INVESTIGATION OF THE ANTIDIABETIC ACTIVITY OF *CNICUS BENEDICTUS* L. IN RATS.

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A thesis submitted in partial fulfilment of the requirements for the degree of Magister

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UNIVERSITY *of the*
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NOVEMBER 2016

DECLARATION

I declare that the thesis, Investigation of the antidiabetic activity of *Cnicusbenedictus* L. in rats, is my own work, that it has not been submitted before for any degree examination in any other University and that all the sources I have used or quoted have been indicated and acknowledged by complete reference.

Raymonde BAMBOUKOU BEKALE   November 2016

Signed…………………………..

DEDICATION

I dedicate this thesis to my loving mother, Boubwetata Loba Helene and my family for their sacrifices and endless love, care and encouragement that has got me to where I am today. Thank you for believing in me and supporting me to be the best.

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The completion of this work has come as a product of hard work and I would like to express my sincere gratitude to the following individual and organizations, whose involvement in my life enabled me to complete this thesis:

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KEYWORDS

Cnicusbenedictus

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Antinociceptive activity

Acute toxicity

HPLC analysis

Leaf methanol extract

Orthodox medicines

Traditional medicines

Pharmacological evaluation

Mice

Rats

ABBREVIATIONS

AMP   Adenosine monophosphate

CNS   Central nervous system

DKA   Diabetic Ketoacidosis

DNA   Deoxyribonucleic acid

GIP   Glucose dependent Insulinotropic Polypeptide

(Gastric Inhibitory Polypeptide)

GIT   Gastrointestinal tract

GLP-   Glucagon-Like Peptide

GLUT   Glucose Transporters

HbA1C   HemoglobinA1c

Hb   Haemoglobin

HLA   Human Leukocyte Antigen

HONK   Hyperosmolar non-Ketotic Acidosis

HPLC   High Performance Liquid Chromatography

IDDM   Insulin-Dependent Diabetes Mellitus

IDF   International Diabetes Federation

i.p   Intraperitoneal

LD50   Median lethal dose

LDL   LDL Low-density Lipoprotein

NIDDM   Noninsulin-Dependent Diabetes Mellitus

NOAEL	No-observed-adverse-effect-level
NPH	Neutral Protamine Hagedorn (Isophane Insulin)
NSAIDs	Non-steroidal Anti-inflammatory Drugs
OGTT	Oral Glucose Tolerance Test
p.o	Oral administration
PPAR,γ	Peroxisome Proliferator Activated Receptor Gamma
RNA	Ribonucleic acid
SEM	Standard error of the mean
SGLT-	Sodium-Glucose co-transporter
STZ	Streptozotocin or Streptozocin
UV	Ultraviolet
WHO	World Health Organization
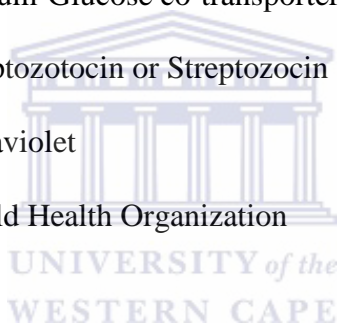
ABSTRACT

Investigation of the antidiabetic activity of *Cnicusbenedictus L.* in rats.

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Diabetes Mellitus, one of the major diseases affecting human population all over the world has caused significant morbidity and mortality. The management of this condition has raised the demand of safe and cost effective remedial measures due to several side effects associated with the present use of modern medicines. Thus, it is crucial to explore other options for diabetes management such as the use of medical plants. *Cnicus benedictus L.* is one of the known plant species used by traditional medicine practitioners in South Africa for the treatment of various ailments including inflammatory conditions, pain and diabetes. Even though the plant species has been extensively studied, scientifically, no evidence exists in literature to corroborate the claim made by traditional medicine practitioners of its therapeutic success in the treatment of diabetes and pain.

Therefore, the objectives of this present study were: to investigate the antidiabetic activity of *C. benedictus* using leaf methanol extract of the plant species on animal model involving male and female Albino rats; to investigate the antinociceptive activity of the plant species on mice; to determine the safety profile of the plant by

investigating the acute toxicity and to carry out HPLC study in order to characterize the plant species.

Animals were divided into groups of six each and fasted overnight prior to the induction of diabetes in rats using Streptozocin (STZ). The plasma glucose was measured at intervals of 30 min for 4 hours by means of a glucometer. *Cnicus benedictus* (100 – 400 mg/kg, i.p.) significantly reduced the blood glucose concentrations of fasted normal rats with percentage maximum reduction ranging from 46 to 79% and chlorpropramide (250 mg/kg, i.p.) significantly reduced the blood glucose concentrations of fasted normal rats by 84%. *Cnicus benedictus* (100 – 400 mg/kg, i.p.) significantly reduced the blood glucose concentrations of STZ-induced diabetic rats with percentage maximum reduction ranging from 44.82 to 66.04% and chlorpropramide (250 mg/kg, i.p.) significantly reduced the blood glucose concentration of STZ-induced diabetic rats by 71.71%.

In the oral glucose tolerance test, administration of leaf methanol extract of *Cnicus benedictus* (100 – 400 mg/kg, i.p.) following oral glucose load on fasted normoglycaemic rats significantly reduced the increased blood glucose concentrations with percentage maximum reduction ranging from 42.45 to 70.75%. Chlorpropramide (250 mg/kg, i.p.) following oral glucose load on fasted normoglycaemic rats significantly reduce the increased blood glucose concentration with a percentage maximum reduction of 79.04%.

In acetic acid writhing test, animals were divided into groups of eight per dose. *Cnicus benedictus* (25-400 mg/kg, i.p.) significantly reduced the number of writhes in mice with percentage inhibition of the writhes ranging from 67.95 to 73.71%. Indomethacin (20 mg/kg, i.p.) and paracetamol (500 mg/kg, i.p.) significantly reduced the number of writhes in mice with percentage inhibition of 75.44 and 69.18% respectively. Combined treatment of lowest and sub-effective doses of *C.benedictus* (12.5 mg/kg, i.p.) and indomethacin (10 mg/kg, i.p.) significantly reduced the writhes with a percentage inhibition of 58.32%.

In hot plat test, animals were divided into groups of eight per dose. *Cnicus benedictus* (25-400 mg/kg, i.p.) significantly delayed the reaction times of the mice to hot-plate thermal stimulation. Morphine (10 mg/kg, i.p.) significantly delayed the reaction time of the mice to the hot-plate stimulation.

The no-adverse-effect-level (NOAEL) of leaf methanol extract of *Cnicus benedictus* was obtained at 3200 mg/kg (p.o.) and the LD50 value of the plant species was found to be 4000 mg/kg (p.o.).

The HPLC fingerprint of the leaf methanol extract of *Cnicusbenedictus* showed distinct peaks at the following retention times of 6.387, 14.628, 18.431, 23.228 and 29.829 min.
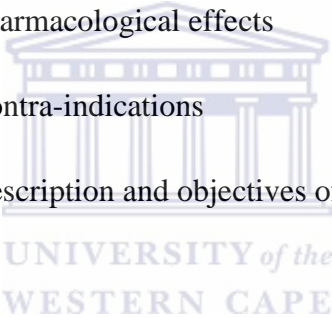
In conclusion, the data obtained showed that leaf methanol extract of *Cnicus benedictus* possesses both antidiabetic and antinocipetive activities.

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CHAPTER 1

INTRODUCTION

According to Kinghorn et al. (1993), natural products and their derivatives represent more than 50 % of all drugs in clinical use in the world and higher plants contribute no less than 25 % of the total. The world is currently undergoing a rapidly growing interest in natural and traditional herbal remedies as a source of new commercial products in health shops and pharmacies. Commonly known examples of plant-derived medicines include atropine, codeine, colchicine, digoxin, morphine, quinine, reserpine and new cancer drugs such as taxol and vincristine (Kinghorn et al., 1993).

The curative actions of medicinal plants or phytomedicines ("*phyto*" means plant) and derivatives are due to the presence of mixture of different chemical entities. Chemical entities are defined as pure chemical compounds used for medicinal purposes. The active chemical compounds in medicinal plants are called secondary metabolites. A secondary metabolite can have more than one pharmacological activity because they are multifunctional compounds. These metabolites act individually or in synergy in direct or indirect pathways to treat diseases or improve and maintain health. In order to work as therapeutic agents, secondary metabolites need to interfere with a molecular target in the human body. Secondary metabolites are commonly classified in groups such as amino-acids, alkaloids, flavonoids, essential oils, glycosides, lectins, glycoproteins tannins, quinones, coumarins, terpenoids and so on(Van Wyk et al., 1997 ; Das Prajapati et al., 2003 ; Light et al.,2005 ; Van Wyk et al.,2012).

Several people all over the world still rely on medicinal plants or plant-derived medicine for daily healthcare needs as natural substitute to synthetic chemicals. Approximately 80% of the populations in Africa still rely on traditional medicinal plants to cure many kinds of ailments especially in rural areas. These herbal remedies are an important part of African cultural heritage transmitted from one generation to the next based on trial and error over many centuries (Das Prajapati et al., 2003).

South Africa is known to have a remarkable biodiversity of over 30 000 species of higher plants with approximately 3 000 of them used for medicinal purposes and some are commonly traded as medicinal plants. It is estimated that around three million South Africans in rural and urban areas are reliant on traditional medicinal plants only or in combination with modern medicine (Van Wyk et al., 1997).

According to the world health organization (World Health Organization, 2008), traditional medicine is the combination of skills, knowledge and practices based on the theories, believes or experiences indigenous to different cultures and used for diagnosis or treatment of illness but also for prevention, improvement or maintenance of health. The adaptation of traditional medicine in different regions is done regardless of advanced international standards and methods of evaluation. Several countries do not have national policies to regulate practitioners, practices and traditional medicine remedies. For example, most traditional medicines prepared by traditional healers in South Africa are likely to develop microbial contamination at any stage of the preparation process due to the lack of proper hygienic settings during

preparation, handling and storage of herbal products (Ernest., 2002 ; Govender et al., 2006 ; World Health Organization,2008).

Since there is less stringent regulatory authority for medicinal plants products, South Africa is moving towards basic research on herbal medicinal remedies to ensure the quality, safety and authenticate the therapeutic claims made by traditional healers or herbalists(Govender et al., 2006 ; Van Wyk et al.,2012).

The therapeutic claims made by traditional healers range from curative diseases to chronic diseases such as diabetes. Diabetes Mellitus, one of the major diseases affecting human population all over the world, has caused significant morbidity and mortality. The condition can be defined as an endocrine disorder characterized by glycosuria and hyperglycaemia. Diabetes Mellitus is due to either the inability of the pancreas to produce enough insulin or the inability of the body to correctly use the insulin produced. According to the annual report of International Diabetes Federation (IDF), 382 million people were living with diabetes in 2013 from which 80% of people live in low and middle-income countries. IDF predicts this number to rise to 592 million people by 2035 (IDF, 2013).

Medical researchers are still working on the management of Diabetes Mellitus which is still a big challenge for them. The management of this condition has raised the demand of safe and cost effective remedial measures because of several side effects associated with the present use of modern medicines (Preethi, 2013).Thus, it is crucial to explore other options for diabetes management such as the use of medicinal

plants. Traditional plant based remedies are still the first choice in the developing countries because of their cost effectiveness, easy availability and minimum or no side effects (Watt et al., 1962 ; Okigbo et al., 2006).

In this present study, we are investigating the antidiabetic activity and safety profile of a medicinal plant called *Cnicus benedictus*.L. The plant species is known to have pharmacological properties such as antimicrobial, anti-inflammatory and antiproliferative effects. Those pharmacological effects have been proven with animal studies but no clinical outcomes have been published. The antimicrobial activity has been proven against *Bacillus subtilis, Brucella species, Escherichia coli, Proteus species, Streptococcus faecalis, Staphylococcus aureus and Pseudomonas aeruginosa* due to the presence of sesquiterpene lactone cnicin (Vanhaelen-Fastre, 1968; Vanhaelen-Fastre, 1974; Chevalier, 2000; Van Wyk et al., 2004).

The pharmacokinetics studies show that cninin, the main component of *Cnicus benedictus* has a mild antiinflammatory activity due to inhibitory effects on cyclic AMP, phosphodiesterase and histamine release in mast cells (Mascolo et al., 1987; Schneider et al., 1987). Studies investigating the antineoplastic and cytotoxic effects demonstrate that the constituent, cninin and arctigenin, show anti-tumour activity via inhibition of cellular DNA, RNA or protein synthesis (Vanhaelen-Fastre, 1971 ; Cobb, 1973 ; Ryu et al., 1995 ; Moritani et al., 1996).

Historically, *Cnicus benedictus*.L was used in the middle ages as a cure for bubonic plague with the main constituent, amaroidcninin. The amaroids are known to

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stimulate the secretion of saliva and gastric juices. The herb has been used as anti-diarrhoeal, antihaemorrhagic, expectorant and for wound healing (Bradley, 1992; Bisset, 1994; Chevalier, 2000; Van Wyk et al., 2004). The plant species has also been extensively used by traditional healers for anorexia, pain, flatulence, dyspepsia, diabetes, bronchial catarrh and topically for gangrenous and indolent ulcer (Bisset, 1994; Van Wyk., 1997).

