PHARMACOLOGICAL EVALUATION OF ANTIDIARRHOEAL AND ANTIDIABETIC ACTIVITIES OF *SYZYGIUM CORDATUM* Hochst. ex C.Krauss.

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DECLARATION

I declare that the thesis, Pharmacological evaluation of antidiarrhoeal and antidiabetic activities of *Syzygium cordatum* 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.

MzonkeDeliwe   May 2011

Signed ……………………………………….

DEDICATION

I dedicate this thesis to my loving wife, Aidy Deliwe and kids Daniel and Justin Deliwe for their sacrifices and undying love, care and encouragement that has got me to where I am today. Thank you for believing in me.
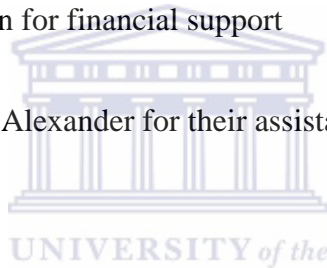
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KEY WORDS

Syzygium cordatum

Myrtaceae

Antidiarrhoeal activity

Antidiabetic activity

Acute toxicity

Aqueous leaf extract

Traditional medicines

Phytochemical analysis

Pharmacological evaluation

Mice

Rats

ABBREVIATIONS

WHO	World Health Organisation
GIT	gastrointestinal tract
CNS	central nervous system
MAOI	monoamine oxidase inhibitors
DM	diabetes mellitus
IDDM	insulin dependent diabetes mellitus
NIDDM	non insulin dependent diabetes mellitus
GDM	gestation diabetes mellitus
DKA	diabetic ketoacidosis
HLA	human leukocyte antigen
HbA1C	glycatedheamoglobin
STZ	streptozotocin
TZD	thialidinediones
mRNA	messenger Ribonucleic acid
GLP-1	Glucagon-like peptide-1
GIP	Gastric inhibitory peptide
PPRE	Peroxysome Proliferator Response Elements
DPP-4	Dipeptidyl peptidase-4
EMEA	European Medicine Agency
FDA	Food and Drug Administration
BMI	body mass index

ABSTRACT

Pharmacological evaluation of antidiarrhoeal and antidiabetic activities of *Syzygium*

cordatum Hochst.exC.Krauss.

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Syzygium cordatum is a medicinal plant indigenous to South Africa and Mozambique,

commonly used to treat stomach aches, diabetes, respiratory problems and tuberculosis. In

spite of the folklore use, adequate scientific data to credit its widespread traditional use is

lacking.

The objectives of this study were: to evaluate and validate scientifically the successful

therapeutic claims by traditional medicine practitioners that *Syzygium cordatum* is effective

in treating diarrhoea and diabetes; to determine the effects of the plant extract on

gastrointestinal transit of a charcoal meal in mice; to determine the effects on castor oil-

induced intestinal fluid accumulation; to determine the safety profile of the plant by carrying

out acute toxicology study and to carry out preliminary screening of the active compounds

present in the plant using standard phytochemical analytical procedures.

The aqueous leaf extract of *Syzygium cordatum* (3.125 -50mg/kg, p.o) significantly reduced

the faecal output caused by castor oil (0.7ml). All the doses used, reduced faecal output from

100% produced by castor oil to between 40 and 61%. *S.cordatum* (6.25 – 50mg/kg, p.o)

significantly and in a dose dependent manner, delayed the onset of castor oil-induced

diarrhoea. Doses 3.25 – 50mg/kg, p.o siginicantly reduced castor oil-induced diarrhoeal

episodes but did not significantly alter the number of animals exhibiting diarrhoea. Loperamide (20mg/kg, p.o) significantly reduced the faecal output from 100% produced by castor oil to 8.7%. Loperamide profoundly delayed the onset of diarrhoea, reduced the incidence of castor oil-induced diarrhoea by protecting 83% of animal and reduced the number of castor oil-induced diarrhoeal episodes by 96%.

The aqueous leaf extract of *Syzygium cordatum* inhibited the propulsion of the charcoal meal by 21.25 – 50.93% and loperamide reduced the propulsion of the charcoal meal by 79.06%. The aqueous leaf extract reduced the fluid accumulation caused by castor oil (1.5ml) by 41.55 – 59.15% and loperamide (20mg/kg, p.o) reduced the castor oil (1.5ml)- induced gastrointestinal fluid accumulation by 52.82%.

Syzygium cordatum (12.5 – 50mg/k, p.o) significantly reduced the blood glucose concentration of normal fasted rats by 28.60 – 32.79% and chlorpropamide (250mg/kg, p.o) significantly reduced the blood glucose concentration of normal fasted rats by 43.26%.

Syzygium cordatum (6.25 – 50mg/kg, p.o) significantly reduced the blood glucose concentration of streptozotocin-induced diabetic rats with a maximal percentage reduction of 35.54% and chlorpropamide (250mg/kg, p.o) profoundly reduced the blood glucose concentration of streptozotocin-induced diabetic rats with a maximal reduction of 93.42%.

The phytochemical studies revealed the presence of tannins, saponins, alkaloids, flavonoids, triterpenesteroids and reducing sugars in the aqueous extract of *Syzygium cordatum*.

The aqueous leaf extract of *Syzygium cordatum* extract was found to be non-toxic in mice via the oral route with a maximum LD50 to be above 4000 mg/kg.

In conclusion, the results showed that the aqueous leaf extract of *Syzygium cordatum* possesses both antdiarrhoeal and antidiabetic activities

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CHAPTER 1

INTRODUCTION

Plants have, for generations, been a source of various kinds of remedies and been used for medicinal purpose to cure all kinds of ailments and will continue to provide remedies for these ailments, especially in rural areas in developing countries in the African continent (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997).

It is estimated that around 70 000 plants species, from lichens to towering trees, have been used at one time or another for medicinal purposes. Medicinal plants or herbs are providing the starting material for the isolation or synthesis of conventional drugs. Herbs are becoming popular throughout the developed world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in concert with the body's own defenses (Das Prajapati et al., 2003).

According to Das Prajapati et al. (2003), medicinal plants have curative properties due to the presence of various complex chemical structures of different composition, which are found as secondary plant metabolites in one or more parts of the plant. Plant metabolites are grouped according to the composition as alkaloids, glycosides, corticosteroids, essential oils and so on.

South Africa has a rich floral biodiversity with an estimated 30 000 species of higher plants, with 3000 of these plants used for medicinal purposes (Van Wyk et al., 1997; Light et al., 2005). This rich cultural diversity in the country greatly, also, contributes to the blending of the cultures and is reflected in the systems of medicines practiced (Van Wyk et al., 1997).Approximately, three million South Africans in both urban and rural areas are reliant

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on traditional medicine exclusively or in combination with western medicine (Van Wyk et al., 1997; Amabeoku et al., 1998; Jager et al., 2003; Light et al., 2005; Govender et al., 2006).

Most traditional remedies in South Africa are prepared in unhygienic settings by herbalist or traditional healers therefore microbial contamination is likely to occur at any stage during preparation, handling and storage of the herbal product hence affecting the quality of the final product. Since South African lack stringent regulatory authority for herbal products therefore, basic research on herbal medicine should be geared towards toxicity and efficacy and authenticate the successful therapeutic claims as asserted by traditional healers, respectively (Ernst 2002; Govender at al., 2006).

According to WHO (2008), traditional medicines are adopted in different cultures and regions without parallel advance of international standards and methods of evaluation. Not many countries have national policies and, regulating traditional medicine products, practices and practitioners is difficult due to variations in definitions and categorization of traditional medicine therapies. A single herbal product can either be defined as food, dietary supplement or a herbal medicine depending on the country. Scientific evidence from tests done to evaluate safety and effectiveness of traditional medicine products is limited (WHO, 2008).

This, therefore, necessitates comprehensive research on traditional medicine products vis-à-vis plant medicines including pharmacological evaluation to verify and/or validate pharmacological activities; phytochemical analysis to determine chemical components; HPLC study to characterize the plants, toxicological studies and so on. The main purpose of the above studies may be to enhance the efficacy and the safety profile of the medicinal plants and to increase the acceptability of this type of medicine in the society. In the current study, some of the above mentioned research methods were employed.

CHAPTER 2

LITERATURE REVIEW

2.1   Diarrhoea

2.1.1   General

Diarrhoea is a condition of having three or more loose or liquid stools per day (Kasper et al., 2005). It is a common cause of death in Third World Countries and the second most known cause of children deaths worldwide (Weber, 1996; WHO, 2009). The loss of fluid and electrolytes through diarrhoea can cause dehydration and electrolyte imbalances. In 2009, diarrhoea was estimated to have caused 1.1million deaths in people aged 5 years and over, and 1.5 million deaths in children under the age of 5 years. Oral rehydration solutions are the treatment of choice and have been estimated to have saved 50 million children in the past 25 years (Kasper et al., 2005; Wilson,2005; WHO, 2009a).

Diarrhoea due to infection, may last a few days or several weeks, as in persistent diarrhoea. Severe diarrhoea may be life threatening due to fluid loss in watery diarrhoea, particularly in infants and young children, the malnourished and people with impaired immunity. The impact of repeated or persistent diarrhoea on nutrition and the effect of malnutrition on susceptibility to infectious diarrhoea, can be linked to a vicious cycle amongst children, especially in developing countries. It is also associated with other infections such malaria and measles. Chemical irritation of the gastrointestinal tract or non-infectious bowel disease can also result in diarrhoea (Kasper et al.,2005; Wilson,2005; WHO, 2009).

