no significant change in the same parameters for the SNE groups ($P > 0.05$, Table 9). There were also no significant changes in other parameters (MCV, MCHC, MHC, PLT etc) for all animal groups.

Table 9: The effect of SNE (100–1000 mg/kg, *p.o*) on haematological indices in STZ - induced diabetic SD rats after 8 week administration compared to control and vehicle.

Parameter	Control	Vehicle	100 mg/kg	300 mg/kg	1000mg/kg	P-value
WBC #	2.29±0.09	2.640±0.36	4.81±1.67	8.03±1.02	8.29±1.68	0.01
Neut %	22.05±4.35	23.35±14.85	38.80±0.40	29.95±5.65	30.62±2.89	0.33
Lymp%	75.60±4.60	48.55±0.55	57.10±1.40	68.00±6.08	66.24±2.46	0.04
Eos%	1.15±0.05	8.00±2.00**	1.90±0.10	1.17±0.51	1.68±0.54	0.01
Mon %	0.90±0.60	0.95±0.05	0.80±0.10	0.53±0.20	0.56±0.14	0.38
Bas%	0.30±0.20	1.60±0.10	1.40±0.80	0.35±0.12	0.96±0.14	0.08
Neut#	0.50±0.08	0.83±0.05	1.92±0.62	2.24±0.15	3.97±0.33*	0.01
Lym#	1.74±0.18	1.21±0.09	2.81±0.81	5.64±1.17	8.90±1.05*	0.01
Mon#	0.02±0.01	0.02±0.01	0.04±0.02	1.04±1.02	0.08±0.06	0.67
Eos#	0.02±0.01	0.19±0.04	0.09±0.04	0.08±0.03	0.22±0.09	0.31
Bas#	0.01±0.01	0.02±0.01	0.08±0.07	0.03±0.03	0.12±0.03	0.13
RBC(×10¹²)	6.59±0.16	2.49±0.16****	7.99±0.48	7.92±0.31	7.74±0.61	0.01
MCV (fL)	59.65±0.25	60.95±1.25	61.20±1.40	61.58±0.68	63.18±1.81	0.62
MCH pg	19.15±0.35	19.60±0.60	18.95±0.65	18.88±0.16	20.08±0.76	0.58
MCHC (g/dL)	32.15±0.45	32.10±0.30	30.95±1.85	30.68±0.48	31.72±0.29	0.41
HGB (g/dL)	12.75±0.45	5.00±0.30**	15.15±1.45	14.95±0.61	15.38±0.6	0.01
PLT#	879.50±20.50	157.00±8.00	476.50±8.50	737.00±321.2	880.60±132.20	0.28

Data are mean ± SEM (n = 5). *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001 compared to control

group (one –way ANOVA followed by a Bonferroni’s posthoc test). **NB # = (×10⁹/L)** for counts.

4.7 ANTIOXIDANT ACTIVITY

4.7.1 SOD and MDA assay

For SOD activity, there was dose dependent increase in SOD activity for extracts of SNE. The SNE 1000 mg/kg gave a significant increase ($P < 0.05$, $F_{4,25} = 4.050$, Fig 5A) in SOD activity compared to the vehicle. The increase in activity by 100 and 300 mg/kg was not significant ($P > 0.05$). MDA also showed a dose dependent decrease in the amount of TBARS substances. The highest decrease was in the SNE 1000 mg/kg group which was the only significant decrease ($P < 0.01$, $F_{3,18} = 3.650$, Fig 5B). The other groups showed no significant decrease ($P > 0.05$).