Even though scientific evidence exists for some pharmacological activities such as the anti-inflammatory, antitumour and antimicrobial effects of the plant species, none has been found in any literature to corroborate the claim by traditional medicine practitioners of therapeutic success of the plant species in the treatment of diabetes and pain.
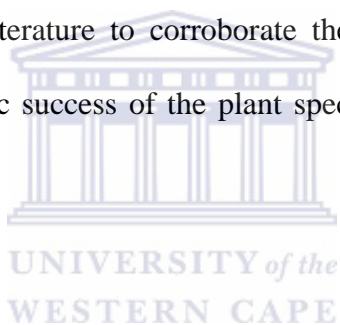
CHAPTER 2

LITERATURE REVIEW

2.1. Overview of Diabetes mellitus

2.1.1. Description

Diabetes mellitus is a widely known chronic metabolic disorder characterized by deficiency of insulin secretion or the inability of the body to use effectively the insulin produced or a combination of these. Diabetes mellitus involves fat, carbohydrate and protein metabolism. The condition is generally divided into two main types, idiopathic and secondary. Idiopathic diabetes is classified as insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). IDDM, previously known as juvenile onset diabetes mellitus, is commonly called type 1 diabetes mellitus and NIDDM, previously known as adult onset diabetes mellitus is called type 2 diabetes mellitus. Despite that common classification, other types of diabetes such as pancreatic diabetes and gestational diabetes mellitus are also identified (Anon, 2010; World Health Organization 1999; Standard treatment guidelines, 2012; King, 2014)

2.1.2. Pathophysiology and Pathogenesis of diabetes mellitus

Type 1 diabetes mellitus

Insulin is a polypeptide hormone known to regulate carbohydrate metabolism. It comes from the Latin word insula which means "island" because of its production by beta cells called Islets of Langerhans. Those cells are located in the pancreas. During

a meal, the food is transformed through the digestive system where nutrients, fats, proteins and carbohydrates are absorbed into the bloodstream. The presence of carbohydrates stimulates the pancreas to secrete insulin. Insulin works by enabling the entry of glucose from blood into muscles and other tissues where the glucose is metabolized for energy production. Deficiency of insulin secretion is the major defect in type 1 diabetes while type 2 diabetes has two main defects, insulin resistance and the impaired insulin secretion. (Anon, 2010)

Type 1 diabetes develops when pancreatic beta cells are unable to produce insulin because of progressive destruction of beta cells. The process of beta-cell destruction starts when macrophages and dendritic cells present beta cell antigens to naive CD4+ T-cells through the major histocompatibility complex. Through a series of interleukin signaling, CD4+ T-cells are activated, which in turn activate CD8+ T-cells directly responsible for causing beta-cell death. The progressive autoimmune destruction of pancreatic beta cells results in deficiency of insulin secretion which leads to uncontrolled lipolysis and high levels of free fatty acids in plasma, causing metabolic disorders such as impaired glucose(increase of glucose concentration in the blood), protein, and lipid metabolism (Al Homsi et al., 1992; Yoon et al., 2005; Ahmad, 2014; Ogbera, 2014; Ozougwu, 2013)

Type 1diabetes mellitus is not only a result of autoimmune factors but also an interaction of genetic and environmental factors. The genes involve in type 1 diabetes are multiple but the most susceptible one is located on chromosome 6 specifically on the human leukocyte antigen (HLA) region of the chromosome. Polymorphisms

occurring in the HLA region increase the genetic risk of developing diabetes mellitus type 1 more, strongly individuals with genotyping showing the haplotypes DQA1*0301, DQB1*0302, and DQB1*0201. (Ulbelen et al., 1977; Powers, 2015a)

Type 2 diabetic mellitus

In most cases, diabetes mellitus type 2 usually involves insulin resistance. Insulin is known to act by biding to its specific receptor on plasma membrane through a series of intracellular protein phosphorylation mechanisms that result in glucose uptake.

Previously, insulin resistance was thought to be the main abnormality in type 2 diabetes mellitus and that the inability to secrete insulin was a very late manifestation (Reaven et al., 1988). This concept has changed because scientific studies have shown that there is a feedback mechanism ensuring the integration of glucose homeostasis to maintain glucose concentration in a narrow range. This feedback regulation involves beta cells and insulin sensitivity (Kahn et al., 1993). Several studies show that insulin resistance precedes the defect in insulin secretion but individual will develop diabetes only when insulin secretion is inadequate to maintain glucose concentration close to normal (Triplittet al 2014; Khan et al., 2014).

Type 2 diabetes mellitus is more characterized by a combination of various factors related to insulin resistance, relative deficiency or diminished effectiveness of insulin and environmental factors including obesity, over eating, sedentary lifestyle and stress (Kahn et al., 1993; Khan et al., 2014; Kaku, 2010).

2.1.3. Diagnosis

The following criteria are applied to diagnose diabetes mellitus in patients:

a) Patients with classic symptoms of diabetes mellitus, plus a random plasma glucose concentration at or above 11.1 mmol/L (200mg/dL). Random plasma glucose is defined as a glucose test done anytime of the day regardless of the time since the last meal consumption. (Anon, 2010; Standard treatment guidelines , 2014; Powers, 2015a).

b) Fasting plasma glucose concentration at or above 7.0 mmol/L (126mg/dL) or fasting blood glucose at or above 6.1 mmol/L. 6.1. Fasting is defined as no caloric intake at least 8 hours before the test is performed. (Anon, 2010; Standard treatment guidelines , 2014; Powers, 2015a).

c) 2 hours plasma glucose concentration at or above 11.1 mmol/L (200mg/dL) during an oral glucose tolerance test (OGTT). OGTT is performed according to the WHO description, using a 75 g of oral glucose load. (Anon, 2010; Standard treatment guidelines , 2014; Powers, 2015a).

d) Glycated hemoglobinA1c test (HbA1C) at or above 6.5 % ($\geq$0.065; $\geq$48 mmol/molHb) which represent the percentage of haemoglobin that has glucose attached and should be performed in laboratory (Anon, 2010; Powers, 2015a).

2.1.4. Signs and symptoms

Classic symptoms of diabetes mellitus are excess and frequent urination or polyuria, excessive hunger or polyphagia and excessive thirst or polydipsia. These symptoms

are more common in diabetes mellitus Type 1 compare to Type 2. The polyuria is characterized by increased plasma glucose concentrations exceeding the limit for glucose reabsorption in the kidney. As a result, glucose is spilled into the urine and creates a hyperosmolar environment causing patients to urinate more frequently. Excessive plasma glucose increases the chance of dehydration. In the attempt to correct this excess, hyperosmolar state and dehydration will stimulate excessive thirst. Finally, the excess plasma glucose cannot be used efficiently which results in lack of energy to be used by the body. In the attempts to correct the energy deficiency, the body will stimulate excessive eating (polyphagia). The most common symptom of diabetes mellitus Type 2 is fatigue followed by nocturia because symptoms of Type 2 diabetes occur more slowly (Standard treatment guidelines , 2014)
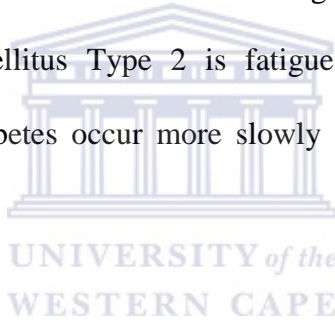
2.1.5. Screening

Screening for diabetes mellitus Type 1 is recommended in high risk people while Type 2 is recommended in people with body mass index at or above 25 kg/m2 and experiencing at least one risk factor of Type 2 diabetes. The risk factors for developing diabetes mellitus Type 2 include: first degree family history of diabetes, physical inactivity, history of cardiovascular disease, hypertension and high triglycerides. Adults from the age of forty should be screened for diabetes even without any risk factors of developing diabetes Type 2 as age itself can be a risk factor for diabetes Type 2.(Standard treatment guidelines , 2012; Standard treatment guidelines , 2014; Herrier et al., 2015).

Children losing weight despite a good appetite, experiencing polyuria, polydipsia, tiredness, abdominal pain and a sweet smell on their breath with a positive ketones urine test should be screened for diabetes. Children above the age of 10 or with physical signs of puberty and experiencing hypertension, hyperlipidaemia, polycystic ovarian syndrome together with family history of diabetes and body mass index above 85 % for age and gender should be screened for diabetes. (Standard treatment guidelines , 2012; Standard treatment guidelines , 2014; Herrier et al., 2015).

2.1.6. Clinical presentation

Table 1 Clinical presentation of diabetes Mellitus Type 1 and 2

Criteria	Diabetes mellitus Type 1	Diabetes mellitus Type 2
Age of onset	Juvenile onset , commonly below age of 30	Maturity onset, commonly above age of 30
Body weight	Non-obese	Patients usually obese or have history of obesity
Symptoms (refer to signs and symptoms section)	Symptomatic	Often asymptomatic
Insulin resistance	Absent	Present
Acute complications	Associated with diabetes ketoacidosis	Associated with hyperosmolar hyperglycaemic state
Insulin therapy	Immediate after diagnosis	Years later after diagnosis

2.1.7. Management of diabetes mellitus

2.1.7.1. Non-drug therapy

General measures for non-drug treatment include lifestyle changes, nutrition control and patient education. Patient education is an important key to maintain an ideal adherence to therapy. Patients should be advised to wear a disease identification bracelet, card or necklace in case of emergency assistance. Lifestyle changes focus on losing weight for overweight patients and also moderate physical exercises. Physical activity such as walking for 30 minutes for three days a week, climbing stairs etc are recommended (Standard treatment guidelines ,2014; Powers, 2015b).

Diet control is more complex, patients should have a nutrition rich in fruit and vegetables and a low fat diet. The daily meal regimen should include only one fresh fruit at a time and the following:

- One portion containing vitamin C such as cabbage family, guavas and tomatoes.

- One portion of dark green vegetables such as green beans, baby marrow and broccoli.

- One portion of dark yellow-orange vegetables such as pumpkin and carrots.

- One fresh fruit at a time.

Healthy diet including low fat dairy products such as two cups of milk daily for adults and limited consumption of cheese. It is preferable to eat chicken without skin and fish compared to red meat. Food containing high level of cholesterol and saturated fat should be restricted. Adequate fluid intake per day (women at least 4

glasses of 250 ml and men at least 6 glasses of 250 ml of water) and increase fibre consumption (Standard treatment guidelines ,2014; Powers, 2015b)

2.1.7.2. Drug therapy

Diabetes mellitus is a chronic condition difficult to cure but the management focuses on keeping the plasma glucose concentrations as close as possible to normal range with the help of glucose lowering medications, exercises and healthy nutrition. Target goals should be individualized with each patient after consultation with a medical practitioner. To reach the treatment goal, the HbA_{1c} is the marker used to monitor patient glycaemia. The target HbA_{1c} differs from young adult to elderly close to normoglycemia without significant hypoglycaemia. A key factor is patient education and understanding of the disease and complications associated with diabetes such as risks of cardiovascular disease. Treatment of diabetes depends on the type of diabetes. In general, Type 1 diabetes mellitus is caused by absence of insulin therefore; insulin should be administrated to a patient suffering with Type 1diabetes. Type 2diabetes is more characterized by insulin resistance and treatments include oral anti-diabetic medications (Standard treatment guidelines, 2014; Powers, 2015b; Triplitt et al., 2014).

a) Insulin therapy

Insulin is used for treatment of diabetes mellitus Type 1 and involve sadministration of combined split-mixed injections. It is also used in acute diabetes emergencies such as diabetic ketoacidosis (DKA) and pregnancy. It can be used as a supplement in

diabetes Type 2 when oral therapy fails to control blood glucose concentrations. The common adverse reactions observed with insulin therapy are hypoglycaemia and rebound hyperglycaemia. Hypoglycaemia can lead to brain damage. Usually, patients with chronic renal impairment required less insulin therefore, regular control of blood glucose levels must be done to minimize the risk of hypoglycaemia. Rebound hyperglycaemia also known as somogyi effect, occurs after an episode of hypoglycaemia following insulin administration especially at night. This is caused by release of counter regulatory hormones such as glucagon, adrenaline, cortisol and growth hormone. Currently, 4 types of insulin injections are available: rapid acting insulin, short acting insulin, intermediate acting insulin and long lasting insulin (Powers, 2015b; Triplitt et al., 2014).

Rapid acting insulins

They have a very fast onset of action that permits more replacement of physiologic prandial insulin. Their duration of action is short with less risk of hypoglycaemia after meal. Insulin aspart, insulin lispro and insulin glulisine are the three commercially used ones in this class. Insulin aspart is obtained from substitution of the B28 proline with aspartic acid and therefore, prevents insulin self-aggregation. Insulin lispro is formulated by exchanging the position of proline and lysine (Proline at B28 goes to B29 while lysine at B29 goes to B28) which permits a very low propensity compared to human insulin. Insulin glulisine is created from substitution of the B3 lysine with asparagine and the B29 lysine with glutamic acid (Powers, 2015b; Triplitt et al., 2014).

Short acting insulins

Recombinant DNA techniques are recent techniques used to produce short acting soluble insulin molecule very close to human insulin. Short acting soluble insulin is a regular insulin molecule which is administrated intravenously 30 to 45 minutes before meal. If given at meal time, short acting insulin can cause early postprandial hyperglycaemia and late postprandial hypoglycaemia. It is very useful in diabetic ketoacidosis management (Powers, 2015b; Triplitt et al., 2014).

Intermediate acting and long lasting insulins

Neutral protamine hagedorn (NPH) insulin, intermediate acting insulin regularly used in combination with rapid and regular insulins. NPH action is unpredictable therefore is used in combination with insulin analogs having predictable action. The morning administration of intermediate acting insulin provides basal insulin for the day and covers the midday meal whereas the evening dose covers basal insulin for the evening and the rest of the day. Glargine and detemir are long acting insulin providing peakless basal insulin lasting more than 20 hours. Insulin glargine is often administrated once a day but insulin resistant patients can have split dose (twice a day). Insulin detemir is the most recent developed insulin and has less risk of hypoglycaemia than NPH insulin (Powers, 2015b; Triplitt et al., 2014).

   b) **Oral anti-diabetic drugs**

The main goal of the treatment is to reduce and maintain blood glucose concentrations close to normal range and for as long as possible, in order to prevent

complications associated with increase glucose concentrations in human body. Oral therapy is prescribed in Type 2 diabetes mellitus when diet alone has failed and also as an adjunct to continued diet restriction (Powers, 2015b; Triplitt et al., 2014; Sumrall et al., 2009).