Diarrhoea is caused by a host of bacterial, viral and parasitic organisms most of which can be spread by contaminated water. It is more common where there is a shortage of clean water for

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drinking, cooking and cleaning, and basic hygiene is important in prevention. Water contaminated with human faeces, for example, from municipal sewage, septic tanks and latrines is of special concern. Animal faeces also contain micro-organisms that can cause diarrhoea. It can also spread from person to person, aggravated by poor hygiene. Food is another major cause of diarrhoea when it is prepared or stored in unhygienic conditions. Water can contaminate food during irrigation, and fish and seafood from polluted water may also contribute to the disease. Worldwide, around 1.1 billion people lack access to improved water and 2.4 billion people have no basic sanitation. These will increase susceptibility to diarrhoea (Kasper et al., 2005; Wilson, 2005; WHO, 2009).

2.1.2 Types and causes of Diarrhoea

Secretory diarrhoea means that there is an increase in the active secretion, or there is an inhibition of absorption. There is little or no structural damage. The most common cause of this type of diarrhoea is cholera which stimulates the secretions of fluids into the gastrointestinal tract (GIT) following an increase in salt secretion like sodium chloride. The increase in the fluid buildup in the GIT lumen stimulates peristalsis resulting in watery diarrhoea (King et al.,2003;Wilson, 2005; Kasper et al., 2005).

Osmotic diarrhoea occurs when too much water is drawn into the GIT lumen. This can be the result of malabsorption caused by pancreatic disease, in which the nutrients and salts like sodium chloride are left in the GIT lumen to pull in water. Osmotic diarrhoea can also be caused by osmotic laxatives used in the treatment of constipation (Wilson, 2005; Kasper et al., 2005).

Exudative diarrhoea occurs with the presence of blood or pus in the stools. This occurs with inflammatory diseases such as Crohn's disease or ulcerative colitis (Wilson, 2005; Kasper et

al., 2005).

Inflammatory diarrhoea occurs when there is damage to the mucosal lining, which leads to passive loss of protein rich fluids and a decreased ability to absorb these loss fluids. Features of all three types of diarrhoea can be found in this type of diarrhoea. This type of diarrhoea can be caused by bacterial, viral, parasitic infections or autoimmune problems such as inflammatory bowel syndrome. It can also be caused by tuberculosis, colon cancer and enteritis (Kasper et al., 2005; Wilson, 2005).

Generally, if there is blood visible in the stools, it is referred to as dysentery which is a symptom of bacterial invasion of the colon by a shigella organism (Kasper et al., 2005;Wilson, 2005).

2.1.3   Treatment of Diarrhoea

Most episodes of diarrhoea are acute and self limiting and may reflect food intolerance, bacterial toxin infection or enteric infection. The first line management is the prevention or treatment of fluid and electrolyte depletion. This is particularly important for infants, children and the frail elderly and may be achieved by either homemade or commercially available oral rehydration solutions (Kasper et al., 2005;Wilson, 2005).

2.1.4   Drug treatment

Drugs may be used in the management of diarrhoea. The different kinds of antidiarrhoeal drugs include anti-propulsives, anti-infectives; intestinal absorbents and anti-inflammatory drugs (Kasper et al., 2005;Wilson 2005).

2.1.4.1 Anti-propulsive

2.1.4.1.1   Loperamide

Loperamide is an opioid-receptor agonist and acts on the μ-opioid receptors in the myenteric plexus of the large intestine; by itself it does not affect the central nervous system like other opioids. It works by decreasing the activity of the myenteric plexus, which, like morphine, decreases the tone of the longitudinal smooth muscles but increases tone of circular smooth muscles of the intestinal wall. This increases the amount of time substances stay in the intestine, allowing for more water to be absorbed out of the feacal matter. Loperamide also decreases colonic mass movements and suppresses the gastrocolic reflex. It may also reduce gastrointestinal secretions. It is given by mouth as an antidiarrhoeal drug and as an adjunct in the management of acute or chronic diarrhoea and is usually obtainable as 2 mg tablets or 1 mg/5 ml syrup. About 40% of the dose of loperamide is reported to be absorbed from the gastrointestinal tract to undergo first-pass metabolism in the liver and excretion in the faeces via the bile inactive conjugate, there is slight urinary excretion. Little intact drug reaches the system circulation. The elimination half life is reported to be 1 hour (Altman, 2001; Kasper et al., 2005;Wilson, 2005, Waller et al., 2005)**.**

In acute diarrhoea, the usual dose for adults is 2 tablets immediately followed by 1 tablet after each loose stool to a maximum of 8 tablets per day, the usual daily dose is 3 to 4 tablets. In children the dose is 5 ml three to four times a day up to 3 days (Kasper et al., 2005; Wilson, 2005; SAMF, 2010).

In chronic diarrhea, the usual dose for adults is 2 to 4 tablets daily in divided doses subsequently adjusted as necessary. The major side effects for loperamide are abdominal pain, bloating, nausea, dry mouth, dizziness, and fatigue and hypersensitivity reactions like

skin rashes. Loperamide has been associated with paralytic ileus particularly in infants and young children and death has been reported. Depression of CNS, to which children may be more sensitive, may be seen in over dosage and naloxone hydrochloride has been recommended for its treatment (Martindale, 2005, SAMF, 2010).

Loperamide should not be used when inhibition of peristalsis is to be avoided, in particular where ileus or constipation occurs, and should be avoided in patients with abdominal distension, acute inflammatory bowel disease or antibiotic-associated colitis. It should not be used alone in patients with dysentery because dysentery is caused by bacteria and therefore, an antibiotic use is necessary. Loperamide should be used with caution in patients with hepatic impairment because of its considerable first-pass metabolism in the liver and should not be used in infants. Concomitant use with co-trimoxazole increases the bioavailability of loperamide, apparently by inhibition of the first-pass metabolism (Martindale 2005).It is considered safe for use by breast feeding mothers as there has not been any report of clinical effect on the infant associated with its use (Kasper et al., 2005;Wilson,2005).

2.1.4.1.2 Diphenoxylate

Diphenoxylate hydrochloride is a synthetic derivative of pethidine with little or no analgesic activity. It reduces the intestinal motility and is used in the symptomatic treatment of acute or chronic diarrhea. It is well absorbed from the gastrointestinal tract and is rapidly and extensively metabolized in the liver principally to diphenoxylic acid, which has antidiarrhoeal activity. Other metabolites include hydroxydiphenoxylic acid. It is excreted mainly as metabolites and their conjugates in the faeces and lesser amounts in urine. It may be distributed in breast milk (Martindale, 2005; SAMF, 2010).

In acute diarrhea, the usual dose for adults is 10mg by mouth followed by 5mg every 6 hours, later reduced as the diarrhoea is controlled. Suggested initial doses for children 4 to 8 years of age, are 2.5mg three times a day; 9 to 12 years, 2.5mg four times a day and over 12 years, 5mg three times a day. Similar initial doses are used for chronic diarrhoea and subsequently, reduced as necessary (Martindale, 2005; SAMF, 2010).

Diphenoxylate is related to opioid analgesics and its adverse effects and their treatment are similar, particularly in overdosage. The reported side effects include anorexia, nausea, vomiting, abdominal distension or discomfort, paralytic ileus, toxic megacolon, pancreatitis, headaches, drowsiness, restlessness, euphoria, depression, numbness of extremities, angioedema, pruritus and swelling of the gums. Signs of overdosage may be delayed and patients should be observed for at least 48 hours. Young children are particularly susceptible to effects of overdosage (Kasper et al., 2005; Wilson, 2005; Martindale, 2005).

The presence of subclinical dose of atropine in preparation containing diphenoxylate may give rise to the side effects of atropine, such as blurred vision, constipation, flushing, urinary retention, tachycardia, mental confusion and agitation, in susceptible individual or in overdosage, thereby, acting as a preventive measure to potential abuse (Kasper et al., 2005; Wilson, 2005; Martindale, 2005; SAMF, 2010).

Diphenoxylate hydrochloride should be avoided in patients with jaundice, intestinal obstruction; antibiotic associated colitis or diarrhoea associated with enterotoxin-producing bacteria and should be used with caution in patients with hepatic impairment. It should also be used with caution in young children because of a greater variability of response in this age group and is not generally recommended in infants. Patients with inflammatory bowel disease receiving diphenoxylate should be carefully observed for signs of toxic megacolon

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and it should be discontinued immediately should abdominal distention occur (Martindale, 2005).

There is a theoretical risk of hypertensive crisis if it is used with monoamine oxidase inhibitors (MAOI's) due to its structural relationship with pethidine (Kasper et al., 2005; Wilson, 2005; Martindale, 2005).

2.2   Diabetes mellitus

Diabetes mellitus, often simply referred to as diabetes, is a chronic condition in which a person has a high glucose level as a result of the body either not producing enough insulin or because the body cells do not properly respond to the insulin that is produced. Insulin is a hormone produced by pancreatic beta cells in the Islet of Langerhans, which enables body cells to absorb glucose which is turned into energy. If the body cells do not absorb the glucose, the glucose accumulates in the blood leading to various potential medical complications. (WHO, 1999; Wild et al., 2004; Rother, 2007)

There are many types of diabetes, the most common of which are:

a) Type 1 Diabetes or Insulin Dependent Diabetes Mellitus (IDDM) results from the body's failure to produce insulin, and presently, requires the person to inject insulin.

b) Type 2 Diabetes or Non insulin Dependent Diabetes Mellitus (NIDDM) results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency.

c) Gestational Diabetes is when pregnant women, who have never had diabetes before,

have a high blood glucose level during pregnancy. It may precede development of

Type 2 diabetes mellitus.

Other forms of diabetes include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes and steroid diabetes induced by high doses of glucocorticoids (WHO, 1999; Wild etal., 2004; Rother, 2007).