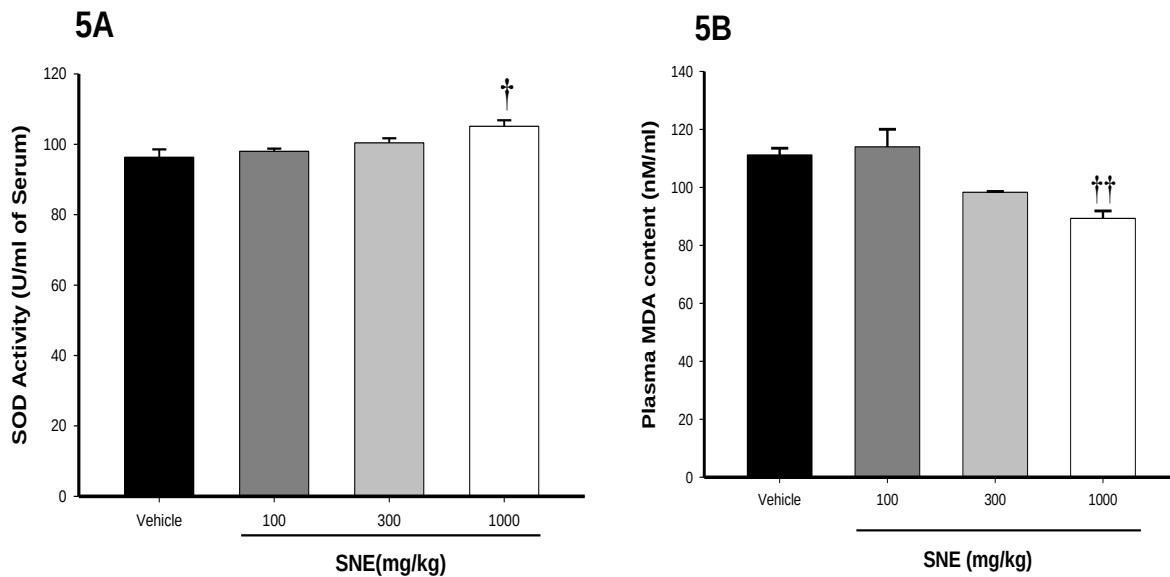


Figure 5. The effect of SNE (100, 300 and 1000 mg/kg, p.o) on SOD activity (A) and MDA levels (B) in STZ -induced diabetic male SD rats after 8week of administration. Data are mean $\pm$ SEM (n=5). [†]P $\leq$ 0.05, ^{††}P $\leq$ 0.01, ^{†††}P $\leq$ 0.001 compared to vehicle group (one –way ANOVA followed by a Bonferroni's post hoc test)

4.8 HISTOPATHOLOGY

Liver histopathology showed a distortion in the microstructure for the vehicle group. There were large interstitial spaces, dilated central veins and increased blood congestion compared to the SNE groups. These changes were not obvious in the SNE which had a few slightly dilated central veins and blood congestion.

In the kidneys there were mild leucocyte infiltrations and a few patches of blood congestion in the kidneys for both extract treated and vehicle groups. There was however no serious pathological conditions observed like tubular or glomerular necrosis. Similarly, lung histopathology showed thickened alveolar with mild leucocyte infiltrations for all groups. Brain histopathology showed mild astrogliosis for all groups treated and untreated. However there was no significant demyelination or any abnormalities seen in all subjects.

4.8.1 Liver

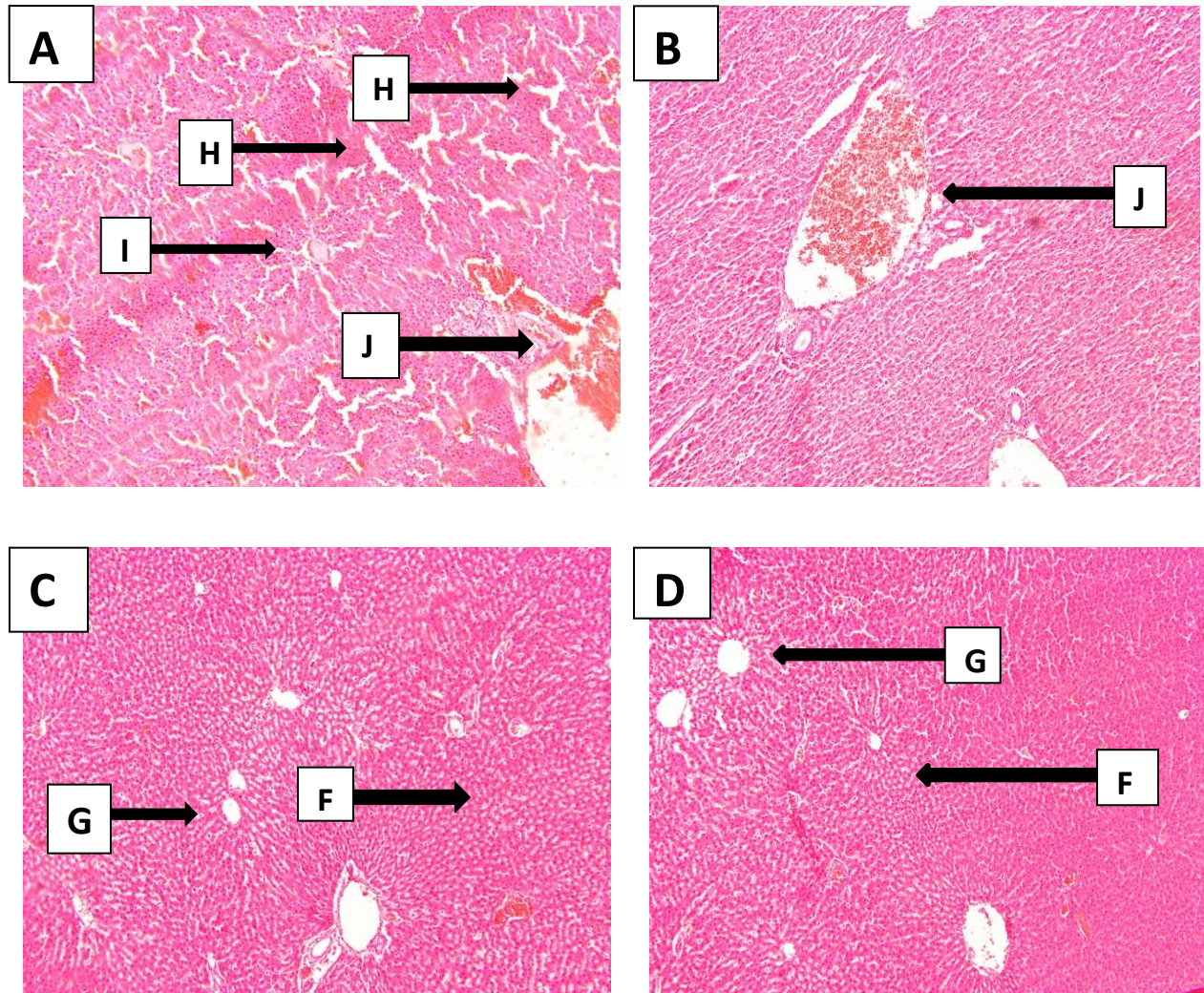


Figure 6: Photomicrographs of livers isolated from rats after 8 week continuous administration of (A) Vehicle, (B) SNE 100 mg/kg, (C) SNE 300 mg/kg and (D) SNE 1000 mg/kg. Arrows show normal central veins (G) and normal distribution of hepatocytes (F). The arrows also indicate distorted central vein (I), blood congestion (J) and increased interstitial spaces (H) (H&E staining, 20×).