Biguanides

Biguanides are known to reduce hepatic output of glucose and increase glucose uptake by peripheral muscle tissues. Commonly used drug is metformin with no direct effect on pancreatic beta cells. Metformin reduces endogenous insulin requirements but also reduces plasma triglycerides and LDL-C by 8 to 15%. Amongst other oral antidiabteic medications, metformin is useful in reducing macrovascular complication in obese patients and does not stimulate appetite or cause weight gain. Metformin reduces HbA1c level by 1.50 to 2.0%. Metformin can be used in combination with other oral antidiabetic drugs especially sulphonylureas. It is and usually given as the first line treatment for Type 2 diabetes mellitus in conjunction with lifestyle modification (such as weight loss or exercise). Metformin has no risk of serious hypoglycaemia but is associated with mild gastrointestinal side effects such as abdominal discomfort, diarrhoea and stomach upset. In order to reduce these side effects, the drug should be taken with meals or after meals. Metformin should be used with caution in patients suffering from impaired kidney or liver function. Hypoglycaemia is observed when metformin is used in combination with sulphonylureas (Powers, 2015b; Triplitt et al., 2014; Sumrall et al., 2009).

Sulphonylureas

They are insulin secretagogues widely used as oral antihyperglycaemic drugs. They work by binding to sulfonylurea receptor on beta cell surface to stimulate secretion of insulin from the pancreatic beta cells. Sulphonylureas are divided into two groups, 1st generation and 2nd generation. Second generartion sulphonylureas have fewer side effects and are more effective than the first generation. First generation has a shorter duration of action compared to the second generation. Commonly used first generation drugs are tolazamide, tolbutamide, acetohexamide, and chlorpropamide. Commonly used second generation drugs are glibenclamide glimepiride, glipizide and gliclazide. They can be used as monotherapy or in combination with other antidiabetic drugs. Average reduction of HbA1c level by sulphonylueras is 1.50 to 2.0%. Drug interactions occur when given with drugs such as salicylates, warfarin or sulphonamides. Hypoglycaemia and weight gain are the most common side effects associated with sulphonylureas administration (Powers, 2015b; Triplitt et al., 2014; Sumrall et al., 2009).

Alpha glucosidase inhibitors

They do not have a direct effect on insulin sensitivity or insulin secretion but they act by competitively inhibiting α-glucosidase enzymes in the intestine. The inhibition causes a reduction in postmeal glucose excursions by slowing the digestion and absorption of starch in the small intestine. Acarbose is the most commonly used drug in this class. It inhibits carbohydrate digestion and lowers the postprandial glucose concentration by 30 to 50%. These drugs are useful as monotherapy in early stages of

impaired glucose tolerance but can also be used in combination with other antidiabetic drugs. These drugs reduce HbA1C level by 0.5 to 1.0%. The major side effects associated with this class of drugs are abdominal pain, flatulence and diarrhoea. Hypoglycaemia is observed when they are used in combination with sulphonylureas or insulin (Sumrall et al., 2009; Kennedy et al., 2015).

Thiazolidinediones (TZDs)

Pioglitazone and rosiglitazone are the two drugs currently used for diabetes mellitus Type 2. TZDs are referred as glitazones. They work by binding to the peroxisome proliferator activated receptor gamma (PPAR-γ), a type of nuclear regulatory protein found in fat, muscle and liver. These PPAR-γ receptors are involved in modulating the expression of the genes regulating insulin signal transduction and glucose and lipid metabolism. TZDs are used to improve insulin sensitivity, reduce cellular resistance and improve glycaemic control based on the adequate production of insulin by pancreatic beta cells. TZDs can be used as monotherapy or in combination with other oral antidiabetic medications. TZDs reduce HbA1C level by 1.5 to 2.0%. The use of this class of drugs has been reduced due to safety concern and side effect shown by multiple retrospective studies. The result from forty two randomized clinical trials suggested an increased risk of myocardial infarction or angina pectoris associated with rosiglitazone administration. Some side effects associated with these drugs are fluid retention, heart failure, hypoglycaemia and liver injury. Combination of metformin with TZDs has the advantage of not causing hypoglycaemia (Sumrall et al., 2009; Kennedy et al., 2015).

Meglitinides

They are non-sulphonylurea insulin secretagogues often called short acting secretagogues and are rapidely absorbed. The commonly known drugs under this class are vateglinide and repaglinide. These drugs work by stimulating insulin secretion through interaction with potassium channels on pancreatic beta cells to boost insulin response after meal. These medications are given orally just before meals to compensate for the defect in insulin response after meal consumption and if a meal is skipped, the drugs should also be skipped. Repaglinide has a faster onset of action and more appropriate for controlling postprandial glucose excursion. Meglitinides reduce the HbA1c level by 0.5 to 1.0%. Both drugs can cause hypoglycaemia and weight gain as side effects but the risk of hypoglycamia is less compared to sulphonylureas (Triplitt et al., 2014).

Incretin mimetics

Incretins are gut hormones released to intensify the glucose induced insulin secretion. Glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) are the major incretins involved in increased secretion of insulin. Diabetes mellitus Type 2 is more sensitive to GLP-1 compared to GIP because of the deficiency in GLP-1 levels in Type 2 whereas GIP levels are usually normal or high. GLP-1 infusion in diabetes Type 2 causes stimulation of insulin release and reduction of glucose concentrations. All GLP-1 receptor agonists can increase the risk of pancreatitis and patients are advised to seek medical care in case of severe abdominal pain after administration. GLP-1 agonists are not recommended to be used in patients

with family history or past medical history of Type 2 multiple endocrine neoplasia syndrome or medullar thyroid cancer (Triplitt et al., 2014; Sumarall et al., 2009; Kennedy et al., 2015).

Exenatide is a GLP-1 agonist and the first one in this class of drugs to be approved for treatment of diabetes type 2. It is given as a fixed dose by subcutaneous injection within one hour before breakfast and dinner. Exenatide used in mono or combined therapy reduces the HbA1c by 0.2 to 1.2%. The common side effect is nausea and the drug is contraindicated in patient with glucose filtration rate below 30 ml/min (Triplitt et al., 2014; Sumarall et al., 2009; Kennedy et al., 2015).

Pramlintide is a soluble and non-aggregating peptide analogue of human amylin and administrated subcutaneously twice daily in Type 1 and Type 2 diabetes patients unable to reach their targeting postprandial blood glucose concentrations. The drug is given immediately before eating and reduces A1C in diabetes Type 1 by 0.1 to 0.4 % and in Type 2 by 0.3 to 0.7%. The side effects include hypoglycaemia, anorexia, nausea, and vomiting. The drug is contraindicated in patients suffering from gastroparesis (Triplitt et al., 2014; Sumarall et al., 2009; Kennedy et al., 2015).

Liraglutide is a GLP-1 agonist analogue (97% homology to GLP-1) which increased glucose dependent insulin secretion and reduced the high glucagon secretion in presence of increased blood glucose levels. It is administered once daily in diabetes mellitus Type 2 concurrently with metformin, TZDS or sulphonylureas administration. Liraglutide reduces HbA1c level by 0.8 to 1.50% with nausea and

vomiting as the most common side effects (Triplitt et al., 2014; Sumarall et al., 2009; Kennedy et al., 2015).

Dipeptidyl peptidase-4 (DPP-4) inhibitors

They are called incretin enhancers as they increase the blood concentration of GLP-1 and prolong its half-life by preventing the natural breakdown of incretins by DDP-4. The examples of DDP-4 inhibitors approved in the United States are linapliptin, sitagliptin and saxagliptin. The average reduction of the HbA1C level by DPP-4 is around 0.7 to 1%. Incretin enhancers are very well tolerated. They do not increase the risk of hypoglycaemia and do not cause gastrointestinal disturbances (Triplitt et al., 2014; Sumarall et al., 2009).

Bile acid sequestrants

Colesevelam is the only one drug in this class to be approved for treatment of diabetes mellitus Type 2. Colesevelam is a cholesterol lowering medication also used as an antihyperglycemic agent that works by binding to the bile acid in the lumen of the intestine resulting in reduction of the bile acid. Colesevelam reduces the HbA1C by 0.3 to 0.5%. Some side effects occurring with colesevelam administration include constipation, indigestion and dyspepsia. The drug is contraindicated in patients with past medical history of major gastrointestinal surgery and those suffering from gastroparesis and bowel obstruction (Kaku, 2010; Kennedy et al., 2015).

Dopamine agonists

Bromocriptine mesylate is the dopamine agonist in this class currently approved to be used in treatment of diabetes mellitus Type 2. The mechanism of action of bromocriptine is not yet clear but the drug improves the glycaemic control and hepatic insulin sensitivity. The drug reduces the HbA1C level by 0.3 to 0.6% from baseline. The side effects include fatigue, vomiting, nausea, dizziness and headache (Triplitt et al., 2014; Kennedy et al., 2015).

c) **Combined therapy in diabetes mellitus Type 2**

Failure to maintain good glucose concentrations despite monotherapy for long term treatment may require multiple medications to achieve glycaemic control. Diabetic Type 2 treatment usually starts with metformin unless there is a contraindication. In the case of clinical failure with metformin, a second agent is added. Second agents include sulphonylureas, pioglitazone, DDP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors and insulin. Before adding the second agent, considerations are given to efficacy of the agent, risk of hypoglycaemic, weight gain, side effects and cost. Insulin therapy is introduced when oral agents combined with injectable GLP-1 receptor agonists fail to maintain reasonable glucose concentrations. If two agents still can't maintain a good response to therapy, a third agent cannot be added (Kennedy et al., 2015).

2.1.8. Monitoring

The rate of mortality and morbidity related to diabetes mellitus can be significantly reduced by consistent monitoring of the treatment therefore, decreasing the risk of complications associated with diabetes. Patients need to receive a comprehensive education of the condition including nutrition, regular exercise and therapeutic regimen. For optimal diabetic care, continuous self-monitoring of blood glucose levels is required together with a long term glycemic control by a medical practitioner. Blood glucose levels should be perfectly monitored at home in patients receiving more than two doses of insulin per day. Patients who meet treatment targets should have an annual HbA1c test whereas patients whose treatment has changed need the test every 3 to 6 months. Each hospital visit should take into account the weight and blood pressure of patients. An annual examination of potassium, lipids and creatinine must be done. Depending on the dilatation of the pupils, a fundoscopy must be done annually (Standard treatment guidelines, 2014; Powers, 2015b)

2.1.9. Complications

Diabetes mellitus can lead to acute and chronic complications affecting several organ systems. Acute complications of diabetes include hypoglycaemia and hyperosmolar hyperglycaemicnon-ketotic syndrome. Chronic complications related to diabetes are divided into two main groups, nonvascular and vascular complications. The nonvascular complications include conditions such as hearing loss, gastroparesis, infections and skin changes. The vascular complications are divided into microvascular and macrovascular complications. The microvascular complications

result from chronic hyperglycaemia and include neuropathy, retinopathy and nephropathy diseases. Retinopathy may cause blindness while nephropathy may lead to renal failure. Diabetes neuropathies play an important role in mortality and morbidity related to diabetes. Hyperglycaemia also leads to impairment of white blood cell function and complication of wound healing which may result in amputation of the lower limb. Rigorous glycaemic control can reduce the risk of microvascular complications.The macrovascular complications include peripheral vascular disease, atheroma, high blood pressure, coronary artery disease, myocardial infarction, obesity and hyperlipidemia (Triplitt et al., 2014; Powers, 2015b; Standard treatment guidelines , 2012).
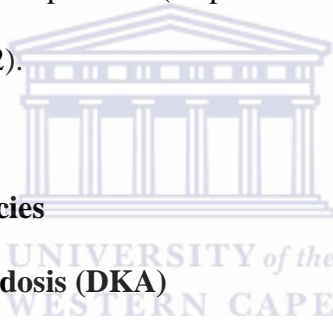
2.1.10. Diabetes emergencies

2.1.10.1. Diabetes ketoacidosis (DKA)

DKA is a diabetic emergency and life threatening condition often precipitated by increased insulin demands. It often occurs in younger people and developed over hours to days. DKA is caused by insulin omission, inadequate administration of insulin and intercurrent diseases such as pancreatitis or myocardial infarction. It usually occurs in diabetes mellitus Type 1 when cells in the body cannot get the glucose they need for energy. In normal situation, cells in the body need glucose to produce the energy they need. Insulin enables glucose (sugar) entry from blood into muscles and other tissues. In case of insulin deficiency, the body is unable to use glucose for energy needed. In order to produce energy, the body starts to breakdown fat and proteins leading to production of ketones and free fatty acids. The produced

ketone and fatty acids enter the bloodstream causing chemical imbalance (metabolic acidosis) called diabetic ketoacidosis. Severe ketoacidosis can cause breathing difficulties, brain swelling, increased risk of coma and even death (Triplitt et al., 2014; Powers, 2015b).

Symptoms

The symptoms of DKA include strong fruity breath odour, blurred vision, drowsiness, confusion, dry skin, loss of appetite and vomiting. These symptoms are often noticed only after a very high level of blood sugar. Patients present with glucose level usually higher than 40 mmol /L, a serum osmolality greater than 350 mosm /L and a positive blood ketones test (Triplitt et al., 2014; Powers, 2015b).

Treatment

The goal treatment is to stabilize the patient at glucose levels around 8.3 to 13.9 mmol /L (150 to 250 mg /dL) and resolved the acidosis state.

- Oral or intravenous fluid replacement to dilute the increased blood glucose and rehydrate the body (too much fluid lost through excessive urination).

- Electrolyte replacement to maintain muscle, nerve and heart. Electrolytes are measured (particularly potassium, bicarbonate and phosphate) every 4 hours for the first 24 hours particularly.

- Intravenous insulin therapy to reverse DKA until the blood becomes non acidic. Insulin is administrated only after potassium level is corrected.

Close monitoring of blood pressure, respiration, pulse, mental state and fluid intake is required every 1 to 4 hours (Triplitt et al., 2014; Powers, 2015b).