Acute complications of diabetes include hypoglycaemia, ketoacidosis and long term complications like cardiovascular diseases, retinal complications and angina. Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as smoking ceasation and maintaining a healthy body weight (Wild etal., 2004; Rother, 2007)

As of 2000, at least 171 million people worldwide suffered from diabetes, or 2,8 % of the population. Type 2 diabetes is by far the most common, affecting 90 to 95% of the US diabetes population (WHO, 1999).
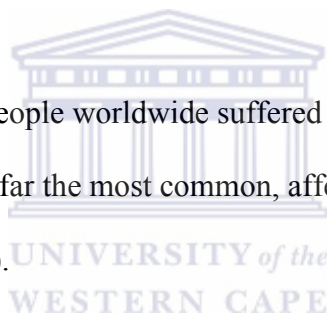
2.2.1   Classification

Most cases of diabetes mellitus fall into the three broad categories of Type 1 or Insulin Dependent Diabetes Mellitus (IDDM), Type 2 or Noninsulin Dependent Diabetes Mellitus (NIDDM) and Gestational diabetes.

2.2.1.1 Type 1 Diabetes Mellitus (IDDM)

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the Islets of Langerhans in the pancreas which leads to insulin deficiency. The loss of these cells

can be the results of autoimmune disease where the body's T-cell attack the pancreatic cells or the results of which is unknown, thereby classified as idiopathic in origin. IDDM causesapproximately10% of DM cases worldwide and there is no known preventive measures against it and it affects people of all ages including children (WHO, 1999; Wild et al., 2004; Rother,2007).

2.2.1.2 Type 2 Diabetes Mellitus(NIDDM)

Type 2 diabetes mellitus is characterized by insulin resistance which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. In the early stage of Type 2 diabetes, the predominant abnormality is reduced insulin sensitivity. The early stages of type 2 diabetes may be managed by medications such as metformin and diet, and as the disease progresses, insulin therapy may be necessary (WHO, 1999; Wild etal., 2004; Rother, 2007).

2.2.1.3 Gestation Diabetes Mellitus (GDM)

Gestation diabetes mellitus resembles Type 2 diabetes in several respects, involving combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2 to 5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy and if left untreated, can damage the health of the foetus or mother resulting in high birth weight, congenital cardiac or nervous system abnormalities and skeletal muscle malformation. About20 to 50% of affected women develop Type 2 diabetes later in life. Increased foetal insulin may inhibit foetal surfactant production and cause Respiratory Distress Syndrome (Wild etal., 2004).

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2.2.1.4   Other Types of Diabetes.

Pre-diabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of Type 2 diabetes. Many people destined to develop Type2 diabetes spend many years in a state of pre-diabetes. Some very uncommon cases of diabetes are caused by the body's tissue receptors not responding to insulin (WHO, 1999; Rother, 2007).

2.2.2 Signs and symptoms

The classical symptoms of diabetes are hyperglycaemia, polyuria, polydipsia and polyphagia. The symptoms may develop quite rapidly in Type 1 diabetes particularly in children, however, in Type 2diabetes, symptoms usually develop much more slowly and may be subtle or completely absent. Type 1 diabetes may also cause a rapid yet significant weight loss and persistent mental fatigue. All of these symptoms except weight loss can manifest in Type 2 diabetes in patients whose diabetes is poorly controlled, although unexplained weight loss may be experienced at the onset of the disease (Wild et al., 2004).

Final diagnosis is made by measuring the blood glucose concentration. When the glucose concentration in the blood is high (hyperglycaemia), reabsorption of glucose in the proximal convoluted tubule of the kidneys is incomplete resulting in high concentration of glucose in the urine (glycosuria).This highconcentration of glucose in the urine results in high osmotic pressure which inhibits the reabsorption of water and thereby, resulting in increase urine production or polyuria. The resultant lost blood volume will be replaced osmostically from water held in body cells and other body compartments causing dehydration and increased thirst or polydipsia (Wild et al., 2004).

Prolonged high blood glucose causes glucose absorption, which leads to changes in the retinal cells, resulting in vision acuity and eventually blindness; sustained sensible glucose control usually returns the lens to its original shape. Blurred vision is a common complaint leading to a diabetes diagnosis. Type 1 diabetes should always be suspected in cases of rapid vision change, whereas with Type 2 diabetes, change is generally more gradual but should still be suspected (WHO, 1999; Wild et al., 2004; Rother, 2007).

Patients, usually with Type 1 diabetes, may also initially present with diabetic ketoacidosis (DKA), an extreme state of metabolic dysregulation characterized by the smell of acetone on the patient's breath, rapid and deep breathing, polyuria, nausea, abdominal pain and any of many altered states of consciousness or arousal such as hostility or equally, confusion and lethargy. In severe DKA, coma may follow progressing to death. Diabetic ketoacidosis is a medical emergency and requires immediate hospitalization (WHO, 1999; Wild et al., 2004; Rother, 2007).

2.2.3   Aetiology

Type 2 diabetes is determined primarily by lifestyle factors and genes. A number of lifestyle factors is known to be important to the development of Type 2 diabetes. People who had high levels of physical activity, eat a healthy diet, do not smoke and consumed alcohol in moderation have reduced chances of developing Type 2 diabetes. Medical conditions associated with Type 2 diabetes are Cushings Syndrome and hypogonadism (Wild etal., 2004; Rother, 2007).

Both Type 1 and Type 2 diabetes are partly inherited. Type 1 diabetes may be triggered by certain infections with some evidence pointing at Coxsackie B4 virus. There is a genetic

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element in individual susceptibility to some of these triggers which has been traced to a particular HLA (Human Leukocyte Antigen) genotype. However, even in those who have inherited the susceptibility, Type 1 diabetes mellitus seems to require an environmental trigger. There is a stronger inheritance pattern in Type 2 diabetes. Those with first degree relatives with Type 2 diabetics have a much higher risk of inheriting the disease (WHO, 1999; Wildet al., 2004).

Gene expression promoted by a diet of fat and glucose as well as high levels of inflammation related cytokines found in obese patients results in cells that "produce fewer and smaller mitochondria than is normal" and are thus, prone to insulin resistance (Kasper et al., 1998; Wild et al., 2004; Rother, 2007).

2.2.4   Pathophysiology

Insulin production is more or less constant within the beta cells, irrespective of blood glucose levels. It is stored within vacuoles pending release, which is primarily triggered by food, chiefly food containing absorbable glucose. The chief trigger is a rise in blood glucose levels after eating. Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus (Nathan et al., 2005; Santaguida, 2008).

Humans are capable of digesting some carbohydrates, in particular those most common in food; starch, and some disaccharides such as sucrose, are converted within a few hours to simpler forms most notably the monosaccharide glucose, the principal carbohydrate energy source used by the body. The most significant exceptions are fructose, most disaccharides, except sucrose and in some people lactose, and all more complex polysaccharides, with the

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outstanding exception of starch. The rest are passed on for processing by gut flora largely in the colon. Insulin is released into the blood by beta cells found in the Islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage (Nathan, et al 2005; Santaguida, 2008).

Insulin is also the principal control signal for conversion of glucose to glycogen for internal storage in liver and muscle cells. Low glucose levels result both in the reduced release of insulin from the beta cells and in the reverse conversion of glycogen to glucose when glucose levels fall. This is mainly controlled by the hormone, glucagon, which acts in the opposite manner to insulin. Glucose thus forcibly produced from internal liver cell stores (as glycogen) re-enters the bloodstream because muscle cells lack the necessary export mechanism to transport glucose to other body tissues. Normally, liver cells do this when the level of insulin is low (Nathan et al., 2005; Santaguida, 2008).

Higher insulin levels increase some anabolic processes such as cell growth and duplication, protein synthesis, and fat storage. Insulin (or its lack) is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and vice versa. In particular, a low insulin level is the trigger for entering or leaving ketosis. If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin, insulin insensitivity or resistance, or if the insulin itself is defective, then glucose will not have its usual effect and will not be absorbed properly by those body cells that require it nor will it be stored appropriately in the liver and muscles. The net effect is persistently high

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levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis(Nathan et al., 2005; Santaguida, 2008).

2.2.5   Diagnosis

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following:

a)   Fasting plasma glucose level at or above 7.0 mmol/l.

b)   Plasma glucose at or above 11.1 mmol/l two hours after a 75 g oral glucose load as in a glucose tolerance test.

c)   Symptoms of hyperglycemia and casual plasma glucose at or above 11.1 mmol/l.

d)   Glycated hemoglobin (HbA1C) at or above 6.5%.

About a quarter of people with new Type 1 diabetes have developed some degree of diabetic ketoacidosis by the time the diabetes is recognized. The diagnosis of other types of diabetes is usually made in other ways. These include ordinary health screening; detection of hyperglycemia during other medical investigations; and secondary symptoms such as vision changes or unexplainable fatigue. Diabetes is often detected when a person suffers a problem that is frequently caused by diabetes, such as a heart attack, stroke, neuropathy, poor wound healing or a foot ulcer, certain eye problems, certain fungal infections, or delivering a baby with macrosomia or hypoglycemia. A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above-listed methods on a different day. According to the current definition, two fasting glucose measurements of above 7.0 mmol/l considered diagnostic for diabetes mellitus (WHO, 1999; Santaguida, 2008).

Patients with fasting glucose levels from 5.6 to 6.9 mmol/l are considered to have impaired fasting glucose. Patients with plasma glucose at or above 7.8 mmol/l, but not over 11.1 mmol/l, two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two pre-diabetic states, the latter in particular is a major risk factor for progression to full blown diabetes mellitus as well as cardiovascular disease (Kasper et al., 1998; WHO, 1999; Santaguida, 2008).

2.2.6   Screening

Diabetes screening is recommended for many people at various stages of life, and for those with any of several risk factors. The screening test varies according to circumstances and local policy, and may be a random blood glucose test, a fasting blood glucose test, a blood glucose test two hours after 75 g of glucose, or an even more formal oral glucose tolerance test. Many healthcare providers recommend universal screening for adults at age 40 or 50 years, and often periodically thereafter. Early screening is typically recommended for those with risk factors such as obesity, family history of diabetes and high-risk ethnicity (Kasper et al., 1998; WHO, 1999; Santaguida, 2008).