4.8.2 Kidney

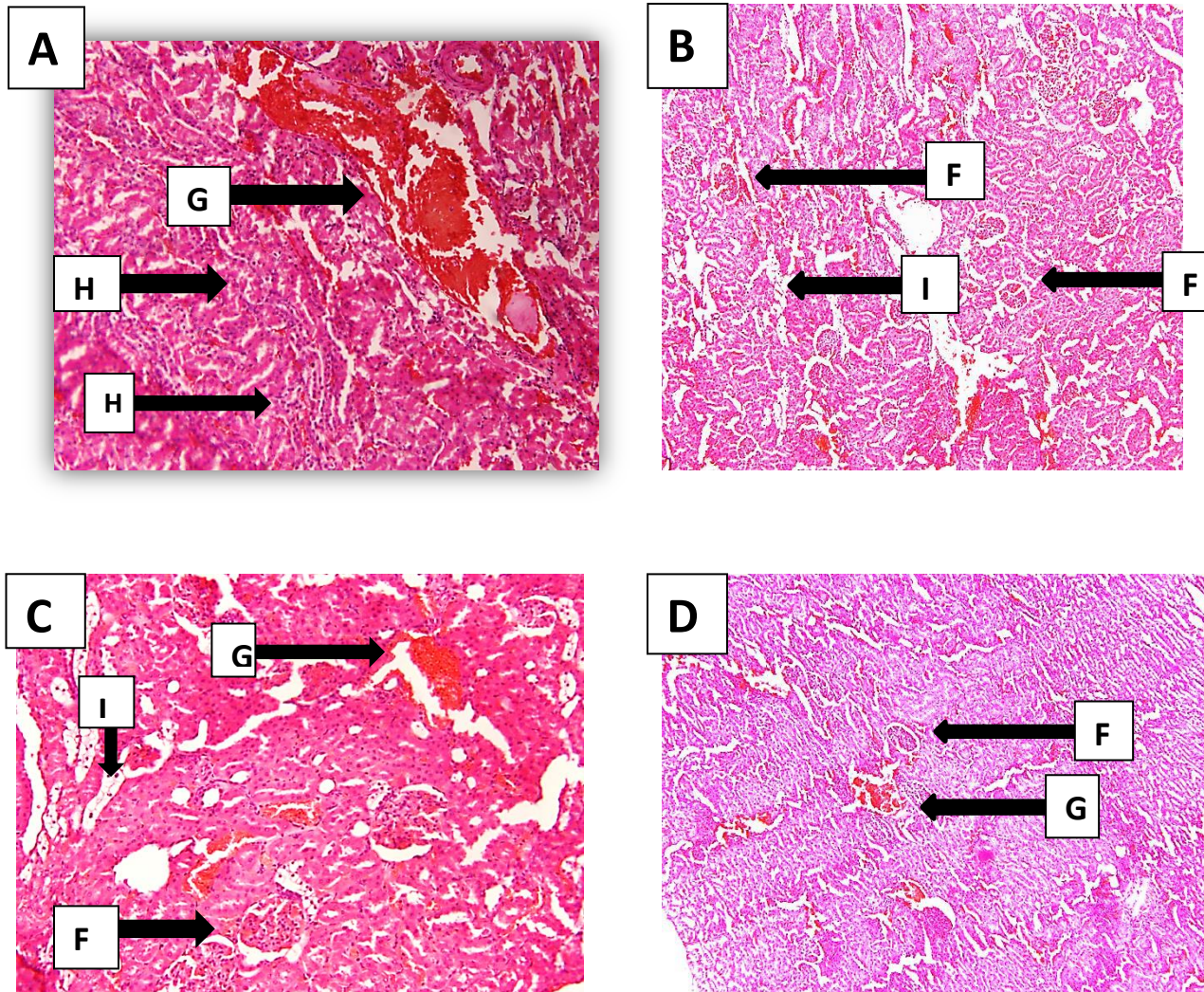


Figure 7: Photomicrographs of kidneys isolated from rats after 8 weeks of continuous administration of (A) Vehicle, (B) SNE 100 mg/kg, (C) SNE 300 mg/kg and (D) SNE 1000 mg/kg. Arrow F shows normal glomeruli, G shows blood congestion, I for normal tubules and H for mild leucocytic infiltrations (H&E staining, 40× for A and C, 20×for B and D).