2.1.10.2. Hyperosmolar nonketotic diabetic coma (HONK)

Hyperglycaemic hyperosmolar state is a syndrome that usually occurs in elderly people with diabetes mellitus Type 2. It can also appear in younger diabetic patients presenting with serious renal insufficiency or prolonged hyperglycemia and dehydration. The underlying causes are insufficient fluid intake and relative insulin deficiency (Powers, 2015c; Standard treatment guidelines , 2012)

Symptoms

Patients present with impaired consciousness, extreme dehydration and severe hyperglycaemia not accompanied by severe ketoacidosis. The condition develops over days to weeks with blood glucose level greater than 40 mmol/L, serum osmolality higher than 320 mOsm/L and blood ketone test usually negative to slightly elevated. Ketonemia and acidosis are rarely present but a small quantity of anion gap metabolite may be present (Powers, 2015c; Standard treatment guidelines , 2012).

Treatment

Fluid replacement is the first step to stabilize the hemodynamic status and reverse the hyperosmolar state as dehydration and fluid loss are more pronounced compared to DKA. Patient's fluid must be closely monitored and electrolyte replacement is also required. Intravenous insulin therapy is necessary (Powers, 2015c; Standard treatment guidelines , 2012).

2.1.11. Other types of diabetes

2.1.11.1. Gestational diabetes

According to Harrison's principles of internal medicinal, 4% of pregnant women develop gestational diabetes. Glucose tolerance occurring during pregnancy is usually categorized as gestational diabetes whereas insulin resistance is related to the metabolic changes affecting the body during late pregnancy (Powers, 2015a; Barbieri et al., 2015).

In attempts to maintain glucose concentration in normal ranges, the body increases the insulin secretion but increased insulin may lead to impaired glucose tolerance or diabetes. Gestational diabetes increases the risk of preeclampsia, stillbirth and delivery complication due to baby weight. Assessment of risks of developing gestational diabetes should take placeduring the first prenatal visit. However, these risks can be reduced by close monitoring of blood glucose through the whole pregnancy. The diagnosis is done with 75 g of oral glucose tolerance test (OGTT) at 24 to 28 weeks of gestation and applying the following criteria:

- Fasting plasma glucose test greater or at 92 mg /dL (5.1 mmol /L)

- 1 hour plasma glucose test greater or at 180 mg /dL (10 mmol /L)

- 2 hours plasma glucose test greater or at 153 mg /dL (8.5 mmol /L)

Gestational diabetes mellitus is confirmed when patients meet any one of the diagnosis criteria listed above. In the event where diabetes is diagnosed before pregnancy, this is not classified as gestational diabetes but rather as pregnancy with pre-existing diabetes mellitus (Triplitt et al., 2014; Barbieri et al., 2015).

2.1.12. Summary of streptozotocin-induced diabetes

Streptozotocin (STZ) is one of the most prominent cytotoxic glucose analogue used in diabetic research. STZ is an antimicrobial agent with diabetogenic properties and also used as an alkylating agent in chemotheray. The effects of STZ on insulin and blood glucose concentrations reflect its toxic induced abnormalities in beta cell functions. The low affinity of GLUT2 glucose transport in the plasma membrane causes STZ to be selectively accumulated in the pancreatic beta cells resulting in inhibition of insulin secretion (Schein et al., 1967; Rakieten et al., 1963; Bergevin, et al., 1974). In the present study, streptozotocin (STZ) was used to induce diabetes in rats.
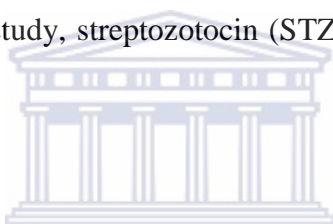
Table 1: Summary of chemical characteristics of streptozotocin (Lenzen et al., 2008)

	Chemical Characteristics of Streptozotocin
Chemical name	2-Deoxy-2-([(methylnitrosoamino)carbonyl]amino)-D-glucopyranose
Chemical structure	It has a cytotoxic methylnitrosourea moiety (*N*methyl-*N*-nitrosourea) attached to the glucose (2 deoxyglucose) molecule; glucosamine derivative (see figure below)
Chemical properties	Hydrophilic, beta cell-toxic glucose analogue. It is relatively stable at pH 7.4 and 37^OC (at least for up to 1 h)
Chemical reactivities	DNA alkylating agent and protein alkylating agent
Toxicity mode	DNA alkylation
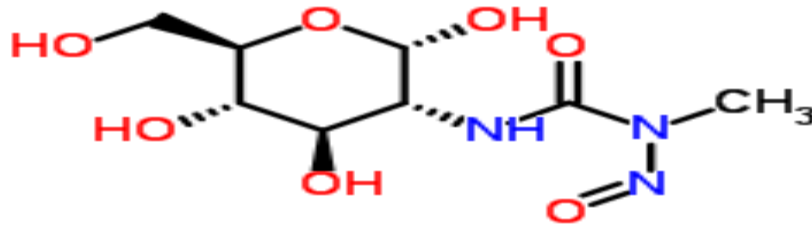
Figure 1: Chemical structure of STZ(Lenzen et al., 2008)

2.2. Overview of nociception and pain

2.2.1. Description

Pain can be described as an unpleasant complex sensation caused by tissue damage that leads to breakdown of cells followed by the release of biochemical substances that provoke autonomic responses. Nociception can be defined as the mechanism by which noxious peripheral stimuli are transmitted to the CNS (central nervous system). Pain is a subjective experience not always associated with nociception and involves strong emotional components whereas nociception is the perception of noxious stimuli (Li et al., 2015; Berry et al., 2009).

Considering the symptoms presented, pain can be classified as acute, chronic and cancer pain. Acute pain is a physiologic process often nociceptive in nature and warning individuals of possible disease or potential harmful situations. If not treated or undertreated, acute pain can develop in to chronic pain development. Chronic pain

is usually a result of changes occurring in the nerve function and transmission therefore, making it difficult to treat. Pain associated with high risk of life threatening conditions is often referred to as malignant pain or as cancer pain in the case of cancer. This type of pain sometimes has several aetiologies and comes from the disease itself. The pain includes components of both chronic and acute pain (Hanley et al., 2007; Latremoliere et al., 2009; Berry et al., 2006)

The nature of pain varies and the amount of pain produced by a particular stimulus depends on several factors than the stimulus itself. Pain arising from joints, connective tissue of the skin, muscles and bones injury (somatic pain) is usually described as aching, sharp, throbbing and well localized. Pain arising from internal organs, gastrointestinal tract, stomach, liver and pancreas (visceral pain) is usually described as dull, cramping, deep, stabbing and sometimes diffuse. Pain arising from damaged peripheral or central nerves is usually described as tingling and burning (Li et al., 2015; Baumann et al., 2014).

Nociception is well explained in terms of transduction, transmission, perception and modulation. Transduction can be summarized as the activation of receptors (known as nociceptors) by conversion of noxious stimuli into electrical activity. Noxious stimuli are activated by mechanical, chemical or thermal impulses. After receptor activation, the action potentials are transmitted along A-δ and C-afferent nerve fibres and this is known as nociceptive transmission. Stimulation of the A-δ afferent nerve fibres conveys well localized pain whereas stimulation of the C-afferent nerve fibre conveys a poor localized pain. The action potentials along afferent nerves are transmitted to

the spinal cord where the pain can be processed further. At that point, the pain becomes a conscious experience taking place in higher cortical components. Physiology around the perception of pain is complex and not well understood but associated with behaviours and cognitive functions. Modulation of pain is done by the body through several complex systems such as endogenous opiate systems (Li et al., 2015; Baumann et al., 2014; Berry et al., 2006).

2.2.2. Pain management

2.2.2.1. Non pharmacological therapy

Some of the non-pharmacological therapies have been found to be useful in acute and chronic pain such as massage, application of cold or heat, relaxation, acupuncture, cognitive behavioural therapy, exercise and physical manipulation. Patient education about expected discomfort after certain medical procedures can reduce patient's distress (Baumann et al., 2014; Chou et al., 2007; Benzon et al 2008; Simpson et al., 2009).

2.2.2.2.Pharmacological therapy (Analgesics)

a) Non-steroidal anti-inflammatory Analgesics/drugs (NSAIDs)

NSAIDs are used for somatic pain and in the treatment of pain due to inflammatory response. Common examples are acetylsalicylic acid (known as aspirin), indomethacin, ibuprofen, diclofenac and naproxen. Aspirin is contra-indicated in children with symptoms of flu. Aspirin used particularly in children presenting with symptoms of flu caused by virus can lead to Reye's syndrome. This syndrome is

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characterized by hepatic failure and encephalopathy (Baumann et al., 2014; Roelofs et al., 2008).

NSAIDs work on central and peripheral systems to relieve pain by inhibiting the prostaglandins synthesis by blocking the activity of cyclooxygenase enzymes. They are more effective in mild to moderate somatic pain compared to visceral pain (Baumann et al., 2014;Li et al ., 2015).

b) Non opioid analgesics

These agents are used in the treatment of mild to moderate somatic pain. Acetaminophen (known as paracetamol), is commonly used as first line treatment. Acetaminophen has both analgesic and antipyretic actions but also produced very little and no significant anti-inflammatory effects. The mechanism of action regarding its analgesic property is not well understood. Paracetamol produces its antipyretic effect by acting as a prostaglandin synthesis inhibitor in the brain with. It has fewer adverse effects when administrated in recommended doses. However, in higher doses it may cause nephrotoxicity, hepatotoxicity and thrombocytopenia. (Baumann et al.,2014; Li et al., 2015).

c) Opioid analgesics

Any substances whether endogenous or synthetic producing morphine like effects and antagonized by narcotic antagonists are referred to opioids. Opioid analgesics include both natural and semi synthetic alkaloid derivatives (from opium) and synthetic forms. They are classified as second line treatment in acute pain, chronic pain and cancer related pain. Opioids are used for pain treatment of any type or duration but

they are most effective in nociceptive pain. There is no pharmacological dose but the dose depends on side effects and patient's response. The side effects can be reversed with administration of narcotic antagonist such as naloxone. Opioids are generally divided into three main groups. The first group includes strong opioid analgesics used for severe painful conditions such as visceral and chronic pain. Common examples of strong opioids are diamorphine (Heroin), morphine, methadone and pethidine. Heroin is not used clinically. The second group includes mild to moderate opioid analgesics used in mild to moderate pain especially when the patient is not responding to paracetamol or aspirin. Some examples of second group are codeine, dextropropoxyphene and dihydrocodeine. The third group includes mixed agonist and antagonist (partial) opioid analgesics used to produce analgesia in severe pain. In this group, the drugs were initially developed as antagonists but when administrated alone, they produced effects similar to morphine effect but with less analgesic potency compared to morphine (Baumann et al., 2014; Li et al., 2015).

2.3. Overview of *Cnicus benedictus*.L

2.3.1. Description of*Cnicus benedictus*.L

Cinicus benedictus, a plant commonly known as Bitter Thistle, Blessed Thistle, Holy Thistle, Spotted Thistle or St. Benedict's Thistle, is a thistle like plant belonging to the family Asteraceae and grows annually (Van Wyk et al., 2004; Llyod et al., 1983). *C.benedictus* usually grows to 30 to 50cm high but can also grow up to 70cm. The plant has a strong bitter taste. The stems are heavily branching and thistle-like, and

the leaves are amplexicaul with irregular teeth having wavy margin ending in spines. The upper leaves are sessile while the lower ones are petioled (Van Wyk et al., 2004; Lloyd et al., 1983; Vogel, 2005)

Figure 2 : Picture of *Cnicus Benedictus***http://www.uniprot.org/taxonomy/50282**

2.3.2. Origin and distribution

*C. benedictus.*L originated in the Mediterranean region and has been introduced in most parts of Europe, South Africa and Central and South America. The word 'thistle' comes from the old nomenclature under which the plant was formerly classified and 'blessed' refers to the healing powers of the plant while 'Cnicus' refers to the plant's thorns and derived from the Greek word, Knizein meaning 'to torment'.

. In South Africa *C.benedictus* is known locally as Karmedick in Afrikaans (Van Wyk et al., 2004; Lloyd et al., 1983; Vogel, 2005).

2.3.3. Uses and properties

C. benedictus is used to treat several ailments such as cancers, gout, diabetes and also applied externally to wounds and ulcers. The plant is mainly used as a bitter tonic and stomachic to treat dyspepsia and lack of appetite. The recorded uses in South Africa include the treatment of internal cancers, diabetes, arthritis and pain (Chevalier, 2000; Schneider et al., 1987; Van Wyk et al., 2004; Bisset et al., 1994; Natural Standard Monograph, 2008)
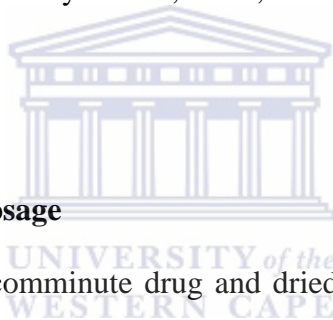
2.3.4. Preparation and dosage

C. benedictus is used as comminute drug and dried extract for infusion as tincture, liquid extract or tea. The tea is prepared by pouring 150 ml boiling water over 1.5 to 2 gm of drug, allowing the drug to set for 5 to 10 minutes. The aromatic bitter dosage is given as 1 cup ½ hour before meals. One cup of tea is taken three times daily (Van Wyk et al., 2004; Chevalier, 2000; Bradley, 1992; Llyod et al., 1983). *C.benedictus* is the component of several herbal teas.