Many medical conditions are associated with diabetes and warrant screening. A partial list includes: subclinical Cushing's syndrome, testosterone deficiency, high blood pressure, elevated cholesterol levels, coronary artery disease, past gestational diabetes, polycystic ovarian syndrome, chronic pancreatitis, fatty liver, hemochromatosis, cystic fibrosis, several mitochondrial neuropathies and myopathies, myotonic dystrophy, Friedreich's ataxia and some of the inherited forms of neonatal hyperinsulinism. The risk of diabetes is higher with chronic use of several medications, including long term corticosteroids, some

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chemotherapeutic agents (especially L-asparaginase), as well as some of the antipsychotics and mood stabilizers, especially phenothiazines and some atypical antipsychotics.People with a confirmed diagnosis of diabetes are tested routinely for complications. This includes yearly urine testing for microalbuminuria and examination of the retina of the eye for retinopathy (Kasper et al., 1998; Santaguida, 2008).

2.2.7   Prevention

2.2.7.1 Type 1 Diabetes Mellitus

Type 1 diabetes risk is known to depend upon a genetic predisposition based on HLA types, an unknown environmental trigger and an uncontrolled autoimmune response that attacks the insulin producing beta cells. Children with antibodies to beta cell proteins but no overt diabetes, and treated with vitamin B3 (niacin), had less than half the diabetes onset incidence in a 7-year time span as did the general population, and an even lower incidence relative to those with antibodies as above, but who received no vitamin B3(Kasper et al., 1998, WHO 1999; Santaguida, 2008).

2.2.7.2 Type 2Diabetes Mellitus

Type 2 diabetes risk can be reduced in many cases by making changes in diet and increasing physical activity. There is inadequate evidence that eating foods of low glycaemic index is clinically helpful despite recommendations and suggested diets emphasizing this approach. Diets that are very low in saturated fats reduce the risk of becoming insulin resistant and diabetic. Some studies have shown delayed progression to diabetes in predisposed patients through prophylactic use of metformin, rosiglitazone or valsartan. Lifestyle interventions are,

however, more effective than metformin at preventing diabetes regardless of weight loss
(Kasper et al., 1998, WHO, 1999; Santaguida, 2008).

2.2.8   Management of diabetes mellitus

Diabetes mellitus is a chronic disease which is difficult to cure. Management concentrates on keeping blood sugar levels as close to normal as possible without presenting undue danger to the patient. This can usually be with close dietary management, exercise and use of appropriate medications. There are roles for patient education, dietetic support, sensible exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure in patients with hypertension, cholesterol in those with dyslipidaemia, as well as exercising more, smoking less or ideally not at all and consuming a recommended diet. Patients with foot problems are also recommended to wear diabetic socks and possibly diabetic shoes(Waller et al., 2005a; Rother, 2007; Rang et al., 2008).

2.2.8.1 Drug therapy in the management of diabetes mellitus

Anti-diabetic drugs treat diabetes mellitus by lowering glucose levels in the blood. With the exceptions of insulin, all are administered orally and are thus, also called oral hypoglycemic agents or oral anti-hyperglycemic agents. There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors(Waller et al., 2005a; Rother, 2007; Rang et al., 2008).

Type 1diabetes mellitus is a disease caused by the lack of insulin. Insulin must be used in

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Type I diabetes, which must be injected mostly subcutaneously. Type 2 diabetes mellitus is a disease of insulin resistance by cells. Treatments include agents that increase the amount of insulin secreted by the pancreas, agents which increase the sensitivity of target organs to insulin, and agents which decrease the rate at which glucose is absorbed from the gastrointestinal tract(Waller et al., 2005a; Rother, 2007; Rang et al., 2008; SAMF, 2010a).

Several groups of drugs, mostly given by mouth, are effective in Type 2 diabetes mellitus, often in combination. The therapeutic combination in Type 2 diabetes mellitus may include insulin, not necessarily because oral agents have failed completely, but in search of a desired combination of effects. The great advantage of injected insulin in Type 2 diabetes mellitus is that a well-educated patient can adjust the dose, or even take additional doses, as determined by the blood glucose levels measured by the patient, usually with a simple meter.(Waller et al., 2005a; Rother, 2007; Rang et al., 2008; SAMF, 2010a).
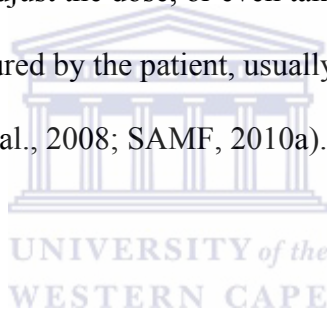
2.2.8.1.1 Insulin

Type 1 diabetes mellitus treatments usually include combinations of regular or NPH insulin, and/or synthetic insulin analogues. Insulin can be classified into three broad categories; fast acting like Novarapid, Apidra, Humalog; intermediate acting like Novomix, Humalog Mix and long acting like Levemir and Lantus. The fast acting insulin preparations are usually given directly after a meal to provide a bolus dose and the long and intermediate acting insulin preparations are given to provide a basal dose for control throughout the day (Rother, 2007, SAMF, 2010a).

2.2.8.1.2   Oral anti-diabetic medications

i)   Sulphonylureas

Sulphonylureas were the first widely used oral anti-hyperglycaemic medications and are insulin secretagogues, triggering insulin release by direct action on the pancreatic beta cells. The "second-generation" drugs are now more commonly used. They are more effective than first-generation drugs and have fewer side effects. All may cause weight gain. Sulphonylureas bind strongly to plasma proteins. Sulfonylureas are only useful in Type 2 diabetes, as they work by stimulating endogenous release of insulin. They work best with patients over 40 years old, who have had diabetes mellitus for underten years. They cannot be used in Type 1 diabetes, or diabetes in pregnancy. They can be safely used with metformin or glitazones. The primary side effect is hypoglycemia. The reductions in HbA1Cvalues for second generation sulphonylureas are 1.0 to 2.0%.First-generation agents are tolbutamide, acetohexamide, tolazamide, chlorpropamide and the second generation agents are glipizide, glibenclamide, glimepiride and gliclazide(Rother, 2007; SAMF, 2010a).

ii)   Meglitinides

Meglitinides, which include repaglinide and nateglinide,help the pancreas produce insulin and are often called "short-acting secretagogues." They act on the same potassium channels as sulphonylureas, but at a different binding site. By closing the potassium channels of the pancreatic beta cells, they open the calcium channels, hence enhancing insulin secretion. They are taken with or shortly before meals to boost the insulin response to each meal. If a meal is skipped, the medication is also skipped. They reduce the HbA1C by approximately

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0.5 to 1.0%.Adverse reactions include weight gain and hypoglycemia (Waller et al., 2005a; Rother, 2007).

iii) Sensitizers

Insulin sensitizers address the core problem in Type 2 diabetes, insulin resistance. Among oral hypoglycemic agents, insulin sensitizers are the largest category (Rother, 2007).

iv) Biguanides

Biguanides reduce hepatic glucose output and increase uptake of glucose by the periphery, including skeletal muscle. Although it must be used with caution in patients with impaired liver or kidney function, metformin, a biguanide, has become the most commonly used agent for type 2 diabetes in children and teenagers. Amongst common diabetic drugs, metformin is the only widely used oral drug that does not cause weight gain. The reductions in HbA1C values for metformin are 1.5 to 2.0%.Metformin may be the best choice for patients who also have heart failure. It should be temporarily discontinued before any radiographic procedure involving intravenous iodinated contrast as patients are at an increased risk of lactic acidosis. Metformin is usually the first-line medication used for treatment of Type 2 diabetes. It is generally prescribed at the initial diagnosis in conjunction with exercise and weight loss as opposed to in the past, where metformin was prescribed after diet and exercise had failed. Initial dosing is 500 mg once daily, then if need be increased to 500 mg twice daily up to 1000 mg twice daily. It is also available in combination with other oral anti-diabetic medications. There is an extended release formulation available, but it is typically reserved for patients experiencing gastro-intestinal side effects. (Waller et al., 2005; Rother, 2007; SAMF, 2010a).

v) Thiazolidinediones

This class comprises of rosiglitazone, pioglitazone and troglitazone which was withdrawn due to hepatitis and liver damage risk.Thiazolidinediones (TZDs), also known as "glitazones," bind to PPARγ, a type of nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism. These PPARs act on Peroxysome Proliferator Responsive Elements (PPRE). The PPREs influence insulin sensitive genes, which enhance production of mRNAs of insulin dependent enzymes. The final result is better use of glucose by the cells. The typical reductions in HbA1C values are 1.5 to 2.0%. As a result of multiple retrospective studies, there is a concern about rosiglitazone's safety, although it is established that the group, as a whole, has beneficial effects on diabetes. The greatest concern is an increase in the number of severe cardiac events in patients taking it (Rother, 2007; SAMF, 2010a).

vi) Alpha-glucosidase inhibitors

The most commonly used alpha-glucosidase inhibitors is acarbose. Alpha-glucosidase inhibitors are "diabetes pills" but not technically hypoglycemic agents because they do not have a direct effect on insulin secretion or sensitivity. These agents slow the digestion of starch in the small intestine, so that glucose from the starch of a meal enters the bloodstream more slowly, and can be matched more effectively by an impaired insulin response or sensitivity. These agents are effective by themselves only in the earliest stages of impaired glucose tolerance, but can be helpful in combination with other agents in Type 2 diabetes. The typical reductions in HbA1C values are 0.5 to 1.0%.The major side effects are flatulence

and bloating. They do have the potential to cause weight loss by lowering the amount of sugar metabolized (Waller et al., 2005a; Rother, 2007).

viii)   Incretin mimetics

Incretins are insulin secretagogues. The two main candidate molecules that fulfill criteria for being an incretin are Glucagon-like peptide-1 (GLP-1) and Gastric inhibitory peptide(GIP). Both GLP-1 and GIP are rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4). GLP agonists bind to a membrane GLP receptor. As a consequence of this, insulin release from the pancreatic beta cells is increased. Endogenous GLP has a half life of only a few minutes; thus an analogue of GLP would not be practical (Rother, 2007).