4.8.3 Lungs

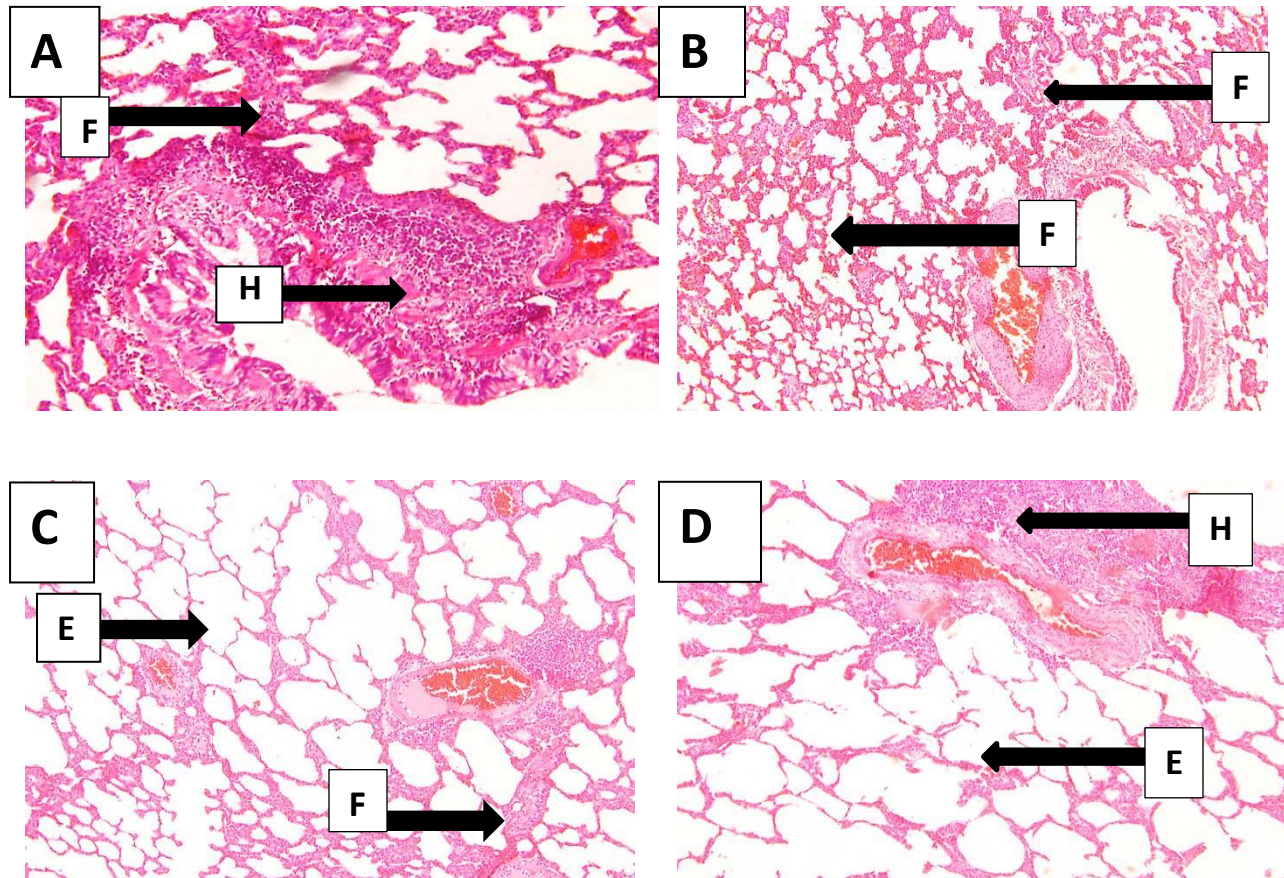


Figure 8: Photomicrographs of lung isolated from rats after 8-week continuous administration of (A) Vehicle, (B) SNE 100 mg/kg, (C) SNE 300 mg/kg and (D) SNE 1000 mg/kg. The arrow shows normal lung tissue (E), slightly thickened alveolar (F) and mild leucocyte infiltrations (H) (H&E staining, 20 $\times$).