2.3.5. Constituent of the plant

*Cnicus benedictus.*L is mainly composed of the principal bitter constituents, sesquiterpene lactone glycosides of the garmacrane type from which the chief component is cnicin (0.2-0.7%). The constituents listed below also are part of the

plant species and are believed to contribute to the pharmacological activity of the plant. (Van Wyk et al., 2004; Chevalier, 2000)

a) Salonitenolide and artemisiifolin (Van Wyk et al., 2004; Vanhaelen-Fastre, 1974; Chevalier, 2000; Natural Standard Monograph, 2008; Bradley, 1992)

b) Lignans which are known to contribute to the bitter charachteristics of the plants; arctigenin, trachelogenin, nortracheloside, several polyacetylenes and 2-acetyl nortracheloside (Vanhaelen-Fastre, 1972; Van Wyk et al., 2004; Natural Standard Monograph, 2008)

c) Triterpenoids such as α-amyrenone, α-amyrin, acetate,α-amyrin, multiflorenol, multiflorenol acetate and oleanolic acid (Ulbelen et al., 1997).

d) Flavonoids such as apigenin-7-O-glucoside, luteolinl and astragalin. (Ulbelen et al., 1997).

e) Essential/volatile oils (0.3 %) such as p-cymene, citronellol, cuminal, fenchon, cinnamaldehyde and benzaldehyde) (Van Wyk et al., 2004; Chevalier, 2000; Natural Standard Monograph, 2008; Vanhaelen-Fastre, 1973).

f) Tannins (8%); phytosterols such as n-nanocosan,stigmasterol. (Van Wyk et al., 2004;).

2.3.6. Pharmacological effects

C C.benedictus is known to have pharmacological activities such as antimicrobial, anti-inflammatory and antiproliferative effects proven with animal studies but no clinical outcomes have been published. The antimicrobial activity was proven against

bacillus subtilis, brucella species, escherichia coli, proteus species, streptococcus faecalis, staphylococcus aureus and *pseudomonas aeruginosa* due to the presence of sesquiterpene lactone cnicin. (Vanhaelen-Fastre, 1968; Mascolo et al., 1987; Ryu et al., 1996; Vanhaelen-Fastre, 1972).

In animal tests, cnicin shows some cytotoxicity in cell cultures and antitumor activity in mice. Use as an aromatic bitter, the plant is believed to increase the secretion of gastric juice and saliva and therefore increases appetite (Vanhaelen-Fastre, 1968; Van Wyk et al., 2004).

2.3.7. Contra-indications

C. benedictus is contraindicated during pregnancy due to its potential emmenagogue and abortifacient properties (Chevalier, 2000; Natural Standard Monograph, 2008). The plant is used traditionally to stimulate lactation but currently, this use is no longer recommended (Natural Standard Monograph, 2008). The plant is believed to increase GIT secretions and therefore, should be used with caution in cases of peptic ulcer (Chevalier, 2000; Natural Standard Monograph, 2008). The toxicity studies show that the plant given in large doses greater than 5 g per cup of tea may cause vomiting (Chevalier, 2000; Vogel, 2005; Natural Standard Monograph, 2008).

2.4. Description and objectives of the project.

Several approved orthodox medicines around the world have been used to manage and treat different kind of ailments. However, various medicinal plants have also been used by traditional practitioners to provide remedies for these ailments especially in rural communities in developing countries. Therefore, the need arises

for the use of alternate medicines which can be easily available, accessible, cheap and relatively safe. Traditional medicines viz-a viz plant medicines have in the past been the major source of drug development. Plant medicines now have wide usage worldwide especially in rural communities of developing countries. The major problem often associated with this type of medicine is that of lack of scientific proof of their effectiveness in therapy. This, therefore, motivated the present study on the antidiabetic and antinociceptive activities of *cnicus benedictus* L. the plant species is used by traditional medicine practitioners to treat cancers, arthritis, diabetes, ulcer, pain and so on. It has been more than 150 years that *Cnicus benedictus* was introduced to South Africa. Since then, the plant has become a weed in the Cape and on the Highveld. However, there is little or no scientific evidence in literature to corroborate the claims made by traditional practitioners regarding the therapeutic successes of the plant species in the treatment of various ailments particularly diabetes and headache. Diabetes and painful conditions such as headache have been well managed with orthodox medicines as mentioned above. However, evidences abound to show that these medicines have problems of side effects which may encourage non-compliance by some patients therefore, worsening their conditions. Secondly, there have been instances of treatment failure with some orthodox medicines.

Therefore, the present study intended to

- Verify and validate mainly the antidiabetic activity of *C.benedictus*.

- Verify the antinociceptive activity

- Investigate the acute toxicity in order to determine the safety profile of *C.benedictus*.

- Carry out HPLC study in an attempt to characterize the plant species.

CHAPTER 3

MATERIALS AND METHODS

3.1. Plant materials

3.1.1. Selection, collection and identification of plant material

Cnicus benedictus.L was selected based on therapeutic claims made by traditional healers of its useful antidiabetic activities. The plant was bought from Montague museum, Cape Town, South Africa. The plant species was authenticated by a taxonomist in the Department of Biodiversity and Conservative Biology, University of the Western Cape. Voucher specimen of the plant (CNI) was deposited in the Herbarium of the University of the Western Cape.
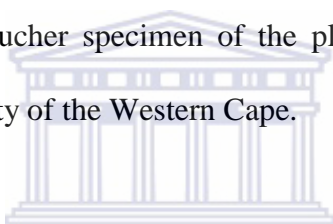
3.1.2. Preparation of leaf methanol extract of *Cnicus benedictus*.L

Fresh leaves of the plant species (5.86kg) were washed with water and sliced into pieces before been dried in a ventilated oven at 35°C for 48 h. The dried plant material (3.44 kg) was ground into fine powder using a Warning Commercial Laboratory blender and a yield of 705 g was obtained. In order to prepare the methanol extract, 90 g of the dried powder was extracted in a soxhlet extractor with methanol as a solvent for 36 h. The methanol filtrate was evaporated to dryness using a Buchi RE IIrotavapor and Buchi 461 water bath. A yield of 11.4 g of crude methanol extract was obtained and preserved in a desiccator until further use. On each day of the experiment, fresh solution of the crude leaf methanol extract was prepared by dissolving weighed quantity of the methanol extract in a small volume of

dimethyl sulfoxide (DMSO) and made up to the appropriate volume with physiological saline. Methanol extract solution was administered intraperitoneally (i.p.) to mice and rats in a volume of 1 ml/100 g of body weight.

3.2. Experimental animals

Male and female albino mice weighing 10-30 g were used for the acute toxicity studies and antinociceptive evaluation of the leaf methanol plant extract. Healthy young adult Wistar rats of both sexes weighing 200-300 g were used for the assessment of the antidiabetic activity. All animals were bred in the Animal House of the Discipline of Pharmacology, University of the Western Cape, South Africa and kept in perspex transparent mouse or rate cages. They had access to food (standard pellet diet) and water ad libitum. The animals were housed in a quiet laboratory with an ambient temperature of 22 ± 2 °C and a 12 h light/12 h dark cycle was maintained. Animals were fasted for 16 h but still allowed free access to water prior to the commencement of the experiments and each animal was used for one experiment only.

3.3. Drugs and chemicals

Streptozotocin (STZ, Sigma Chemical Co) was dissolved in a small volume of sterile citrate buffer (pH 4.6, 0.1M) and made up to the appropriate volume with physiological saline.

Chlorpropamide (Sigma Co.) was dissolved in physiological saline to an appropriate volume and given orally to rats using a bulbed steel needle.

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Indomethacin (Sigma Chemical Co) was dissolved in a minimum volume of dimethylsulfoxide (DMSO, Sigma Chemical Co.) and adjusted to the appropriate volume with physiological saline.

Paracetamol (4-Acetamidophenol, Sigma Chemical Co.) was dissolved in a minimum amount of propylene glycol and made up to an appropriate volume with physiological saline.

Morphine sulfate (Bodene) was dissolved in physiological saline to the appropriate volume.

Acetic acid (Merck) was dissolved in physiological saline to an appropriate strength.

DMSO solution was prepared by dissolving an equal amount of DMSO used to dissolve the plant extract, in an appropriate volume of physiological saline.

STZ, indomethacin, paracetamol, morphine, acetic acid and DMSO were administered intraperitoneally (i.p) to the animals. Acetic acid was administrated in a volume of 0.25 ml to mice. Fresh drug solutions were prepared each morning of the experiment. All drugs were administered in a volume of 1 ml/100 g of body weight of animals while constant volumes of DMSO, physiological saline and acetic acid were used.

In each set of experiments, the control animals received equal volume injections of the appropriate vehicles. The doses and pre-treatment times of the leaf methanol extract of *C.benedictus,* and the standard drugs and the vehicles used were obtained from preliminary studies conducted in our laboratory; and also from similar studies (Amabeoku et al, 2011)

3.4. Acute toxicity

The method described by Lorke (1983) and modified by El Hilaly et al. (2004) was used to determine the median lethal dose (LD50) of leaf methanol extract of *Cnicus benedictus*. Mice used were fasted for 16 hours prior to the commencement of the experiment and randomly divided into groups of 4 per cage. Graded doses of the plant extract in ascending order (100, 200, 400,600, 800, 1600, 2000, 2400, 2800, 3200, 3600 and 4000 mg/kg) were separately administered orally to mice in each test group by means of a bulbed steel needle. Another group of 4 mice used as control, received 0.3 ml of physiological saline orally each using a bulbed steel needle. The mice in both the test and control groups were then allowed free access to food and water and observed over a period of 5 days for death or acute toxicity symptoms. The median lethal dose (LD50) of leaf methanol extract of *C.benedictus* would be determined from the log dose-response plots constructed for the extract.

3.5.High Performance Liquid Chromatography (HPLC) analysis

Chromatographic system: Agilent 1200 system consisting of degassing system, quaternary pump, auto loading sampler, thermos statted column compartment, diode array detector, fluorescence detector, analyte fraction collector and Agilent ChemStation software ; column: Phenomenex Luna (C18) 5μm and dimensions (250 cm x 4.6 mm).

Chromatographic conditions: Mobile phase degassed with helium, solvent A: water containing 0.1% formic acid; solvent B: Acetonitrile containing 0.1% formic acid, Mode: flow rate, 0.8 ml/min; injection volume, 50 μl, detector, UV at 370 nm. The

eluent was monitored at several wavelengths ranged from 210 to 370 nm, the specific wavelength of interest being 350 nm. The HPLC operating conditions were programmed to give the following: 0 min, solvent B: 18%; 15 min, solvent B: 25%; 20 min, solvent B: 35%, 30 min, solvent B: 90%. The run rate was 30 min.

3.6. Pharmacological assessment

## 3.6.1. Assessment of antidiabetic activity

Modified method of Williamson et al. (1996) and Joy and Kuttara (1999) were used to assess antidiabetic activity in normoglycaemic rats and STZ-diabetic rats.

3.6.1.1.Normoglycaemic rats

The rats were randomly divided into seven groups of 6 each. The control group of fasted rats was treated with 0.3 ml of physiological saline administered orally. Four test groups of fasted rats were treated with intraperitoneal injections of leaf methanol extract of the plant species; one dose per group (50,100, 200, 400 mg/kg). The reference group of fasted rats was treated with chlorpropamide 250 mg/kg administered orally. The last group of fasted rats was treated with DMSO (0.25ml,i.p). The collection of blood samples was done from the tail vein of animals and measured by means of a glucometer. The blood glucose concentrations of normoglycaemic rats at the end of the 16 h fasting period were measured and considered as zero time (0 h) which was the time before treatment. After treatment, the blood glucose concentrations in each group were measured and recorded every 30 min over 4 hours.

3.6.1.2. Induction of diabetes mellitus

The modified method of Williamson et al. (1996) was used to induce diabetes in rats. A group of 6 rats were used in this experiment. A single dose of streptozotocin (STZ, 80 mg/kg) in sterile citrate buffer was administrated to fasted rats by intraperitoneal injections. Following the injection, diabetes was allowed to develop and stabilize in rats over a period of 5 to 7 days. Then, plasma glucose was measured to confirm the diabetic state. The blood glucose measurement was obtained by pricking of the rat tail tip vein. A drop of blood was placed on a glucose test strip and the glucose concentrations were determined by using a compatible Glucometer (Accu-Check®Abbot laboratory). Each glucose test strip was used only for one reading. Fasted STZ-rats with blood glucose concentrations greater than or equal to 18 mmol/l were considered diabetic.
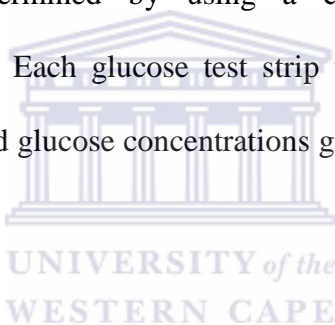
3.6.1.3. STZ-treated rats

The rats were randomly divided into eight groups of 6 each, one group of normoglycaemic rats untreated, one group of rats treated with STZ without the plant extract, four test groups of STZ-diabetic rats treated with intraperitoneal injections of leaf methanol extract of the plant species; one dose per group (50,100, 200, 400 mg/kg), one reference group of STZ-diabetic rats treated with 250 mg/kg of chlorpropamide administered orally and the last group of STZ-diabetic rats treated with 0.25 ml of DMSO administered intraperitoneally. The collection of blood samples was done from the tail vein of animals and measured by means of a glucometer. The blood glucose concentrations of normoglycaemic rats before

induction of diabetes were measured and considered as zero time (0 h) which was the time before treatment. After treatment, the blood glucose concentrations in each group were measured and recorded every 30 min for over 4 hours. The ability of the leaf methanol extract of *Cnicus benedictus* to significantly lower the blood glucose concentrations below 18 mmol/l was taken as an antidiabetic activity in this study (Williamson et al., 1996; Joy and kuttan, 1999).

3.6.1.4. Oral glucose tolerance tests (OGTT)

Modified method of Williamson *et* al. (1996)was used. Rats were randomly divided into seven groups of 6 each. All animals were fasted for 16 hours prior to the commencement of the experiments. One dose of glucose load (1.4 g/kg) was administered orally to fasted rats by means of a bulbed steel tube. The control group of fasted rats was treated with oral administration of glucose load followed by administration of 0.3 ml (i.p.) of physiological saline. Four test groups of fasted rats were treated with oral administration of glucose load followed by administration of leaf methanol plant extract, one dose per group (50,100, 200, 400 mg/kg, i.p.). The reference group of fasted rats was treated with oral glucose load followed by administration of 250 mg/kg (i.p.) of chlorpropamide. The last group of fasted rats was treated with oral glucose load followed by administration of 0.25 ml (i.p.) of DMSO. The collection of blood samples was done from the tail vein of animals and measured by means of a glucometer. The blood glucose concentrations of normoglycaemic rats at the end of the 16 h fasting period were measured and considered as zero time (0 h) which was the time before treatment. After treatment,

the blood glucose concentrations in each group were measured and recorded every 30 min for over 4 hours.