Exenatide (also Exendin-4, marketed as Byetta) is the first GLP-1 agonist approved for the treatment of Type 2 diabetes. It is not an analogue of GLP, but rather a GLP agonist. Exenatide has only 53% homology with GLP, which increases its resistance to degradation by DPP-4 and extends its half-life. The typical reductions in A1C values are 0.5 to 1.0% (Rother, 2007).

Liraglutide, a once daily human analogue (97% homology), is being developed by Novo Nordisk under the brand name, Victoza. The product was approved by the European Medicines Agency (EMEA) on July 3, 2009, and by the U.S. Food and Drug Administration (FDA) on January 25, 2010. Taspoglutide is presently in Phase III Clinical Trials with Hoffman-La Roche. These agents may also cause a decrease in gastric motility, responsible for the common side effect of nausea, and is probably the mechanism by which weight loss occurs (Rother, 2007).

ix) Gastric inhibitory peptide (GIP) analogs

None are approved for use in the management of diabetes (Rother, 2007).

x) DPP-4 inhibitors

Dipeptidyl peptidase-4 (DPP-4) inhibitors increase blood concentration of the incretin GLP-1 (glucagon-like peptide-1) by inhibiting its degradation by dipeptidyl peptidase-4 (DPP-4). The typical reductions in A1C values are 0.5 to 1.0%. The examples are, vildagliptin, sitagliptin and saxagliptin (Rother, 2007; SAMF, 2010a).

2.2.9   Prognosis

Patient education, understanding, and participation are vital since the complications of diabetes are far less common and less severe in people who have well-managed blood sugar levels. Wilder health problems may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. According to one study, women with high blood pressure were three times more likely to develop Type 2 diabetes as compared with women with optimal BP after adjusting for various factors such as age, ethnicity, smoking, alcohol intake, body mass index (BMI), exercise, family history of diabetes, etc. Except in the case of Type 1 diabetes, which always requires insulin replacement, the way Type 2 diabetes is managed may change with age. Insulin production decreases because of age-related impairment of pancreatic beta cells (Nathan et al., 2005; Rother, 2007).

Additionally, insulin resistance increases because of the loss of lean tissue and the accumulation of fat, particularly intra-abdominal fat, and the decreased tissue sensitivity to

insulin. Glucose tolerance progressively declines with age, leading to a high prevalence of Type 2 diabetes and post-challenge hyperglycemia in the older population. Age-related glucose intolerance in humans is often accompanied by insulin resistance, but circulating insulin levels are similar to those of younger people. Treatment goals for older patients with diabetes vary with the individual, and take into account health status, as well as life expectancy, level of dependence, and willingness to adhere to a treatment regimen. Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause (Nathan et al., 2005; Rother, 2007).

In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double. Diabetes mellitus occurs throughout the world, but is more common (especially Type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030. The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation, some of it most compellingly presented (Nathan et al., 2005; Rother, 2007; WHO, 2010).

2.2.10. Description and objective of the project.

Though there are various effective orthodox medicines used to treat diarrhea and diabetes, plant medicines have also been used, especially in rural communities, by traditional medicine practitioners for the management and treatment of these ailments. One of such plants is

Syzygium cordatum Hochst.ex C.Krauss, also known as 'water berry'. This plant belongs to the family, Myrtaceae, and is locally known as 'umdoni' in isiXhosa and Zulu and 'water bessie' in Afrikaans and 'montlho' in South Sotho. It is a medium size tree which can grow up to 15 m in height. It has broad leaves which are sometimes, almost circular with a bluish-green colour (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997).

The tree is often found near streams, on forest margins or in swampy spots. The leaves, barks and roots are used in the form of infusion by traditional medicine practitioners to treat diarrhoea, stomach aches, diabetes, respiratory problems, and tuberculosis and so on. It flowers from August to November and bears fruits with oval berries which turn from red to dark-purple when ripe. It occurs along stream banks from KwaZulu-Natal northwards to Mozambique (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997).

However, little or no scientific evidence exists to corroborate the claims by traditional medicine practitioners of the therapeutic successes of the plant species in the treatment of the various ailments particularly diarrhoea and diabetes. The present study, therefore, investigated the effects of the leaf aqueous extract of *Syzygium cordatum* on castor oil-induced diarrhoea in mice and streptozotocin-induced diabetes in rats. The effects of the leaf aqueous extract of the plants species on the gastrointestinal transit of charcoal meal and castor oil-induced intraluminal fluid accumulation in mice were also investigated. The phytochemical analysis of the plant species was carried out to determine the chemical components and the acute toxicity investigated to determine the safety profile of the plant species.

CHAPTER 3

MATERIALS AND METHODS

3.1   Plant materials

Fresh leaves of *Syzygium cordatum* were collected from Kirstenbosch National Botanical

Gardens, Cape Town, South Africa in February 2009. The identification of the plant species

was done by both the curator of the Gardens and Mr Franz Weitz, a taxonomist in the

Department of Biodiversity and Conservative Biology, University of the Western Cape and a

voucher specimen (SC8231) was deposited in the Herbarium of the University.

3.2   Preparation of leaf aqueous extract of *Syzygium cordatum*

The fresh leaves of the plant species were weighed (1.4 kg), washed with distilled water and

dried at $35^{O}C$ for 4 days. The dried leaves (857 g) were ground to fine powder. 80 grams of

the fine powder was refluxed in 1 l of boiling water, allowed to cool and filtered. The filtrate

was then frozen at $-80^{O}C$ and freeze-dried for 5 days. A yield of 19.57g of dried leaf

aqueous extract was obtained and stored in a dessicator for future use. Fresh extract solutions

were prepared every day for the experiment by dissolving weighed quantities of the extract in

physiological saline. The solutions were administered orally to mice or rats in a volume of

1ml/100g of animal using bulbed steel needle.

3.3   Drugs and Chemicals

Castor oil (GR Pharmaceuticals (Pty) Ltd, Atlantis, South Africa) was administered orally in

a constant volume to mice throughout the experiments using bulbed steel needle. Lopermaide

(4[p-chlorophenyl] – 4 – hydroxy-N,N-dimethyl- diphenyl-1-piperidine-butyramine)

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hydrochloride (Sigma Chemical Co) was dissolved in a minimum amount of 10% ethanol (Merck (Pty) Ltd) and made up to the appropriate volume with physiological saline. Activated charcoal (Sigma Chemical Co), an aqueous suspension of 5% charcoal and 5% acacia, was prepared. Both the loperamide solution and the charcoal meal were given orally to mice in volume of 1ml/100g of animal using a bulbed steel needle. Streptozotocin (STZ, Sigma Chemical Co.) was dissolved in a small volume of 0.1 M citrate buffer pH 4.5, and made up to the appropriate volume with physiological saline, and administered intraperitoneally to rats in a volume of 1ml/100g animals. Chlorpropamide (Sigma Co.) was dissolved in physiological saline and administered orally to rats in a volume of 1ml/100g of animal using a bulbed steel needle.

3.4   Animals

Male albino mice bred in the Animal House of the Discipline of Pharmacology, University of the Western Cape, South Africa and weighing 18 – 30g were used for the antidiarrhoeal activity and the acute toxicity testing of the plant species. Young adult male Wistar rats, bought from the University of Cape Town, South Africa and weighing 160 – 210g were used for the antidiabetic activity. The animals were housed in a quiet laboratory with an ambient temperature of 22 $\pm$ 2^{O}C and a 12- h light/12-h dark cycle was maintained. They were all maintained on a standard pellet diet and water ad libitum. All the animals were fasted for 16 h during which they had free access to water prior to the commencement of the experiments.

3.5   Assessment of antidiarrhoeal activity

Male albino mice, weighing between 18 and 30g, were used in groups of six per dose of plant extractor loperamide, a standard antidiarrhoeal drug, throughout the experiments after

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fasting for 16 hours. The method described by Williamson et al. (1996) was modified and used to assess the antidiarrhoeal activity of the plant extract. Castor oil, a laxative, known to cause diarrhoea within 4 h, was used to induce diarrhoea in a control group of mice pre-treated with 0.3ml (p.o.) of physiological saline for 15 min prior to the oral administration of 0.7ml of castor oil. The onset of diarrhoea, the number of diarrhoeal episodes, stool mass and the number of mice exhibiting diarrhoea were obtained over a 5 h period of observation. Experiments were repeated with other groups of animals (test groups), pretreated for 15 min with either leaf aqueous extract (3.125 – 50 mg/kg) of the plant species or the standard antidiarrhoeal drug, loperamide (20 mg/kg), both given orally in a volume of 1 ml/100g of animals prior to the administration of 0.7 ml (p.o.) of castor oil. The ability of the plant extract to reduce the number of animals exhibiting diarrhoea and /or the number of diarrhoeal episodes is taken as an antidiarrhoeal activity (Williamson et al., 1996). The doses and pretreatment times used were obtained from preliminary studies in our laboratory. All experiments were carried out between 080:00 and 17:00 in a quiet laboratory with an ambient temperature of $22 \perp 2\,^{O}C$.

3.6   Assessment of gastrointestinal propulsion of charcoal meal.

The methods used to assess the effect of the plant extract on the gastrointestinal transit of charcoal meal were those described by Williamson et al. (1996) and Kitano et al. (1994).Animals were used in groups of six per dose of leaf aqueous plant extract (3.125 – 50 mg/kg, p.o.) or loperamide (20 mg/kg, p.o.), a standard antidiarrhoeal drug after fasting for 16 h. Control group was pretreated with 0.3ml of physiological saline given orally, for 20 min and then given 0.4ml of charcoal meal (aqueous suspension of 5% charcoal and 5% gum acacia)

orally. 20 min after the charcoal meal, the animals were killed by ether inhalation and the intestine was removed from the cardia to the rectal end. The distance travelled by the charcoal meal was measured and expressed as a percentage of the total length of the intestine. Experiments were repeated with other groups of animals (test groups) pretreated with the leaf aqueous plant extract (3.125 – 50 mg/kg) or loperamide (20 mg/kg), both given orally in a volume of 1ml/100g of animals, prior to the administration of 0.4ml of charcoal meal. All experiments were carried out between 08:00 and 17:00 in a quiet laboratory with the ambient temperature of 22 ± 2^{O}C.