4.7.4 Brain (cerebral cortex)

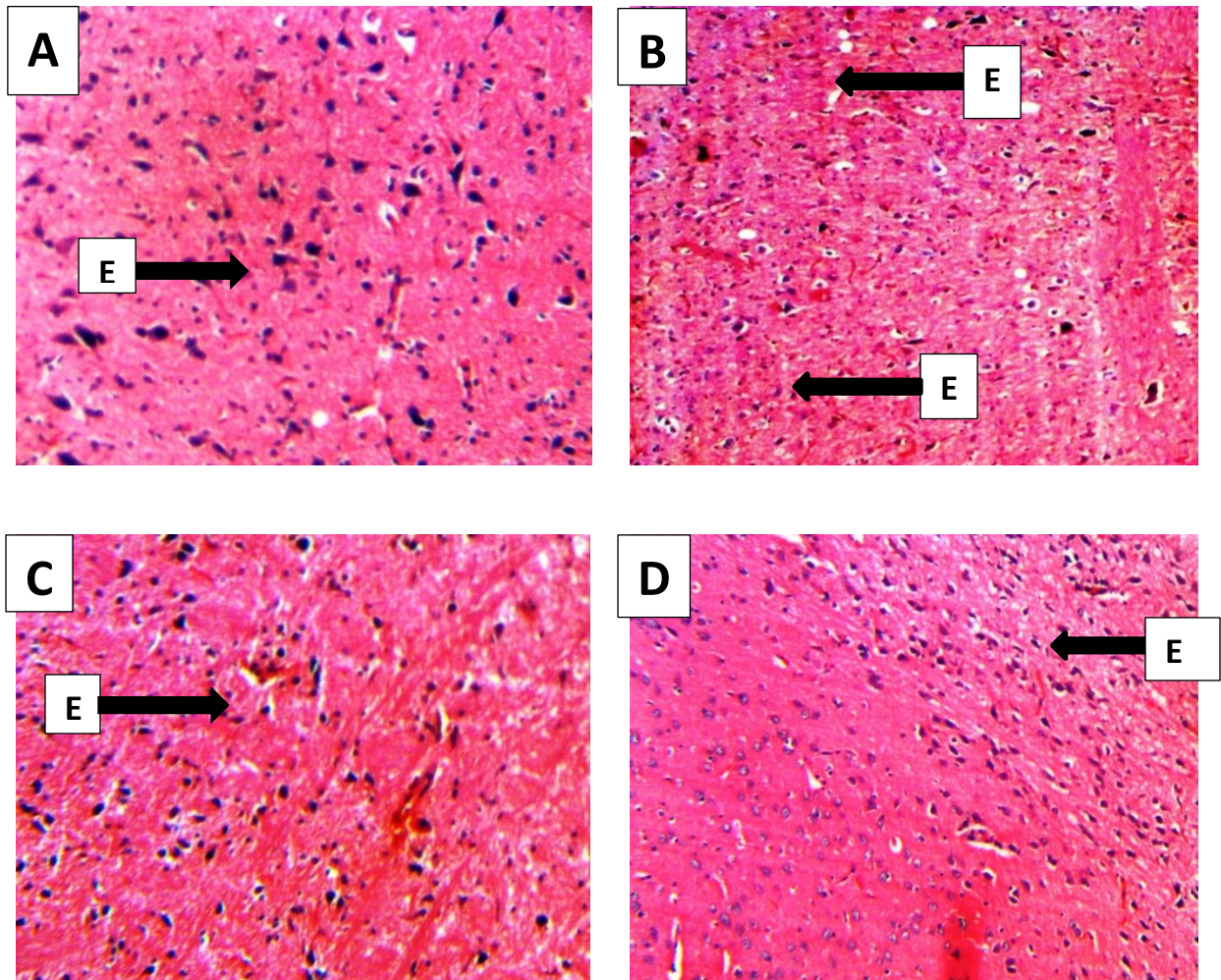


Figure 9: Photomicrographs of cerebral cortex of brains isolated from rats after 8-week continuous administration of (A) Vehicle, (B) SNE 100 mg/kg, (C) SNE 300 mg/kg and (D) SNE 1000 mg/kg. The arrow shows the presence of astrocytes (mild astrogliosis) (E). (H&E staining, 40×).