3.6.2. Assessment of the antinociceptive activity

3.6.2.1. Acetic acid writhing test

Acetic acid writhing test was used to assess the antinociceptive activity of *C. benedictus*. Mice were used in groups of eight per dose of plant extract, standard drugs, physiological saline or DMSO. The animals were kept individually in transparent perspex mouse cages before the commencement of the experiment. Control mice were pre-treated with physiological saline and for 15 min, after which each mouse was injected with 0.25 ml (i.p.) of 3 % acetic acid. The mice were left for 5 min, and the writhes were counted for 30 min. The experiment was repeated using other groups of animals which were pre-treated for 15 min with graded doses of plant extract (i.p.), paracetamol (i.p.) or DMSO (i.p.) and for 30 min with indomethacin (i.p.) prior to injecting them with 0.25 ml (i.p.) of 3 % acetic acid. The experiment was also done using combined treatment of the lowest and sub-effective doses of the plant extract and indomethacin. The ability of the plant extract to significantly reduce the number of acetic acid-induced writhes was taken as antinociceptive activity (Garcia et al., 2004; Williamson et al., 1996; Koster et al., 1959).

3.6.2.2. Hot-plate test

Hot plate test was also used to assess the antinociceptive activity of *C. benedictus*. Mice were used in groups of eight per dose of plant extract, physiological saline,

morphine or DMSO. The animals were kept individually in transparent perspex mouse cages before the commencement of the experiment. Control mice were pre-treated with physiological saline (i.p.) and after 15 min, each mouse was placed in an analgysiometer (TIIC, USA) maintained at 55°C. The pain threshold is considered to be reached when the animals lift and/or lick their hind paws or attempt to jump out of the animal enclosure. The time taken for animals to exhibit these characteristics, known as the reaction time, was noted by means of the 'STOP' button on the analgysiometer. The animals were tested before and then 15 min, 30 min, 45 min and 60 min after administration of physiological saline (i.p.). The experiment was repeated using other groups of animals which were tested before and then 15 min, 30 min, 45 min and 60 min after the intraperitoneal administration of graded doses of plant extract, morphine or DMSO. The ability of the plant extract to delay the reaction time was taken as antinociceptive activity (Williamson et al., 1996; Eddy et al., 1953).

3.7. Statistical analysis

The data obtained from the antinociceptive activity and antidiabetic activity as well as oral glucose tolerance tests were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (GraphPad Prism, version 5.0, GraphPad software, Inc., San Diego CA p2130, USA) and presented as mean ± Standard Error Mean (SEM). To determine the effects of leaf methanol extract of *Cnicus benedictus*, data were presented separately for the non-diabetic and STZ-

diabetic rats. Data for control non-diabetic and STZ-diabetic were used as baseline. P value less than 5% (P<0.05) was considered to be statistically significant.

3.8. Ethical considerations

The ethics committee of the University of the Western Cape, South Africa approved the experimental protocol (15/6/96) used in the present study and this conforms to the "Guide to the care and use of animals in research and teaching" of the University.

CHAPTER 4

RESULTS

4.1 Acute toxicity

Oral administration of leaf methanol extract of *Cnicus benedictus* given to mice at graded doses (200, 400, 800, 1600, 2000, 2400, 2800 and 3200 mg/kg) did not cause any death or acute toxicity symptoms in the animals. However, relatively high doses of methanol plant extract of *C.benedictus* (3600 and 4000 mg/kg, p.o.) tested caused 1 and 2 deaths respectively. The no-adverse-effect-level (NOAEL) was obtained at 3200 mg/kg (p.o.). The LD50 value for the plant species was found to be 4000 mg/kg (p.o.)
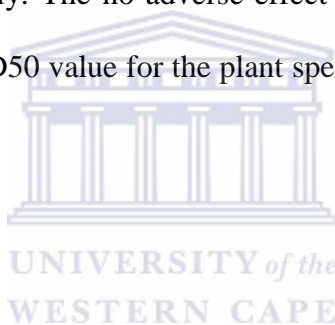
4.2HPLC analysis

The HPLC fingerprint of the leaf methanol extract of *Cnicus benedictus* showed distinct peaks at the following retention times, 6.387, 14.628, 18.431, 23.228 and 29.829 minutes (Figure 3).
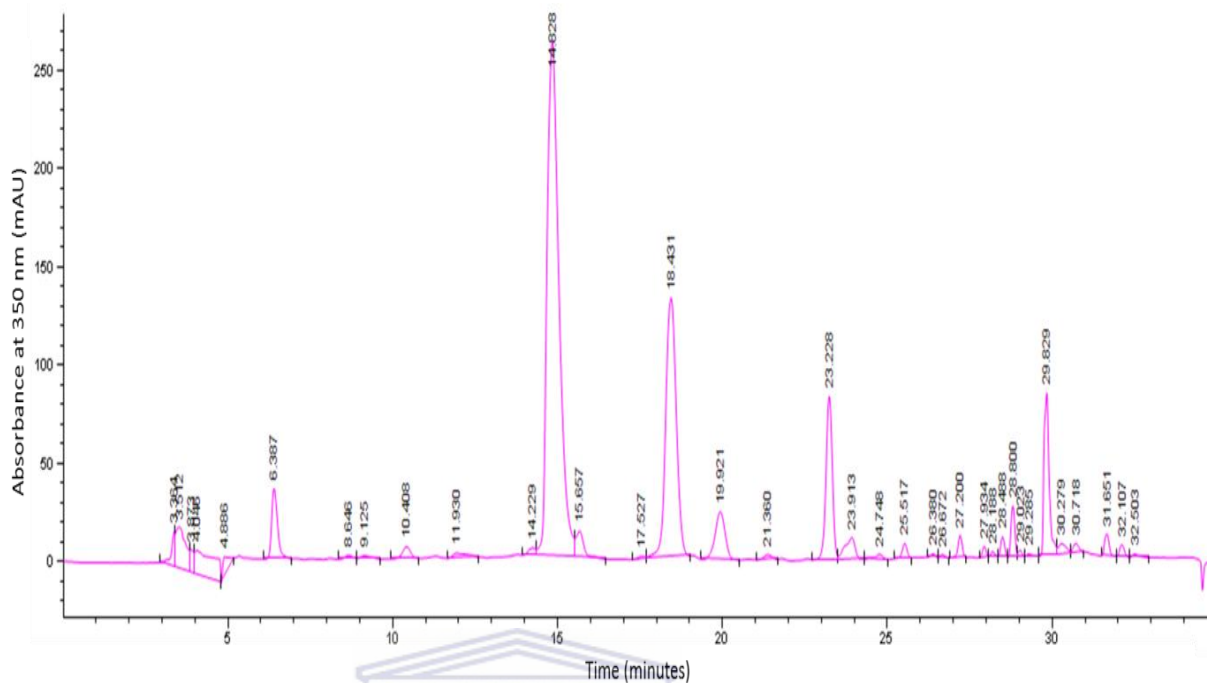
Figure 3 : HPLC fingerprint of methanol extract of *Cnicus benedictus*.

4.3 Effect of leaf methanol extract of *Cnicus benedictus* on blood glucose concentrations (mmol/l) of normoglycaemic rats (Table 3).

Tablet3 shows effect of the plant extract on blood glucose levels of non-diabetic rats. All experiments were done on fasted normal rats.

The blood glucose concentration before the administration of physiological saline was 6.44 ± 0.26 mmol/L. Administration of 0.3 ml (i.p.) of physiological saline did not significantly affect the blood glucose concentrations which ranging from 6.25 ± 0.27 mmol/L to 6.52 ± 0.18 mmol/L over the 4 hr period of observation.

C.benedictus (50 mg/kg, i.p.) did not significantly affect the blood glucose concentrations over the 4 hr of observation compared to the blood glucose

concentration before the administration of the plant extract. The percentage maximum reduction in blood glucose concentration after 4 hr following the administration of 50 mg/kg of plant extract was 11%.

Leaf methanol extract of *C.benedictus* (100 mg/kg i.p.) significantly reduced the blood glucose concentration after 2 ½ hr to 4 hr following the plant extract administration. The blood glucose concentration decreased from 6.08 ± 0.36 mmol/L before treatment to 3.30 ± 0.42 mmol/L, 4 hr after treatment with *C.benedictus* (100 mg/kg, i.p.). The percentage maximum reduction in blood glucose concentrations after 4 hr of plant extract administration was 46%.

Leaf methanol extract of C,benedictus (200 mg/kg, i.p.) significantly reduced the blood glucose concentration after 1 ½hr to 4 hr following the plant extract administration. The blood glucose concentration decreased from 6.00 ± 0.16 mmol/L before treatment to 1.81 ± 0.11 mmol/L, 4 hr after treatment with the plant extract. The percentage maximum reduction in blood glucose concentrations after 4 hr of the plant extract administration was 70%.

400 mg/kg (i.p.) of leaf methanol extract of *C.benedictus* reduced the blood glucose concentration to 1.30 ± 0.22 mmol/L after 4 hr of administration compared to the blood glucose concentration of 6.10 ± 0.26 mmol/L before treatment with the plant extract. The percentage maximum reduction in blood glucose concentrations after 4 hr of the plant extract administration was 79%.

Administration of 250 mg/kg (i.p.) of chlorpropamide (standard drug) (Ojewole, 2006) significantly reduced the blood glucose concentration after 30 min to 4 h of administration. The bloog glucose concentration decreased from 6.30 ± 0.30 mmol/L before treatment to 1.00 ± 0.77 mmol/L, 4 h after chlorpropramide (250 mg/kg, i.p.) administration. The percentage maximum reduction after 4 h of chlorpropramide administration was 84%.

Administration of 0.25 ml (i.p.) of DMSO did not significantly alter the blood glucose concentrations of the normoglycaemic rats during the 4 h period of observation (Table 3).

4.4 Effect of leaf methanol extract of *Cnicus benedictus* on blood glucose concentrations (mmol/l) of streptozotocin-treated (diabetic) rats (Table 4).

Table 4 shows effect of the plant extract on blood glucose levels of diabetic rats. All experiments were done on fasted diabetic rats except the first row where the blood glucose levels were taken from untreated fasted normoglycaemic rats.

The blood glucose concentrations (mmol/L) of untreated rats were not significantly altered during the 4 h period of observation. The blood glucose concentrations of untreated rats ranging from 4.78 ± 0.19 mmol/L to 4.75 ± 0.22 mmol/L over the 4 h period of administration.

Streptozotocin (80 mg/kg, i.p.) significantly increased the blood glucose concentrations from pre-treatment value of 4.94 ± 0.29 mmol/L to 25.47 ± 1.68 mmol/L after 30 min and 24.47 ± 1.17 mmol/L after 4 h of drug administration.

C.benedictus (50 mg/kg, i.p.) did not significantly alter the blood glucose concentrations over the 4 hr of observation compared to the blood glucose concentration before the administration of the plant extract. The percentage maximum reduction in blood glucose concentration after 4 hr following the administration of 50 mg/kg of plant extract was 10.51%.

Leaf methanol extract of *Cnicus benedictus* (100 mg/kg, i.p.) significantly reduced the blood glucose concentrations after 2h30 (17.47± 1.63 mmol/L) up to 4 h (13.80± 1.42 mmol/L) following its administration. The percentage maximum reduction in blood glucose concentration after 4 h of the plant extract administration was 44.82%.

Administration of 200 mg/kg (i.p.) of leaf methanol extract of the plant species significantly reduced the blood glucose concentration after 2 h (16.53± 1.42 mmol/L) up to 4 h (10.43± 1.08 mmol/L) following its administration. The percentage maximum reduction in blood glucose concentration after 4 h of plant administration was 60.04%.

Leaf methanol extract of *C.benedictus* (400 mg/kg, i.p.) significantly reduced the blood glucose concentration after 1 h (16.30± 1.34 mmol/L) up to 4 h (9.10± 1.52 mmol/L) following its administration. The percentage maximum reduction in blood glucose concentrations after 4 h of plant administration was 66.04%.

Chlorphenamine (250 mg/kg,i.p.) (Ojewole, 2006) significantly reduced the blood glucose concentration after 1 h (15.17± 1.64 mmol/L) up to 4 h (7.57± 0.23 mmol/L)

following its administration. The percentage maximum reduction in blood glucose concentration after 4 h of plant administration was 71.71%.

Administration of 0.25 ml (i.p.) of DMSO did not significantly alter the blood glucose concentrations of the streptozotocin-treated rats during the 4 h period of observation (Table 4).

4.5 Effect of leaf methanol extract of *Cnicus benedictus* on oral glucose tolerance test in normoglycaemic rats (Table 5).

Table 5 shows the effect of the plant extract on blood glucose levels of non-diabetic rats after oral glucose load of 1.4 g/kg. All experiments were done on fasted normal rats. An increase in blood glucose concentration was observed in all animals at 30min after oral administration of glucose load.

0.3 ml of physiological saline did not significantly reduce the rise in blood glucose levels during the 4 h period of observation. The same observation was made with 50 mg/kg (i.p.) of the plant extract. 50 mg/kg (i.p.) of methanol plant extract given following the oral glucose load did not significantly reduced the rise in blood glucose levels over the 4 h of observation.

However, 100 and 200 mg/kg (i.p.) of methanol plant extract given following the oral glucose load significantly reduced the increased blood glucose levels. 100 mg/kg (i.p.) of methanol plant extract reduced the increased blood glucose concentrations from the second to the fourth hour of observation with a percentage maximum reduction of 42.45%. 200 mg/kg (i.p.) of methanol plant extract reduced the blood

glucose concentration from 1h 30 min to the fourth hour of observation period with a percentage maximum reduction of 66.20%.