3.7 Assessment of castor oil-induced intestinal fluid accumulation.

The methods of Robert et al. (1976) and DiCarlo et al. (1994) were modified and used to assess the effect of the plant extract on castor oil-induced intestinal fluid accumulation. The animals were used in groups of six per dose of leaf aqueous plant extract (3.125 – 50 mg/kg, p.o.) or loperamide (20 mg/kg, p.o.), a standard antidiarrhoeal drug, after fasting for 16 hours. The control group of six mice was pretreated with 0.3ml (p.o.) of physiological saline for 15 min and then given 1.5ml of castor oil orally. 20 minutes after the administration of castor oil, the animals were killed by ether inhalation and the intestines removed from the pylorus to the caecum. The intestinal contents were then evacuated into a measuring cylinder and the volume measured. Experiments were repeated with other groups of animals pretreated for 15 min with the leaf aqueous plant extract (3.125-50 mg/kg) or loperamide (20 mg/kg), a standard antidiarrhoeal drug, both given orally in a volume of 1ml/100g of animals, prior to the oral administration of 1.5 ml of castor oil.All experiments were carried out between 08:00 and 17:00 in a quiet laboratory with an ambient temperature of 22 ± 2^{O}C.

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3.8   Assessment of antidiabetic activity

Modified methods of Williamson et al. (1996a) and Joy and Kuttan (1999) were used to assess the antidiabetic activity of the leaf aqueous extract of *Syzygium cordatum*. Rats in groups of six each were divided into normoglycaemic and diabetic rats. The normoglycaemic rats were further divided into 'control and test' groups. The control group of six rats received 0.3 ml of physiological saline orally while five groups (of six rats each) of test rats were treated with the leaf aqueous extract of *Syzygium cordatum* (3.125 – 50 mg/kg, p.o.). One group of six test rats received chlorpropamide (250 mg/kg, p.o.), a standard antidiabetic drug. Similarly, the diabetic rats were divided further into 'control and test' groups. Diabetes was induced in all the diabetic rats by intraperitoneal injections of streptozotocin (STZ, 90 mg/kg). Following the injection, diabetes was allowed to develop and stabilize in the rats over a period of 3 – 5 days. The diabetic control group received 0.3 ml of physiological saline orally. Five groups of test diabetic rats received the leaf aqueous extract of the plant species (3.125 – 50 mg/kg, p.o.) whereas one group of test diabetic rats received chlorpropamide (250 mg/kg0, p.o.). In both normoglycaemic and diabetic rats, blood glucose levels were measured first without treatment (0 h) and then, the animals received either 0.3 ml (p.o.) of physiological saline, plant extract (3.125 – 50mg/kg, p.o.) or chlorpropamide (250 mg/kg, p.o.) after which the blood glucose levels were measured at intervals of 1 h, 2 h and 4 h in each treatment group. The blood samples for the measurement of the glucose levels were obtained by pricking of the rat tail tip vein. A drop of blood was placed on a glucose test strip and the glucose level read using a compatible Glucometer (Accu-Check® Abbot laboratory). The blood was obtained from both normoglycaemic and diabetic rats before treatment (0 h) and then 1 h, 2 h,

and4 h after treatment with either physiological saline, plant extract or chlorpropamide. Each glucose test strip was used only for one reading. Fasted streptozotocin–treated rats with blood glucose level greater than or equal to 18 mmol/l were considered diabetic). The ability of the plant extract to significantly lower the blood glucose level below 18 mmol/l is taken as an antidiabetic activity (Williamson et al., 1996a; Joy and Kuttan, 1999).

3.9 Acute toxicity testing

Male albino mice were used in groups of six per dose of plant extract after fasting for 16 h. The method of Lorke (1983) was modified and used to assess the acute toxicity of the leaf aqueous extract of *Syzygium cordatum*. The plant extract was administered orally to mice in graded doses (200, 400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600 and 4000 mg/kg). Another group of six mice used as control, received 0.3 ml physiological saline orally. Both the test and control animals were then allowed access to food and water and observed over a period of 5 days for any deaths or acute toxicity symptoms such as hypoactivity, piloerection, salivation and so on. The log dose/response (% death) curve may be plotted depending on the result obtained from which the median lethal dose (LD_{50}) of the plant extract would be obtained.

3.10 Phytochemical analysis of *Syzygium cordatum*

The phytochemical analysis was performed using the methods of Ikhiri *et al*. (1992) and Harborne (1984) to screen for chemical compounds including alkaloids, flanonoids, saponins,tannins, reducing sugars and triterpene steroids, present in the leaves of *Syzygium cordatum.*

3.10.1 Alkaloids

0.5 g of the dried and powdered *S. cordatum* leaves was boiled with 10 ml of dilute

hydrochloric acid (alcoholic) in a test tube for 5 min. The mixture was cooled and the debris allowed to settle. The supernatant liquid was filtered and 1ml of this filtrate taken into another test tube to which three drops of Dragendorff's reagent were added, shaken and observed for formation of an orange-reddish precipitate which wouldindicate the presence of alkaloids.

3.10.2 Triterpene steroids

1 g of the powdered leaf of *S. cordatum*was extracted for 24 hours in ether. The resultant solution was filtered and I ml of the filtrate evaporated to dryness. The resultant residue was re-dissolved in several drops of acetyl anhydride to which several drops of dilute sulphuric acid were added and observed for a green colour change which would indicate the presence of triterpene steroids.

3.10.3 Reducing sugars

0.2 g of the powdered leaf of *S. cordatum* was boiled with 5 ml water, the mixture cooled and filtered. To the filtrate, an equal amount of Fehling's solutions A and B in a ratio 1:1 was added and the mixture was heated on a water bath, and observed for a red-brown precipitate which would indicate the presence of reducing sugars.

3.10.4 Saponins

0.4 g of the powdered plant material was shaken with 4 ml of water in a clean test tube and observed for a persistent froth (foam) which would indicate the presence of saponins.

3.10.5 Tannins

0.2 g of the powdered plant material was boiled in 5 ml of water, the subsequent mixture was allowed to cool and filtered thereafter. To the filtrate, 2-3 drops of 5% ferric chloride solution were added and observed for a blue-black precipitate which would indicate the presence of tannins.
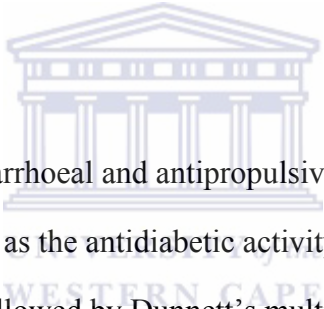
3.10.6 Quinones

10 g of *S. cordatum* leaf powder was moistened with a 10 % hydrochloric acid (HCl) solution and allowed to stand in ether: chloroform mixture (3:1, 40 ml). The mixture was filtered and 1 ml of the resultant extract treated with 1 ml 10 % sodium hydroxide (NaOH) solution. A red discoloration would indicate the presence of quinones.

3.10.7 Flavonoids

5 g of *S. cordatum* powdered leaf was boiled in water bath for 3 min, cooled and filtered. 3 ml of acid-alcohol (ethanol:water:concentrated HCL, 1:1:1), was added to 3 ml of the filtrate. A small piece of solid magnesium and 1 ml of t-amyl alcohol where also added to the mixture. A rose-orange or violet colour would indicate the presence of flavonoids.

3.11 Statistical Analysis

The data obtained from the antidiarrhoeal and antipropulsive activity and intestinal fluid accumulation experiments as well as the antidiabetic activity were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison (GraphPad Prism, version 5.0, GraphPad Software, Inc., San Diego CA 92130, USA) and presented as mean±standard error of mean (SEM). However, the data on the number of animals exhibiting diarrhoea were analysed using the Chi square test. In the above cases, *P* values of less than 5% ($P<0.05$), were considered to be significant.

3.12 Ethics clearance

The Ethics Committee of the University of the Western Cape approved the experimental Protocol (07/04/31) 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 Effect of leaf aqueous extract of *Syzygium cordatum* on castor-oil induced diarrhoea

0.7 ml of castor-oil, given orally, produced diarrhoea within 4 h in all the six animals used and also produced a considerable amount of stool. *Syzygium cordatum* (3.125-50 mg/kg, p.o.) significantly reduced the faecal output produced by castor oil. *S. cordatum* in all the doses used, reduced the feacal output from 100% produced by castor-oil to between 40 and 61%. *S. cordatum*(6.25-50 mg/kg, p.o.) significantly and in a dose dependent manner, delayed the onset of castor oil (0.7 ml, p.o)-induced diarrhoea. Doses of 3.125-50 mg/kg (p.o.) significantly reduced castor oil (0.7 ml, p.o.)-induced diarrhoeal episodes but did not significantly alter the number of animals exhibiting diarrhoea. Loperamide (20 mg/kg, p.o.) significantly reduced the feacal output produced by castor-oil (0.7 ml, p.o.) from 100% produced by castor oil to 8.7%. 20 mg/kg (p.o.) of loperamide profoundly delayed the onset of diarrhoea produced by castor-oil (0.7 ml, p.o.) and profoundly reduced the incidence of castor-oil (0.7 ml, p.o.)-induced diarrhoea by protecting 83% of animals against the diarrhoea. Loperamide (20 mg/kg, p.o.) profoundly reduced the number of castor-oil (0.7 ml, p.o.)induced diarrhoeal episodes by 96% (Table 1).