4.7.5 Spleen

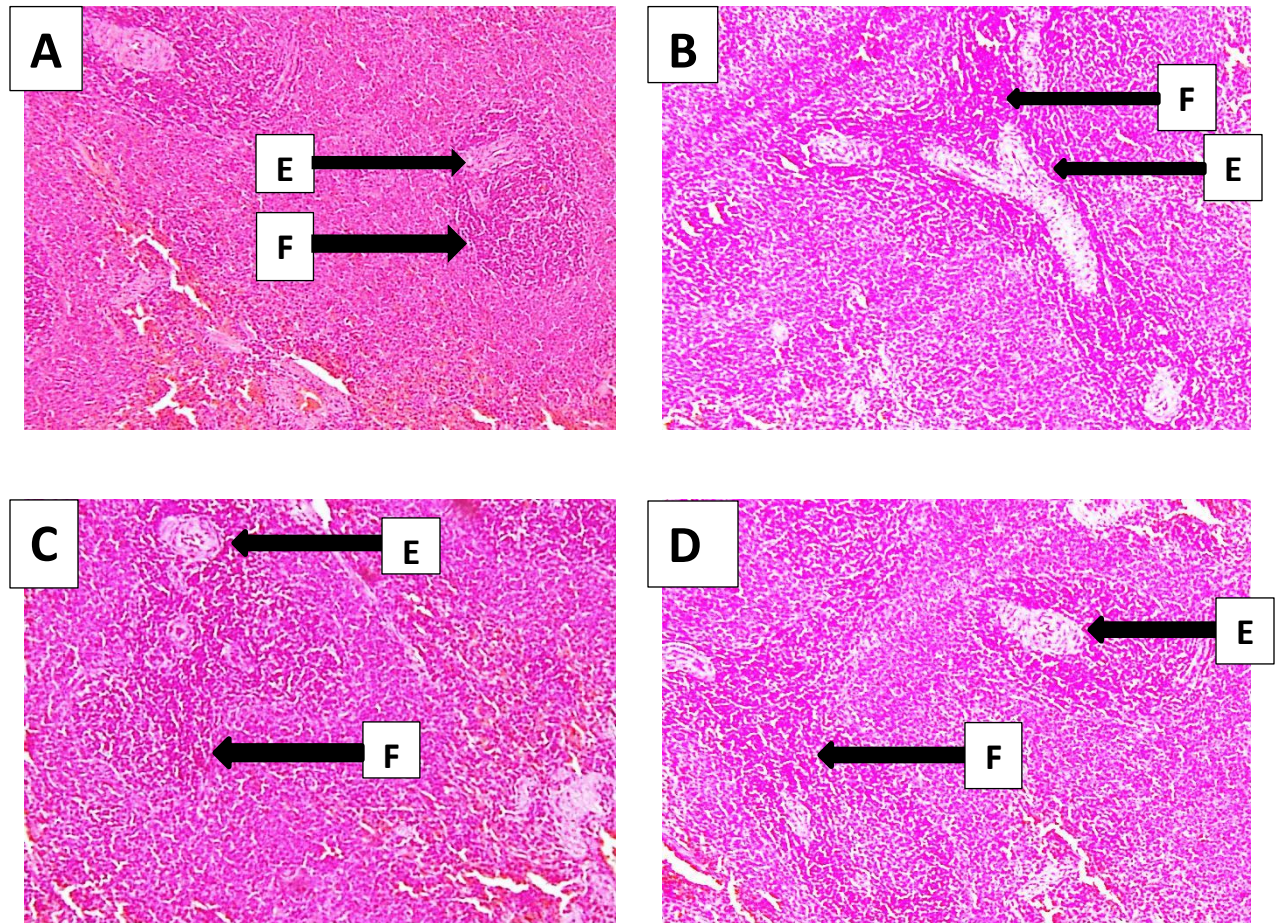


Figure 10: Photomicrographs of spleens isolated from rats after 8-week continuous administration of (A) Vehicle, (B) SNE 100 mg/kg, (C) SNE 300 mg/kg and (D) SNE 1000 mg/kg.

The arrow shows normal spleen tissue with the white pulp (E), and red pulp (F) (H&E staining, 20×).

CHAPTER FIVE

5.0 DISCUSSION

5.1 DISCUSSION

The study described demonstrates that the ethanolic extract of *Synedrella nodiflora* possesses anti-hyperglycaemic, antioxidant and a likely anti-inflammatory activity in STZ-induced diabetic rats. This may corroborate previously discovered properties, that is anti-oxidant (*in vitro*), analgesic and anti-inflammatory properties (Amoateng *et al.*, 2015; Amoateng *et al.*, 2017; Woode *et al.*, 2009). Male Sprague Dawley rats were injected with STZ (55 mg/kg) to induce diabetes. STZ acts as a nitroso-urea alkylating agent that attacks the DNA of beta pancreatic cells. This causes beta cell death leading to a decreased insulin level hence diabetes. After 8 weeks of extract administration the animals were assessed to find out if the extract had any significant effects on biochemical markers for diabetes. A number of effects were observed.

Phytochemical screening identified the presence of alkaloids, saponins, tannins, phenolics, terpenoids, flavonoids and glycosides in the ethanolic extract. This comes as no surprise as ethanol and water are able to extract a wide range of phytochemicals individually. Both solvents are polar and will be very effective in dissolving phytochemicals with hydroxyl groups (-OH) like tannins, phenolics and flavonoids. Thus the hydroethanolic solvent (i.e 30% water, 70% ethanol) had the ability to absorb a lot of bioactive compounds from the whole plant mixture. These phytochemicals could be contributing individually or synergistically to give the observed effects. There are likely to be bioactive compounds which may have anti-oxidant, anti-hyperglycaemic and anti-inflammatory effects in the extract

The SNE dose of 1000 mg/kg reduced fasting blood sugar levels significantly after four (4) weeks of administration. Anti-hyperglycaemic activity of plants as a result the presence phytochemical constituents like polyphenols is well known and well established fact in pharmacology (Abdallah *et al.*, 2011; Manickam *et al.*, 1997). Also an ED₅₀ of 187.9 mg/kg was obtained for the extract. Considering the fact that the extract is a mixture of various phytochemicals, it is possible that the constituents responsible for the anti-hyperglycaemic activity maybe just a fraction of the ED₅₀ value obtained. ED₅₀ represents the dose of extract required to achieve 50% of the desired response. If one particular compound/phytochemical is responsible for this activity could be potent since the ED₅₀ dose is not very high.

It is possible that the extract may be acting via an extra-pancreatic effect since in this experiment most of the β -cells were destroyed. This possible effect the extract may have without stimulating the pancreas has been made mention of (Zahan *et al.*, 2012). The exact mechanisms by which this extract reduced blood sugar will have to be determined in later studies.

Weight gain or loss is an important parameter in the diagnosis and assessment of the progression of the diabetes. Weight loss in early stages of diabetes, especially type 1 is attributed to the lack of insulin. When insulin is lacking, cells do not receive enough glucose for energy and other metabolic processes. Thus the cells send a feed back to the brain, which causes the body to feel hungry. This may cause the patient to eat more (polyphagia). The feedback also causes the body to mobilize glucose from other sources (gluconeogenesis) (Georga *et al.*, 2018). Fats stores and proteins may be used to produce more glucose for the body. This normally causes the loss in weight normally seen in type -1 diabetics. Ketones which are by-products of gluconeogenesis tend to accumulate in the blood leading to diabetic ketoacidosis common in diabetes

(Goldenberg and Punthakee, 2013). From the experiment it could be seen that compared to the control, the diabetic vehicle group and the extract groups had a significant loss in weight by end of the experiment. However the highest dose of the extract (SNE 1000 mg/kg) produced a mild gain in weight. SNE 1000 mg/kg was the only dose of the extract that showed a positive percentage change in bodyweight though significantly lower than the control. It's possible that the extract provided a form of nutritive/supplementary value. The plant has already been known to be used as food by both humans and in livestock (Adjibode *et al.*, 2015). The slight weight gain could also be as a result of its anti-hyperglycaemic effects.