Administration of 400 mg/kg (i.p.) of methanol plant extract following oral glucose load significantly reduced the rise in blood glucose levels from 7.83±0.36 to 3.17±0.33 after 1h30 with a percentage maximum reduction of 70.75%.

Administration of chlorpropamide 250 mg/kg (i.p.) (Ojewole, 2006) following oral glucose load significantly reduced the increased blood glucose concentration from the 1 h through to the fourth hour of observation with a percentage maximal reduction of 79.04%.

Administration of DMSO 0.25 ml (i.p.) did not significantly alter the rise in blood glucose concentration over the 4 h of observation period.
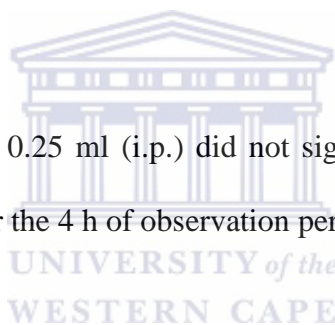
4.6 Effect of leaf methanol extract of *C. benedictus* on acetic acid-induced writhing

Substantial number of writhes was produced by 0.25 ml (i.p.) of 3% acetic acid in mice. Leaf methanol extract of *C. benedictus* (25-400 mg/kg, i.p.) significantly reduced the number of 3% acetic acid-induced writhes in mice. The percentage inhibition of the writhes ranged from 67.95% by 25 mg/kg (i.p.) of the plant extract to 73.71% by *C. benedictus* (400 mg/kg, i.p.). The dose of 12.5 mg/kg (i.p.) of the leaf methanol extract of *C. benedictus* did not affect the number of 3% acetic acid-induced writhes in any significant manner.

Indomethacin (20 mg/kg, i.p.) or paracetamol (500 mg/kg, i.p.) significantly reduced the number of writhes produced by 0.25 ml (i.p.) of 3% acetic acid and the percentage inhibition of the writhes was 75.44 and 6.18% respectively. Indomethacin (10 mg/kg, i.p.) or DMSO (0.25ml, i.p.) did not significantly affect the number of 3% acetic acid-induced writhes.

However, the combined treatment of the lowest and sub-effective doses of leaf methanol extract of *C. benedictus* (12.5 mg/kg, i.p.) and indomethacin (10 mg/kg, i.p.) significantly reduced the number of writhes produced by 3% acetic acid and the percentage inhibition of the writhes was 58.32% (Table6)
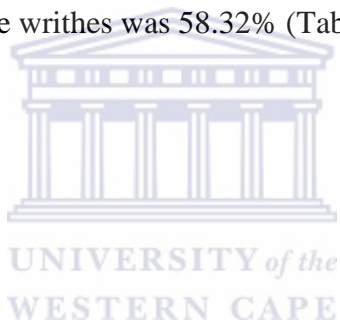
Table 3: Effect of leaf methanol extract of *Cnicus benedictus*.on blood glucose concentration (mmol/L) of normoglycaemic (normal) rats

Treatment groups	Before treatment 0 h	After treatment								Maximal reduction	% Maximal reduction
		30min	1h	1h30	2h	2h30	3h	3h30	4h		
Ps											
0.3 ml	6.44 ±0.26	6.52 ±0.18	6.37 ±0.37	6.30 ±0.29	6.43 ±0.61	6.35 ±0.22	6.29 ±0.17	6.25 ±0.27	6.27 ±0.18	0.19	2.95
Cnicus Benedictus											
50 mg/kg	6.78 ±0.16	7.01 ±025	6.88 ±0.41	6.78 ±0.42	6.76 ±0.42	6.75 ±0.33	6.50 ±0.36	6.43 ±0.35	6.01 ±0.30	0.77	11.36
100 mg/kg	6.08 ±0.36	6.62 ±0.27	6.30 ±0.27	5.93 ±0.28	5.38 ±0.48	4.70 ±0.42*	4.55 ±0.42*	4.43 ±0.40*	3.30 ±0.42*	2.78	45.72
200 mg/kg	6.00 ±0.16	6.83 ±0.33	5.75 ±0.47	4.03 ±0.44*	2.68 ±0.29*	2.18 ±0.23*	1.97 ±0.18*	1.86 ±0.13*	1.81 ±0.11*	4.19	69.83
400 mg/kg	6.10 ±0.26	6.01 ±0.43	5.34 ±0.18	3.12 ±0.25*	2.24 ±0.11*	2.06 ±0.20*	1.82 ±0.35*	1.55 ±0.16*	1.30 ±0.22*	4.8	78.69
Chlorpropamide											
250 mg/kg	6.30 ±0.30	4.43 ±0.40*	3.72 ±0.17*	3.33 ±0.17*	3.03 ±0.21*	3.48 ±0.49*	2.7 ±0.12*	2.3 ±0.10*	1.00 ±0.77*	5.3	84.13
DMSO											
0.25 ml	6.21 ±0.24	6.38 ±0.37	5.98 ±0.50	5.92 ±0.47	6.08 ±0.44	5.95 ±0.47	6.02 ±0.52	6.15 ±0.47	6.12 ±0.45	0.29	4.67

*$p < 0.05$

Values are expressed as mean± SEM. ANOVA (n=6).

PS: Physiological saline

DMSO: Dimethylsulfoxide

Table 4: Effect of leaf methanol extract of *Cnicus benedictus* on blood glucose concentrations (mmol/L) of streptozotocin-treated diabetic rats

Treatment groups	Before treatment	After treatment									Maximal reduction	% Maximal
	0 h	STZ	30min	1h	1h30	2h	2h30	3h	3h30	4h		reduction
Untreated Streptozotocin (STZ)	4.78 ±0.19		4.9 ±0.25	4.1 ±0.42	4.9 ±0.33	4.5 ±0.36	4.88 ±0.24	4.93 ±0.37	4.75 ±0.30	4.75 ±0.22	-	-
80 mg/kg	4.94 ±0.29	25.77 ±1.42	25.47 ±1.68	25.37 ±1.01	25.03 ±1.55	24.38 ±1.28	24.60 ±1.17	24.15 ±1.44	24.55 ±1.76	24.47 ±1.17	1.62	6.29
Cnicus benedictus												
50 mg/kg	4.87 ±0.31	25.78 ±1.39	28.83 ±1.47	28.07 ±1.29	27.95 ±1.10	25.93 ±1.09	26.60 ±1.31	25.70 ±1.17	23.90 ±1.30	23.07 ±1.48	2.71	10.51
100 mg/kg	4.68 ±0.23	25.01 ±1.54	23.48 ±1.31	22.57 ±1.54	20.67 ±1.61	21.22 ±1.28	17.47 ±1.63*	15.62 ±1.55*	14.23 ±1.06*	13.80 ±1.42*	11.21	44.82
200 mg/kg	4.68 ±0.34	26.10 ±1.27	30.30 ±1.88	27.13 ±1.61	24.47 ±1.67	16.53 ±1.42*	14.65 ±1.01*	13.01 ±1.06*	11.90 ±1.45*	10.43 ±1.08*	15.67	60.04
400 mg/kg	4.82 ±0.17	26.80 ±1.32	25.90 ±1.22	16.30 ±1.34*	15.89 ±1.19*	14.78 ±1.22*	13.85 ±1.36*	12.68 ±1.43*	11.11 ±1.21*	09.10 ±1.52*	17.7	66.04
Chlorpropamide												
250 mg/kg	4.97 ±0.26	26.76 ±1.52	28.46 ±1.52	15.17 ±1.64*	13.95 ±1.37*	11.53 ±1.70*	11.85 ±1.77*	10.23 ±1.48*	09.45 ±1.45*	07.57 ±0.23*	19.19	71.71
DMSO 0.25 ml	5.13 ±0.18	24.32 ±1.25	24.32 ±1.25	24.75 ±1.02	24.78 ±1.04	24.65 ±1.20	24.50 ±1.10	24.43 ±1.20	24.10 ±1.14	24.18 ±1.04	0.22	0.90

*$p < 0.05$

Values are expressed as mean± SEM. ANOVA (n=6).

PS: Physiological saline

DMSO: Dimethylsulfoxide

Table 5: Effect of leaf methanol extract of *Cnicus benedictus* on oral glucose tolerance test in normoglycaemic (normal) rats.

Treatment groups	Before treatment 0 h	After treatment								Maximal reduction	% Maximal Reduction
		30min	1h	1h30	2h	2h30	3h	3h30	4h		
Ps 0.3 ml	4.70 ±0.10	6.73 ±0.37	6.08 ±0.22	6.63 ±0.20	6.48 ±0.11	6.38 ±0.22	5.57 ±0.20	5.43 ±0.14	5.55 ±0.23	-	-
Cnicus Benedictus											
50 mg/kg	4.92 ±0.17	9.03 ±0.55	7.32 ±0.67	6.95 ±0.77	6.42 ±0.74	6.28 ±0.68	6.12 ±0.55	5.90 ±0.67	5.40 ±0.59	-	-
100 mg/kg	4.90 ±0.24	7.87 ± 0.76	6.73 ±0.52	6.58 ±0.68	4.08 ±0.52*	3.46 ±0.50*	3.63 ±0.45*	3.12 ±0.58*	2.82 ±0.54*	2.08	42.45
200 mg/kg	5.00 ±0.18	7.58 ±0.61	5.22 ±0.53	3.33 ±0.45*	2.30 ±0.23*	2.05 ±0.25*	1.83 ±0.19*	1.76 ±0.11*	1.69 ±0.09*	3.31	66.2
400 mg/kg	4.65 ±0.11	7.83 ±0.36	6.25 ±0.19	3.17 ±0.33*	3.06 ±0.12*	2.07 ±0.20*	1.63±0.24*	1.58 ±0.31*	1.36 ±0.17*	3.29	70.75
Chlorpropamide											
250 mg/kg	5.01 ±0.08	8.25 ±0.58	3.25 ±0.50*	3.23 ±0.29*	2.55 ±0.18*	1.60 ±0.12*	1.48 ±0.10*	1.23 ±0.17*	1.05 ±0.20*	3.96	79.04
DMSO 0.25 ml	4.90 ±0.06	8.62 ±0.29	7.73 ±0.37	7.97 ±0.43	7.83 ±0.25	7.85 ±0.30	7.30 ±0.39	6.97 ±0.14	6.75 ±0.10	-	-

*p<0.05

Values are expressed as mean± SEM. ANOVA (n=6).

PS: Physiological saline

DMSO: Dimethylsulfoxide

4.7 Effect of leaf methanol extract of *C. benedictus* on hot-plate induced nociception

Mice pre-treated with physiological saline reacted to hot-plate thermal stimulation at 50∘C–55∘C either by lifting and licking their paws or attempting to jump out of the beaker. This manifestation occurred within 5.22± 0.10 sec in the first 15 min and 5.36± 0.77 sec, 60 min later after the intraperitoneal injection of 0.25ml of physiological saline.

Leaf methanol extract of *C. Benedictus* (25–400 mg/kg, i.p.) significantly delayed the reaction times of the animals to hot-plate thermal stimulation 15 min after treatment and over the 1 h period of measurement. Similarly, morphine (10 mg/kg, i.p.) significantly delayed the reaction time of the mice to the hot-plate-induced thermal stimulation over the 1 h period of measurement. Leaf methanol extract of *C. Benedictus* (12.5 mg/kg, i.p.) and DMSO (0.25ml, i.p.) did not significantly alter the reaction time of the mice to the hot-plate-induced thermal stimulation over the 1h period of measurement.
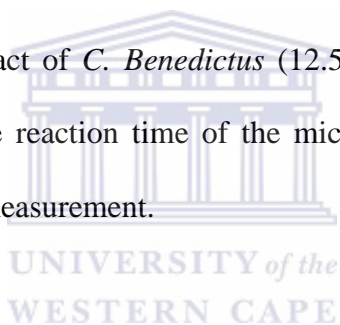
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Table 6: Effect of leaf methanol extract of *C. benedictus* on acetic acid-induced writhing in mice.

Treatment Groups	Dose (mg /kg)	Number of writhes Mean ± SEM		Percentage inhibition (%)
PS		53.67	8.12	-
C.benedictus				
	12.5	46.17	7.34	13.97
	25	17.20*	4.03	67.95
	50	16.31*	3.55	69.61
	100	15.28*	4.46	71.53
	200	15.62*	2.28	70.9
	400	14.11*	1.84	73.71
Indomethacin				
	10	40.39	4.63	24.74
	20	13.18*	1.91	75.44
C.benedictus +indomethacin	12.5 10	22.37*	3.6	58.32
Paracetamol	500	16.54*	1.18	69.18
DMSO	0.25 ml	48.87	9.52	8.94

$*p<0.05$

Values are expressed as mean± SEM. ANOVA (n=6).

PS: Physiological saline

DMSO: Dimethylsulfoxide

Table 7: Effects of leaf methanol extract of *Cnicus benedictus* on hot plate-induced nociception in mice.

Treatment groups	Dose (mg/kg)	Reaction time (s) 0 min	15 min	30 min	45 min	60 min
PS	0.25 ml	5.40 ±0.10	5.22 ±0.64	5.41 ±0.72	6.23 ±0.90	5.36 ±0.77
Cnicus benedictus						
	12.5	5.73 ±0.73	5.92 ±0.25	5.69 ±0.61	5.56 ±0.92	5.88 ±0.34
	25	5.76 ±0.28	12.06 ±0.52*	13.24 ±0.18*	13.04 ±0.25*	12.11 ±0.52*
	50	5.17 ±0.11	12.02 ±0.37*	13.13 ±0.20*	13.36 ±0.10*	12.64 ±0.12*
	100	5.81 ±0.72	13.43 ±0.21*	13.37 ±0.80*	12.54 ±0.18*	11.36 ±0.34*
	200	5.54 ±0.08	13.55 ±0.25*	12.31 ±0.42*	13.66 ±0.28*	12.70 ±0.25*
	400	5.81 ±0.19	13.85 ±0.52*	13.19 ±0.60*	14.22 ±0.87*	12.44 ±0.35*
Morphine	10	5.52 ±0.16	14.11 ±0.44*	14.48 ±0.27*	14.09 ±0.09*	12.85 ±0.30*
DMSO	0.25 ml	5.66 ±0.77	5.72 ±0.29	5.90 ±0.91	5.48 ±0.62	5.39 ±0.37

*$p < 0.05$ compared to physiological saline (0.25 ml, i.p.) control, ANOVA (n = 8).