4.2 Effect of leaf aqueous extract of *Syzygium cordatum* on gastrointestinal transit of charcoal meal

The mean length of intestine travelled by 0.4 ml (p.o.) of charcoal meal in control mice pretreated with 0.3 ml (p.o.) of physiological saline was 87.73 ± 5.01%. Leaf aqueous extract of*Syzygium cordatum* (6.25-50 mg/kg, p.o.) significantly and dose dependently decreased the meanlength of intestine travelled by 0.4 ml (p.o) of the charcoal mean. The propulsion of the charcoal mean was inhibited by 21.25-50.93 at doses of 3.125-50 mg/kg (p.o.) of *S. cordatum*. Loperamide (20 mg/kg, p.o.) significantly reduced the mean length of intestine

travelled by charcoal meal (0.4 ml, p.o.). Loperamide (20 mg/kg, p.o.) inhibited the

propulsion of the charcoal meal by 79.06 (Table 2).

Table 1. Effect of leaf aqueous extract of *Syzygium cordatum* on castor oil-induced diarrhoea in mice

Treatment groups exhibiting (mg/kg)	Mass of stool (g) Mean±SEM	Faecal output (%)	Onset of diarrhoea (min) Mean±SEM	Number of diarrhoeal episode Mean ± SEM	Percentage diarrhoeal episode inhibition (%)	Number of animals diarrhoea
PS						
0.3 ml	3.67 0.29	100	22.17 2.10	25.17 0.93		6/6
Syzygium cordatum						
3.125	2.22**0.14	60.5	42.17 3.90	10.83*** 1.49	56.97	6/6
6.25	2.22**0.39	60.5	70.83* 5.67	12.17*** 2.40	51.65	6/6
12.50	1.80***0.27	49.0	71.50* 4.32	8.00*** 1.05	68.22	6/6
25.00	1.45***0.28	39.5	79.33* 5.97	5.67*** 1.30	77.47	6/6
50.00	1.83***0.21	49.9	105.17***19.60	9.83*** 0.96	60.95	6/6
Loperamide						
20.00	0.32***0.18	8.7	119.83***16.57	1.00*** 0.00	96.02	1/6[+]

*p<0.01, **p<0.005, ***p<0.001 vs castor oil (0.7 ml, p.o.) control. ANOVA (n=6).

[+]p<0.005 vs castor oil (0.7 ml, p.o.) control. Chi-squared test (n=6).

PS: Physiological saline

4.3 Effect of leaf aqueous extract of *Syzygium cordatum* on castor oil-induced intestinal fluid accumulation

The mean intestinal fluid volume produced by castor oil (1.5 ml, p.o.) in control animals

pretreated with physiological saline (0.3 ml, p.o.) was 1.42 ±0.19 ml. *Syzygium cordatum*

Table 2. Effect of leaf aqueous extract of *Syzygium cordatum* on gastrointestinal transit of charcoal meal in mice

Treatment groups (mg/kg)	Length of intestine travelled (%) Mean ± SEM		Percentage inhibition in length of intestine travelled (%)
PS			
0.3 ml	87.73	5.01	
Syzygium cordatum			
3.125	69.09	4.87	21.25
6.25	63.17*	4.57	28.00
12.50	62.34*	7.20	28.94
25.00	58.48**	5.95	33.34
50.00	43.05***	9.84	50.93
Loperamide			
20.00	18.37***	3.14	79.06

*$p<0.05$, **$p<0.005$, ***$p<0.001$ vs charcoal meal (0.4 ml, p.o.) control. ANOVA (n=6). PS: Physiological saline.

(3.125-50 mg/kg, p.o.)significantly reduced the mean intestinal fluid volume produced by castor oil (1.5 ml, p.o.). The mean intestinal fluid volume was reduced or inhibited by 41.55-59.15%. Loperamide (20 mg/kg, p.o.) significantly reduced the mean intestinal fluid volume produced by castor oil (1.5 ml, p.o.). The mean intestinal fluid volume produced by castor oil (1.5 ml, p.o.) was inhibited by loperamide (20 mg/kg, p.o.) by 52.82% (Table 3).

4.4 Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations (mmol/l) of normoglycaemic rats

0.3 ml (p.o.) of physiological saline did not significantly alter the blood glucose concentration

Table 3. Effect of leaf aqueous extract of *Syzygium cordatum* on castor-oil induced intestinal fluid accumulation in mice

Treatment groups (mg/kg)	Intestinal fluid volume (ml) Mean ± SEM		Percentage inhibition (%)
PS			
0.3 ml	1.42	0.19	
Syzygium cordatum			
3.125	0.58**	0.04	59.15
6.25	0.83*	0.15	41.55
12.50	0.80*	0.02	43.66
25.00	0.80*	0.05	43.66
50.00	0.73*	0.05	48.59
Loperamide			
20.00	0.67**	0.07	52.82

*p<0.01, **p<0.001 vs castor oil (1.5 ml, p.o.) control. ANOVA (n=6).

of fasted normal rats throughout the 4 h period of observation. *Syzygium cordatum* (3.125-6.25 mg/kg, p.o.) did not significantly affect the blood glucose concentration of fasted normal rats throughout the 4 h of observation. 12.50-50 mg/kg (p.o.) of *Syzygium cordatum* significantly reduced the blood glucose concentration of fasted normal rats from the second to the fourth hour of observation with a percentage maximal reduction of 28.60-32.79%. Chlorpropamide (250 mg/kg, p.o.) significantly reduced the blood glucose concentration of fasted normal rats from the first hour through the fourth hour of observation and the percentage maximal reduction was 43.26% (Table 4).

Table 4. Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations (mmol/l) of normoglycaemic (normal) rats

Treatment groups (mg/kg)	Before treatment 0 h	After treatment 1 h	2 h	4 h	Maximal reduction	Percentage maximal reduction (%)
PS						
0.3 ml	6.18 ± 0.24	5.82 ± 0.20	5.65 ± 0.18	6.10 ± 0.20	0.53	8.58
Syzygium cordatum						
3.125	6.67 ± 0.21	5.90 ± 0.13	5.57 ± 0.14	5.72 ± 0.27	1.10	16.49
6.25	6.75 ± 0.25	6.03 ± 0.21	5.37 ± 0.18	5.80 ± 0.24	1.38	20.44
12.50	6.33 ± 0.56	5.98 ± 0.22	4.73 ± 0.32*	4.52 ± 0.38**	1.81	28.60
25.00	6.80 ± 0.20	5.92 ± 0.32	4.75 ± 0.15*	4.60 ± 0.20**	2.20	32.35
50.00	6.80 ± 0.29	5.58 ± 0.29	4.62 ± 0.12*	4.57 ± 0.42**	2.23	32.79
Chlopropamide						
250	6.75 ± 0.40	4.85 ± 0.29*	3.83 ± 0.37***	3.87 ± 0.35***	2.92	43.26

*$p<0.05$, **$p<0.01$, ***$p<0.001$ vs physiological saline (0.3 ml, p.o.) control. ANOVA (n=6).

Values are expressed as mean ± SEM. PS: Physiological saline

4.5 Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations (mmol/l) of streptozotocin-treated diabetic rats

Streptozotocin (90 mg/kg, p.o.) raised the blood glucose concentration of fasted animals to 21.40 ± 1.02 mmol/l. 0.3 ml (p.o.) of physiological saline did not significantly affect the blood glucose concentration of fasted diabetic rats throughout the 4 h of observation. 3.125 mg/kg (p.o.) of *Syzygium cordatum* did not significantly alter the blood glucose concentration

of the fasted diabetic rats throughout the 4 h of observation. *Syzygium cordatum* (6.25 mg/kg, p.o.) significantly reduced the blood glucose of the fasted diabetic rats in the 4th h of observation with a percentage maximal reduction of 23.37%. *Syzygium cordatum* (12.50-25 mg/kg, p.o.) significantly reduced the blood glucose concentration of fasted diabetic rats from the 2nd to the 4th h of observation with a percentage maximal reduction of 33.18-35.54%. 50 mg/kg (p.o.) of *S. cordatum* significantly reduced the blood glucose concentration of fasted diabetic rats from the 1st h through to the 4th h of observation with a percentage maximal reduction of 33.37%. Chlorpropamide (250 mg/kg, p.o.) profoundly reduced the blood glucose concentration of fasted diabetic rats from the 1st h through the 4th h of observation with a percentage maximal reduction of 93.42% (Table 5).

4.6 Acute toxicity test

The leaf aqueous extract of *Syzygium cordatum* (200, 400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600 and 4000 mg/kg) administered orally to mice did not cause any death to or any acute toxicity symptoms in the animals in all the doses used. The highest dose tested being 4000 mg/kg should be the no-adverse-effect-level (NOAEL). The LD_{50} value for the plant species should, therefore, be greater than 4000 mg/kg (p.o.).

4.7 Phytochemical analysis

The phytochemical screening methods used to determine the active constituents present in the leaves of *Syzygium cordatum* tested positive for alkaloids, tannins, flavonoids, reducing sugars, triterpene steroids and saponins. However, no quinones were present (Table 6).

Table 5. Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations (mmol/l) of streptozotocin-treated diabetic rats

Treatment groups (mg/kg)	Before treatment 0 h	After treatment 1 h	2 h	4 h	Maximal reduction	Percentage maximal reduction (%)
PS						
0.3 ml	21.40 ± 1.02	23.67 ± 1.26	23.67 ± 0.89	23.88 ± 1.00		
Syzygium Cordatum						
3.125	23.37 ± 1.10	23.78 ± 0.66	23.20 ± 0.60	22.95 ± 0.47	0.83	3.49
6.25	22.68 ± 1.04	22.43 ± 0.52	20.77 ± 0.55	17.38 ± 1.11*	5.30	23.37
12.50	20.32 ± 0.59	21.28 ± 1.14	15.25 ± 0.78*	14.22 ± 1.80*	7.06	33.18
25.00	20.17 ± 0.57	21.41 ± 1.53	14.12 ± 0.12*	13.80 ± 0.93*	7.61	35.54
50.00	20.53 ± 2.08	14.42 ± 0.87*	14.07 ± 0.68*	13.68 ± 0.89*	6.85	33.37
Chloropropamide						
250	23.23 ± 0.94	15.55 ± 1.34*	6.38 ± 1.12*	4.05 ± 0.24*	19.18	93.42

*$p < 0.001$ vs streptozotocin (90 mg/kg, i.p.) control. ANOVA (n=6).