In general all organs form a fraction of the total body weight of all organisms. An enlargement may signify organ inflammation or carcinoma. Hepatomegaly or enlarged liver is a known complication of poorly controlled diabetes (Jardim *et al.*, 2013). It occurs as a result of glycogenosis or increased synthesis of glycogen in the liver due to hyperglycemia (Al-Hussaini *et al.*, 2012; Chatila and West, 1996). Other conditions that may cause enlarged liver in diabetes include fatty liver disease (steatosis) and liver inflammation (hepatitis) (Van Steenberg and Lanckmans, 1995). A relatively large liver was observed in the vehicle group, significantly higher in comparison with the SNE treated groups. The increase in weight of the liver is in direct correlation to an increase in liver size. It can also be inferred that the extract prevented hepatomegaly by preventing liver inflammation or reducing hyperglycemia or both.

Diabetes has been proposed to cause chronic liver disease, liver steatosis (fatty liver), liver inflammation and carcinoma (El-serag *et al.*, 2004). Non-alcoholic fatty liver disease (NAFLD) and diabetes are common conditions that regularly co-exist and can act synergistically to drive adverse outcomes. These damages may be as a result of oxidative stress (Cichoż-Lach *et al.*, 2014; Yaribeygi *et al.*, 2018). Furthermore, the role of free radicals in streptozotocin-induced

tissue damages in diabetic rats has been demonstrated already (Murugana and Pari, 2006). Hepatocytic proteins, lipids and DNA are among the cellular structures that are primarily affected by reactive oxidative species (Brosnan *et al.*, 2012). Liver enzymes (ALT, ALP, AST, and GGT) are also usually released into the blood when such damages occur, and have been measured to assess the ability of certain extracts to reduce liver damage in diabetes (Ashraf and Samad, 2015; Hamadi *et al.*, 2012). In this study there was a significant increase in the levels of these enzymes for the vehicle group. The extract groups however showed no significant enzyme increase, most likely due to the fact that the extract had the ability to ameliorate the danger posed by oxidative stress in the liver (Amoateng *et al.*, 2011).

Creatinine levels were also significantly low for all the diabetic groups both treated and untreated. The lowest level of creatinine was however found in the vehicle group. Creatinine is a by-product of the breakdown of creatine in the muscles. The liver plays a major role in the biosynthesis of creatine (Brosnan *et al.*, 2011). It is entirely possible that the oxidative stress on the liver and a possible inflammation of the liver caused the vehicle group to have the lowest level of creatinine. Another possible explanation for the decrease of creatinine in all the diabetic rats is that the diabetes affected the muscle mass due to gluconeogenesis. The lower the muscle mass of the rats the lower the amount of creatine/creatinine produced. Gluconeogenesis leading to weight loss has already been mentioned above.

A low albumin level is a condition that has been associated with liver inflammation and protein protein-losing nephropathy (Throop *et al.*, 2004). Considering the fact that liver inflammation is a key constituent of chronic diabetes it comes as no surprise that there are reports of decreased serum concentrations of albumin in diabetes (Viswanathan *et al.*, 2004). Microalbuminuria however is still one of the early predictors of nephropathy in diabetes (Yasuda *et al.*, 2014).

Significantly low albumin levels were observed for the diabetic untreated vehicle group. Since the extract group had albumin levels close to normal it suggests that the extract was able to reduce inflammation of the liver. This may be further buttressed by the fact that haematology results also showed a significantly high level of eosinophils in the vehicle group. Eosinophils are associated with allergic and inflammatory reactions (Gleich, 2000; Sampson, 2000). In liver disorders eosinophils are known to release cytokines like IL- 1 β and IL-18 that cause inflammation (Palacios-Macapagal *et al.*, 2017). There was a markedly high amount of eosinophils in the serum of the diabetic vehicle group.

For the haematological indices, the results showed decrease in RBCs and haemoglobin for the vehicle group. Diabetic patients especially T1DM are known to develop erythropoietin-dependent anaemia (Thomas, 2007; Thomas *et al.*, 2005). Erythropoietin is a hormone produced and released by the kidney and it controls the production of RBCs in the bone marrow. It is believed that diabetes may affect the level of erythropoietin in 2 ways. Diabetes may directly damage the kidney (diabetic nephropathy) leading to a decrease in the production of the hormone (Khoshdel *et al.*, 2008). Secondly autonomic nerves are also susceptible to damage in diabetic neuropathy. The release of erythropoietin is controlled by the autonomic nervous system; hence diabetic neuropathic damage may reduce the secretion of erythropoietin (Hadjadj *et al.*, 2001; Spallone *et al.*, 2004). The extract group however showed no significant decrease in RBCs. The extract may be preventing or reducing damage to the nerves and the kidney by reducing hyperglycaemia and/or oxidative stress.

Histopathology also showed a change in the tissue structure of the liver for the vehicle group. The central veins were slightly dilated and distorted. There were also large interstitial spaces seen in liver tissue. Increase in blood supply to the liver usually leads to an increase in

hydrostatic pressure of the blood flowing to the liver. Increase in blood flow to the liver can be associated with inflammation (Bernardi *et al.*, 2015; Mehta *et al.*, 2014). The fluid buildup was responsible for the large spaces seen in between the liver tissues and is characteristic of increased hydrostatic pressure seen in liver inflammation and cirrhosis (Henriksen, 1980; Recker *et al.*, 2015).