The reaction time in seconds was expressed as Mean±SEM.

PS: Physiological saline

DMSO: Dimethylsulfoxide

CHAPTER 5

DISCUSSION

Diabetes is a chronic metabolic condition recognized worldwide as an important cause of premature death and disability, especially in the developing world. According to the WHO, the number of adult people suffering from diabetes has almost quadrupled since 1980 mainly due to the increase number of people living with diabetes mellitus type 2 and related factors driving it including obesity and overweight. Additional 2.2 million of deaths are associated with the increase risk of cardiovascular diseases due to the high blood glucose concentrations. The cost of managing diabetes can be catastrophic in poor population. There is an urge to seek other possibilities of managing diabetes in order to reduce the high rate of mortality. In fact, about 80% of the population relies on herbal medicines especially in developing countries due to the low cost and availability of these medicines. The WHO has requested several governments to include herbal medicines with proven efficacy and safety in their healthcare programs (Marini-Bettelo, 1980; Van Wyk et al., 1997; World Health Organization, 2010; International Diabetes Federation, 2013)

One of such herbal medicines which could be included in the South African healthcare program is *Cnicus benedictus* L, commonly called Karmedick in Afrikaans. The plant species is widely used by traditional healer in rural area of South Africa to treat various ailment including diabetes and pain (Van Wyk et al., 1997). In order to scientifically corroborate the claim made by traditional healers of the therapeutic success of this plant species, this present study was undertaken to

investigate the antidiabetic activity of *C.benedictus* in streptozotocin-induced diabetic rats and to verify the antinociceptive activity of the plant extract.

In the present study, no signs of acute toxicity symptoms or death in mice were observed with dose of leaf methanol extract of *C.benedictus* from 50 mg/kg (p.o.) to 3200 mg/kg (p.o.). However, death was observed at the doses of 3600-4000 mg/kg (p.o.). The LD50 value of the plant extract when given orally was found to be 4000 mg/kg suggesting that it may be safe in mice at relatively high doses. It is pertinent to mention that traditional medicine practitioners administer the plant species orally in form of infusion in the treatment of diabetes and other ailments (Van Wyk et al., 1997).

HPLC study was carried out to characterize *C.benedictus* and the HPLC fingerprint obtained in this study revealed characteristic distinct peaks of the plant species at the following retention times (minutes), 6.387, 14.628, 18.431, 23.228 and 29.829.

Hypoglycaemic effect

In this study, physiological saline (0.3 ml, i.p.) did not significantly alter the blood glucose concentrations of fasted normoglycaemic (non-diabetic) rats. Similarly, DMSO (0.25 ml, i.p.) did not significantly produce changes in the blood glucose concentrations of non- diabetic rats. However, leaf methanol extract of *C.benedictus* (100 and 200 mg/kg, i.p.) significantly reduced the blood glucose concentrations of non-diabetic rats. Further significant reduction in blood glucose concentrations was observed with 400 mg/kg (i.p.) of leaf methanol extract. Chlorpropramide (250

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mg/kg, i.p.), a standard oral antidiabetic drug, significantly reduced the blood glucose concentrations of fasted normoglycaemic rats. Chlorpropramide, a first generation sulphonylurea oral antidiabetic or hypoglycaemic drug, is thought to exert its hypoglycaemic effect by stimulating and increasing the release of endogenous insulin from pancreatic beta cells, and also promoting peripheral tissue glucose update and distribution (Schneider et al., 1987). Like chlorpropramide, the leaf methanol extract (100 – 400 mg/kg, i.p.) was shown in this study to exhibit hypoglycaemic effect.

Streptozocin (STZ, 80 mg/kg, i.p.) was used to induce diabetes in rats in this study. According to Ojewole (2006), STZ-induced diabetic rat model is a good experimental protocol for antidiabetic studies especially Type 2 diabetes. STZ is thought to cause diabetes by destroying the pancreatic beta cells thereby reducing the release of insulin which may lead to hyperglycaemia (Schneider et al., 1987). In the present study, leaf methanol extract of C.benedictus (100 – 400 mg/kg ,i.p.) and chlorpropramide (250 mg/kg, i.p.) separately and significantly reduced the blood glucose concentrations of STZ-induced diabetic rats. This supports the assertion that the leaf methanol extract of the plant species has hypoglycaemic effect. Even though the mechanism of the hypoglycaemic effect of *C. benedictus* is not known but since both the leaf methanol extract and chlorpropramide similarly reduced the blood glucose concentrations of STZ-induced diabetic rats, it is possible to suggest that *C. benedictus* maybe producing its hypoglycaemic effect either by promoting peripheral tissue glucose uptake and utilization or either mechanisms that do not involve insulin considering that STZ is known to selectively destroy pancreatic beta cells thus blocking insulin production.

The phytochemical analysis carried out by previous studies revealed that *C.benedictus* is mainly composed of sesquiterpene lactone glycosides from which the principal component is cnicin. Additionally triterpenoids, ligans, tannins and essential oil such as cinnamaldehyde are present amongst other chemical metabolites. The plant species also contained alkaloids, phenolic compounds, saponins, starch and flavonoids such as apigenin-7-0-glucoside (Hule et al., 2011). Several authors in their studies have reported flavonoids, alkaloids and phenolic compounds to be bioactive antidiabetic chemicals (Oliver-Bever, 1986).

An extensive study on flavonoids has demonstrated that flavonoid compounds have antidiabetic activity (Fawzy et al., 2008). Li et al in their study of *Malus toringoides* showed that flavonoids have hypoglycemic activity in experimental diabetic animals (Li et al., 2014). The study done by Rauter et al showed that after 7 days of treatment, apigenin (a flavoinoid) significantly lowered the blood glucose levels of diabetic animals (Rauter et al., 2010). However, flavonoids are not the only phytochemicals with antidiabetic activity. In fact, Junk et al in their study on antidiabetic agents from medicinal plants showed that flavonoids as well as alkaloids exhibit antidiabetic activity (Junk et al., 2006). A study by babu et al., (2007) showed that cinnamaldehyde, a potential antidiabetic agent, given for one month to streptozotocin-induced diabetic rats significantly reduced the plasma glucose concentrations of the animals. Therefore it is possible to speculate that the presence of flavonoids, alkaloids and cinnalaldehyde may be contributing at least in part, to the

antidiabetic activity of *Cnicus benedictus* observed in normoglycaemic rats and streptozotocin-induced diabetic rats in our study.

In the oral glucose tolerance test done in this project, administration of leaf methanol extract of *C. benedictus* following oral glucose load on fasted normoglycaemic rats significantly reduced the increased blood glucose concentrations. Further supports the claim that the plant species has hypoglycaemic effect on normal fasted animals when compared to the results obtained from chlorpropramide the hypoglycaemic reference drug, used.

Analgesic effect

In this study, the leaf methanol extract of *C. benedictus* significantly antagonized the acetic acid induced writhes in mice. Indomethacin or paracetamol also significantly antagonized the writhes produced by acetic acid in mice. Combined sub effective doses of leaf methanol extract of *C.benedictus* and indomethacin significantly antagonized acetic acid induced writhes in mice. According to Satyanarayana et al. (2004), acetic acid may be inducing writhes by stimulating the production of prostaglandins. Indomethacin and paracetamol, the standard analgesic drugs, have been shown to inhibit prostaglandin synthesis peripherally and in the brain respectively (Rang et al., 2012, Flower and Vane, 1972). Therefore, it is not surprising that both indomethacin and paracetamol significantly antagonized writhes produced by acetic acid. Since *C.benedictus* and also the combined treatment of sub-effective doses of *C.benedictus* and indomethacin antagonized writhes produced by

acetic acid, it is possible to suggest that the antinociceptive activity of the plant extract may be underpinned by prostaglandin mechanism(s).

It is also observed in the study that the leaf methanol extract of *C.benedictus* significantly delayed the reaction time of mice to thermal stimulation produced by the hot plate. Morphine, a known standard centrally acting analgesic drug, was shown in this study to significantly delay the reaction time of mice to thermal stimulation produced by the hot-plate. According to Koster et al. (1959), Eddy and Leimback (1953), acetic acid writhing and hot-plate tests are used to assess analgesic drugs with peripheral and central effects respectively. Since *C.benedictus* significantly antagonized writhes produced by acetic acid and thermal stimulation produced by hot-plate, it is possible to suggest that the plant species has both peripheral and central antinociceptive activities.
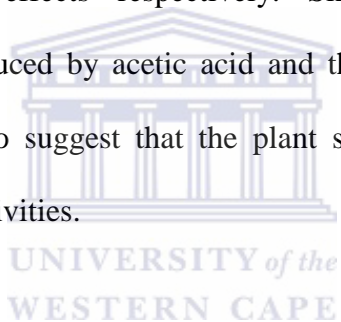
CHAPTER 6

CONCLUSION

The data obtained from this present investigation provide evidence that *Cnicus benedictus* has hypoglycaemic effects on blood glucose concentrations of diabetic rats and may be effective in cases of glucose tolerance impairment. However, it can be speculated that the antidiabetic activity of *C.benedictus* may be due to non-specific mechanism and not due to the stimulation of insulin release from pancreatic beta cells since streptozotocin used in this study is well known to work by depleting the pancreatic beta cells thus reducing the release of insulin from these cells. Also, the synergistic effect of different bioactive chemicals may have a crucial contribution to the potential hypoglycaemic action of the plant species.

Furthermore, the results obtained from this study confirm that *C.benedictus* has antinocieptive activity which may involve prostaglandins mechanism and certain central pain receptors.

The LD50 of the plant revealed that the plant is safe and/ or non-toxic in mice when doses less than 3200 mg/kg of body weight of animals.

All the above data obtained from *C.benedictus* studies indicate that the plant species has both antidiabetic and antinociceptive activities which support or justify the reported folkloric and anecdotal use of *Cnicus benedictus* in the treatment and/ or management of diabetes and pain in some southern Africa regions.

However, we recommend more studies to be done on toxicity of the plant species to further enhance the safety profile of *Cnicus benedictus*. Pharmacological and biochemical studies could be undertaken to elucidate the mechanism of action of the antidiabetic and antinociceptive activities of the plant species.

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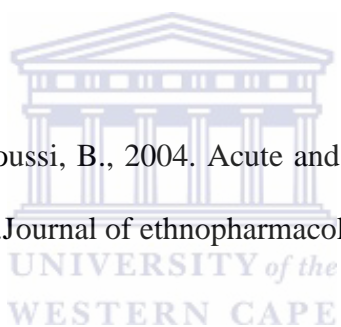
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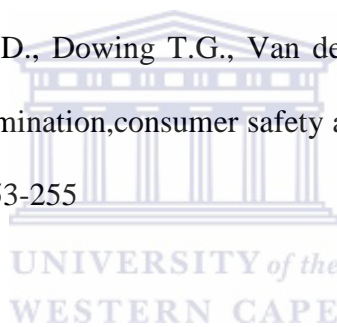
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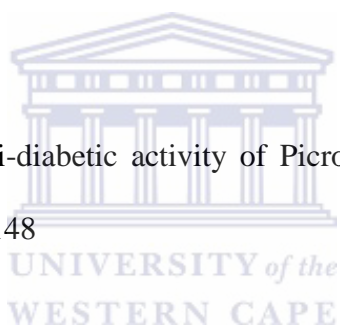

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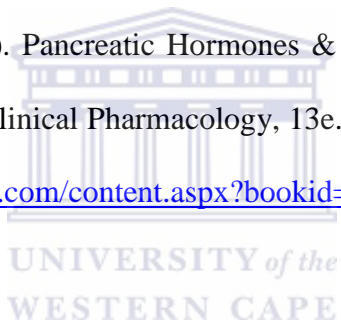
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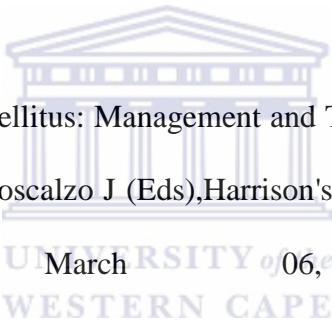
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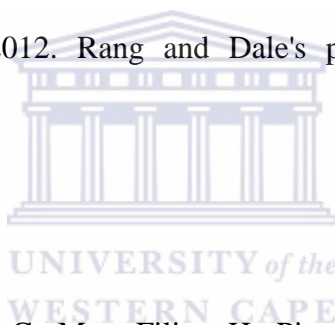
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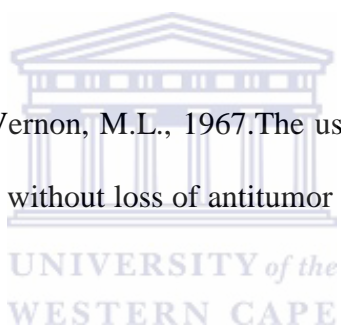
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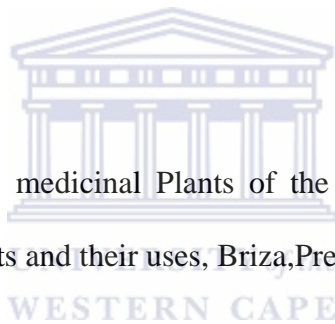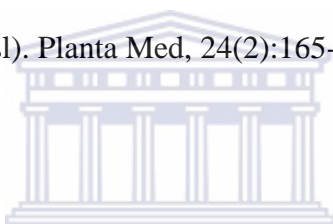
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