Values are expressed as mean ± SEM.

PS: Physiological saline

Table 6. Phytochemical screening of the leaf aqueous extract of *Syzygium cordatum*

Compound	Result
Tannins	+
Saponins	+
Alkaloids	+
Flavonoids	+
Triterpene steroids	+
Quinones	-
Reducing sugars	+

+ (positive): Present, - (negative): Absent

CHAPTER 5

DISCUSSION

Diarrhoea and diabetes are debilitating conditions known to afflict so many people worldwide and especially on the African continent (Weber,1976; Syder and Merson, 1982; Rother, 2007). Modern or orthodox medicines have provided adequate management and treatment of the conditions. It is a well established fact that about 80% of the population especially in developing countries rely on herbal medicines for their healthcare need. The reasons range from low cost to availability of these medicines and their use depends on ancestral experience. WHO has also urged various governments especially those of the developing countries, to include in their healthcare programmes those herbal medicines with proven safety and efficacy (Marin-Bettolo, 1980; Amos et al., 2001). *Syzygium cordatum* is one medicinal plant used by traditional medicine practitioners in South Africa to treat various ailments including diarrhoea and diabetes (Van Wyk et al., 1997). In order to scientifically scrutinized the claims by traditional medicine practitioners of the therapeutic success of the plant species, this project studied the antidiarrhoeal and antidiabetic activities of *Syzygium cordatum* in mice and rats respectively.

In this study, the plant extract, up to the highest dose (4000 mg/kg, p.o.) used in the acute toxicity test, did not cause any death to or acute toxicity symptoms in the mice. The LD_{50}, therefore, may be greater than 4000 mg/kg (p.o.). This relatively high LD_{50} shows that the plant extract is non-toxic and/or safe in mice. Traditional medicine practitioners are known to use the plant for treatment in the form of infusion (Van Wyk et al., 1997). However, this study did not ascertain the doses used by the practitioners for such treatments.

The pharmacological screening results obtained in the present study, show that *S. cordatum*

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(3.125-50 mg/kg, p.o.)antagonized the diarrhoea produced by castor oil (0.7 ml, p.o.).

Loperamide (20 mg/kg, p.o.) also antagonized the castor oil-induced diarrhoea in mice.

Castor oil, an irritant laxative, is thought to produce diarrhoea by being hydrolysed in the

upper small intestine to ricinoleic acid which exerts its effects by irritating the mucosa of the

gastrointestinal tract, resulting in an increase in intestinal motility (Altman, 2001).

Furthermore, ricinoleic acid has been shown to diminish the permeability of sodium and

chloride ions and also stimulate the release of prostaglandin, known to cause diarrhoea

(Gaginella and Phillips, 1975; Zavala et al., 1998).In addition, the works of Capasso et al.

(1994) and Mascolo et al. (1994), on the effect of N^G-nitro-L-arginine methyl esther, an

inhibitor of nitric oxide (NO) synthase, on the dissociation of castor oil-induced diarrhoea

and mucosal injury in rat, showed that nitric oxide may mediate castor oil-induced diarrhoea.

Loperamide, an opioid derivative and a standard antidiarrhoeal drug, is thought to decrease

intestinal motility by binding to mu receptors on neurons in the submucosal neural plexus of

the intestinal wall. This leads to the segmental contractions in the colon increasing, the

propulsive movement of the small intestine and colon being inhibited and the transit time of

the intestinal content being prolonged (Altman, 2001). Loperamide is also known to have an

antimuscarinic activity contributing to the inhibition of peristalsis by inhibiting contractions

in both the longitudinal and circular muscles (Altman, 2001; Camillen et al., 2002; Waller et

al., 2005). In this study, therefore, loperamide may be antagonizing castor oil-induced

diarrhoea by decreasing the intestinal motility. Similarly, *Syzygium cordatum* may be said to

exert its antidiarrhoeal activity by slowing intestinal motility.

In my study, the leaf aqueous extract of *S. cordatum* significantly antagonized the

gastrointestinal transit of charcoal meal and also significantly reduced the castor oil-induced

intraluminal accumulation of fluid volume. Similarly, loperamide significantly antagonized

the gastrointestinal transit of charcoal meal and also significantly reduced the castor oil-induced intraluminal accumulation of fluid volume. According to DiCarlo et al. (1994), agents that reduce intestinal motility and secretion may possess antidiarrhoeal activity. Furthermore, Nwafor et al. (2000) have shown that agents that suppress intestinal fluid accumulation may inhibit gastrointestinal functions. The above reports lend support to the suggestion that *S. cordatum* may be exerting its antidiarrhoeal activity by slowing intestinal motility.

The phytochemical analysis of the powdered leaf of *S. cordatum* carried out in this study showed that the plant species contains the following chemical components, tannins, saponins, alkaloids, triterpene steroids, flavonoids and reducing sugars. Several studies have shown tannins to have antidiarrhoeal activity. Tannin containing drugs have been used for the treatment of diarrhoea and other related disorders (Frei et al., 1998; Bruneton, 1999). Astringents such as tannins have been known since the last century to have antisecretory effect in the gastrointestinal tract and have been used to treat diarrhoea (Farthing). It is probable therefore, that the presence of tannins in the plant species as shown by the phytochemical analysis, may contribute to the antidiarrhoeal activity of *S. cordatum*.

Syzygium cordatum was shown in the present study to have an antidiabetic activity. Chlorpropamide, a sulphonylurea, and an oral antidiabetic agent used for the treatment of Type 2 or non-insulin dependent diabetes (NIDD), was used as a standard drug in this study. Chlorpropamide is thought to act by stimulating and increasing the release of endogenous insulin from the pancreatic beta cells of the Islet of Langerhans (Waller et al., 2005a; Rang et al., 2008). Diabetes, in this study, was induced using streptozotocin (STZ). Streptozotocin is thought to produce diabetes by a rapid depletion of pancreatic beta cells and thereby, reducing insulin release and causing hyperglycaemia (Mahomed and Ojewole, 2003). Thus,

the STZ-induced rat diabetes model has the hallmark of non-insulin dependent diabetes or Type 2 diabetes. In this study, *S. cordatum* (12.5-50 mg/kg, p.o.) and chlorpropamide (250 mg/kg, p.o.) significantly reduced the blood glucose concentration of fasted normal rats while 0.3 ml (p.o.) of physiological saline did not alter the blood glucose concentration of the fasted normal rats. Similarly, both the leaf aqueous extract of *S. cordatum* (12.5-50 mg/kg, p.o.) and chlorpropamide (250 mg/kg, p.o.) significantly reduced the glucose concentration of diabetic rats treated with streptozotocin. Since chlorpropamide used to treat diabetes, acts by stimulating insulin secretion from pancreatic bête cells and also promoting peripheral glucose uptake and utilization (Waller et al., 2005a; Rang et al., 2008), it is probable that *S. cordatum*, may be acting in a similar manner. The result obtained in this study is in agreement with the study of Musabayane et al. (2005) who showed that *S. cordatum* leaf extract significantly lowered the plasma glucose and hepatic glycogen levels in STZ-induced diabetic rats.

In this study, the phytochemical analysis carried out revealed the presence of alkaloids and flavonoids amongst other chemical metabolites in *S. cordatum*. Punitha et al. (2005) in their study with berberine, an alkaloid, and antidiabetic activity, showed that alkaloids have antidiabetic activity. Dineshkumar et al. (2010) in their study on the antidiabetic and hypolipidemic effects of mahanimbin from *Murraya koenigii* leaves also showed that alkaloids have antidiabetic activity. Flavonoids have also been shown by the study of Ghada et al. (2008) which investigated the antidiabetic and antioxidant activities of major flavonoids of *Cynanchum acutum* L., to have antidiabetic activity. Furthermore, Hule et al. (2011) in their study on the evaluation of the antidiabetic effects of *Elaeocarpus ganitrus* in experimental animals, showed that alkaloids and flavonoids have antidiabetic activities. It is probable therefore, that the flavonoids and alkaloids found in *S. cordatum* may be contributing to its antidiabetic activity.

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CHAPTER 6

CONCLUSION

The data obtained in this study indicate that *Syzygium cordatum* have both antidiarrhoeal and antidiabetic activities. This project was not set out to investigate the mechanisms of the antidiarrhoeal and antidiabetic activities of *S. cordatum*. However, various studies as shown above, have shown that castor oil-induced diarrhoea may also involve increase in electrolyte permeability, increased release of prostaglandins and nitric acid mechanism. It is probable therefore, that the antidiarrhoeal activity of *S. cordatum* may involve the inhibition of electrolyte permeability, inhibition of prostaglandin release and inhibition of nitric acid mechanism. The antidiabetic activity may be due to the plant species stimulating the release of insulin from the pancreatic beta cells since streptozotocin used to induce diabetes is known to act by rapidly depleting pancreatic beta cells and thus, reducing insulin release. The role of tannins in the antidiarrhoeal activity and alkaloids and flavonoids in the antidiabetic activity of *S. cordatum* also needs mentioning. The relatively high LD50 of the plant species shows that it is non-toxic and /or safe in mice. These data may justify the use of the plant species by traditional medicine practitioners in the treatment of diarrhoea and diabetes especially Type 2 or non-insulin dependent diabetes. However, further studies on the acute toxicity and the mechanisms of the antidiarrhoeal and antidiabetic activities of *S. cordatum* need to be carried out to enhance the safety and efficacy of the plant species.

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