There were mild leucocytic infiltrations in the kidneys for both extract treated and untreated diabetic (vehicle) groups. Leucocyte infiltrations in the kidney are usually attributed to inflammation (Adalid-Peralta *et al.*, 2008; Akcay *et al.*, 2009). There were also a few blood congestions seen. There was however no serious pathological conditions observed such as tubular or glomerular necrosis. Possible diabetic nephropathy could still be in the nascent stage. Similarly lung histopathology showed thickened alveolar and leucocyte infiltrations for all groups of diabetic rats. Allergic and inflammatory reactions usually results in lung leucocyte infiltrations (Schmiedl *et al.*, 2005). The presence leucocytes in lungs however, may not be unusual as it is part of the natural defense system against infection. Brain histopathology showed mild signs of astrogliosis in all groups. Demyelination or any other abnormalities were however not prominent. Astrogliosis is the mechanism employed by the CNS for defense and repair against injury. It is characterized by an increase in astrocytes in the area of damage (Sofroniew, 2015). Diabetes is known to play a role in CNS neuropathy and gliosis (Reske-Nielsen and Lundbaek, 1968; Reske-Nielsen *et al.*, 1970). The mild astrogliosis seen in all groups may be a sign of developing CNS neuropathy. There was however no significant difference seen amongst all diabetic groups. There was also no significant pathological damage observed.

Plants rich in polyphenols have been known to possess anti-inflammatory properties (García-Pérez *et al.*, 2017). In countries like Malaysia the *Synedrella nodiflora* plant is locally used to

treat inflammatory conditions like rheumatism (Burkill, 1985). The plant's extract has shown anti-inflammatory properties in previous studies (Abad *et al.*, 1996; Haque *et al.*, 2012). Because of the ability of the extract to reduce inflammation at the site of injury, it has been proposed that the extract may have peripheral anti-inflammatory activities. This activity was seen in carrageenan induced paw oedema models (Haque *et al.*, 2012).

SOD (superoxide dismutase) activity and MDA (malondialdehyde) content were also measured to ascertain the ability of the extract to exert its antioxidant activity *in vivo*. SOD activity showed a dose dependent increase in activity. However, only the 1000 mg/kg of SNE, the highest dose, was significantly higher than the control. In the MDA assay the extract's highest dose also gave the only significant reduction in MDA, even though the decrease was dose dependent. SOD and MDA assays are among the most reliable and widely used assays for *in vivo* antioxidant properties. Oxidative stress is a cellular phenomenon which occurs as a result of physiological imbalance between levels of antioxidants and oxidants (free radicals), where there are more oxidants. SOD is one of the primary antioxidant enzymes contained in mammalian cells that serve as a defensive mechanism against oxidative stress (McCord *et al.*, 1971).

SOD basically reacts with the highly reactive superoxide anion (O_2^-) in a reaction known as dismutation to form a hydrogen peroxide and oxygen which are relatively harmless (Birben *et al.*, 2012). The superoxide anion is generated by various biochemical processes and it can damage macromolecules (proteins, nucleic acids, etc.) by oxidizing them (Ighodaro and Akinloye, 2017; Perry *et al.*, 2007). Anti-oxidants neutralize ROS by donating electrons to them and stabilizing them (Nimse and Pal, 2015). A high SOD activity usually means the presence of antioxidants to prevent the natural SOD activity from being overwhelmed by ROS. When SOD activity is low, it most likely means oxidative stress has overwhelmed the natural defense

systems and the proteins that produce SOD have possibly been damaged by oxidation (Fucci *et al.*, 1983; Stadtman, 1990). This goes to show that high dose of the extract was able to protect the SOD activity, thus the significantly high activity observed in comparison with the vehicle group that had a relatively low activity of the enzyme. Preliminary *in vitro* tests have shown that the extract may possess free radical scavenging properties (Onocha *et al.*, 2016).

Free radicals can attack the polyunsaturated fatty acids (PUFA) of cell membranes. The oxidative damage of PUFA, also known as lipid peroxidation is particularly destructive, because it perseveres as self-sustaining chain reaction (Marnett, 1999). MDA is one of the byproducts of the reaction of the ROS with polyunsaturated fatty acids (Ayala *et al.*, 2014). Hence an increase in serum aldehyde is directly proportional to the level of oxidative stress (Gawel *et al.*, 2004). A significantly low amount of aldehydes was seen in the 1000 mg/kg SNE dose compared with the control, confirming the antioxidant and anti-lipid peroxidative effect of the extract *in vivo*. In general there was a dose-dependent decrease in aldehydes for the extract treated animals, but only the 1000 mg/kg SNE was statistically significant. *Synedrella nodiflora* extract possesses polyphenols and flavonoids which may account for its antioxidant activity.

5.2 CONCLUSION

The ethanolic extract of SNE showed anti-hyperglycaemic, anti-oxidant and a possible anti-inflammatory activity in streptozotocin-induced diabetes in rats. It also exerted various effects on blood biochemistry, haematology and histopathology which corroborated its anti-hyperglycaemic, anti-oxidant activity and anti-inflammatory activity.

5.3 RECOMMENDATIONS

- Further investigations should be conducted to fully evaluate the extract for its hepatoprotective activity.
- The potential of the extract to lower or reverse diabetic neuropathy should be evaluated.

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