GENERAL DISCUSSION AND CONCLUSIONS

Diabetes mellitus comprises a collection of heterogeneous diseases that differ in their etiological, clinical, and epidemiological characteristics, but have hyperglycaemia and glucose intolerance in concurrence, which are either due to insulin deficiency or to the impaired effectiveness of insulin's action or a combination of both (Roussel, 1998). Five major categories of diabetes mellitus are identified, namely; insulin dependent diabetes mellitus (IDDM, type 1 diabetes), non-insulin dependant diabetes (NIDDM, type II diabetes), impaired glucose tolerance, gestational diabetes mellitus and undiagnosed diabetes mellitus (Szava-Kovats and Johnson, 1997). The two dominant types are insulin dependent diabetes mellitus and non-insulin dependant diabetes mellitus. It is a chronic disease with major long-term implications, not only for the health and well-being of affected individuals, but also for costs to the society as a whole (Szava-Kovats and Johnson, 1997). As a chronic metabolic disorder, diabetes mellitus can affect all the body's major organ systems leading to complications that are a source of significant morbidity and premature mortality, making it a costly disease (Szava-Kovats and Johnson, 1997). According to the World Health Organization it will affect an estimated 366 million people in 2030 (Motala *et al.*, 2008).

Until the 1980s, the few reported studies on diabetes in Africa indicated a low prevalence of diabetes that is between 0 and 1.0 % in sub-Saharan Africa. However, over the past few decades, type 2 diabetes has emerged as an important medical problem in this region (Motala *et al.*, 2008). Recent estimates by the International Diabetes Federation indicated that the largest increase in the prevalence of diabetes is expected to occur in developing regions of the world, including Africa (Motala *et al.*, 2008). The projected increase in diabetes for Africa is from 3.1% in 2007 to 3.5% in 2025 with the corresponding increase in numbers from 10.4 to 18.7 million (International Diabetes Federation, 2006). Currently there are approximately 6.5 million diabetics in South Africa (Health 24, 2006).

Diabetes is a growing concern as African populations become Westernized, urbanized and adopt a Western diet that often leads to overweight and obesity. It was the sixth leading

natural cause of death in South Africa for the 2004 – 2005 period (South Africa Government Information, 2007).

Unfortunately, there is no cure yet for diabetes, but by controlling blood sugar levels through a healthy diet, exercise and medication, the long term complications of diabetes can be minimized. The progressive nature of the disease necessitates constant reassessment of glycaemic control in people with diabetes, and the appropriate adjustment of therapeutic regimes when glycaemic control is no longer maintained with a single agent. The addition of a second and third drug is usually more effective than switching to another single agent (Gerich, 2001).

The indigenous people of southern Africa have a long history of traditional plant usage for medicinal purposes and primary health care. A large portion of the population relies heavily on traditional healers and herbalist to meet primary health care needs. Although modern medicine may be available, herbal medicine has often maintained popularity for cultural and historical reasons. Four plant species, *S. pinnata*, *E. transvaalense*, *P. divaricata* and *E. undulata*, used for the treatment of diabetes by traditional healers and herbalist in South Africa, were validated for their hypoglycaemic activity and toxicity. The plant species were investigated by executing *in vitro* assays for hypoglycaemic, alpha-glucosidase and alpha-amylase, as well as, C2C12 myocyte, 3T3-L1 preadipocyte and Chang liver cell on the various plant extracts.

The *in vitro* antidiabetic screening of the four acetone and single ethanol plant extracts was carried out using a method developed by Van de Venter *et al* (2008). This method measures glucose utilization, which can be used for long-term exposure of the cells to the sample.

The alpha-glucosidase inhibiting activity of the four acetone plant extracts were tested by making use of the 96-well microplate assay developed by Collins *et al.* (1997). Alpha-glucosidase inhibitory activity was determined by measuring the release of *p*-nitrophenol from *p*-nitrophenyl-α-D-glucopyranose. The released *p*-nitrophenol yielded a yellow colour when the stopping reagent glycine was added.

. An adapted method described by Park and Johnson (1949), Bernfeld (1955) and Slaughter *et al*. (2001) was used to determine the alpha- amylase inhibiting activity of the four acetone plant extracts. The reduction of ferricyanide ions in alkaline solutions followed by the formation of Prussian blue (ferric ferrocyanide) was measured quantitatively as a basis for the estimation of glucose levels.

The alpha-glucosidase inhibitory activity for the four acetone plant extracts at five different concentrations demonstrated that the percentage of inhibition is concentration dependent. Three of the four acetone plant extracts namely *E. undulata*, *E. transvaalense* and *P. divaricata* inhibited alpha-glucosidase while *S. pinnata* showed no inhibition.

The *in vitro* assays in C2C12 myocytes, 3T3-L1 preadipocytes and Chang liver cells indicated that four of the five plant extracts tested, namely *S. pinnata* (ethanol/ acetone), *E. undulata* (acetone) and *E. transvaalense* (acetone), showed positive results in increasing glucose utilization whereas *P. divaricata* showed no ability. The cytotoxicity tests, however, revealed that the *S. pinnata* extracts (ethanol/acetone) were toxic to 3T3-L1 preadipocytes and that the *E. transvaalense* extract was toxic to Chang liver cells. These results were interpreted by making use of the scoring system developed by Van de Venter *et al*. (2008). According to this system *E. undulata* scored a +3 out of a maximum activity score of +6 and was therefore chosen for further analysis.

The crude acetone extract (35 g) of *E. undulata* was subjected to silica-gel column chromatography for the isolation of bioactive principals. Fractions containing the same compounds as determined by thin layer chromatography (TLC) were combined. Nine main fractions were obtained. Fraction II was chromatographed over a silica column and yielded (2500 mg; 71.4% yield)) lupeol **(3)**. Fractions III and IV were combinded and chromatographed over a sephadex column using ethanol and yielded a new α-amyrin-3O-ß-(5-hydroxy) ferulic acid **(1)** (14.28 mg; 0.40% yield) and betulin **(2)** (20.01 mg; 0.57% yield). Fraction VIII was chromatographed over a sephadex column and yielded (12.02 mg; 0.34% yield) epicatechin **(4)**.

An *in vitro* assay was executed on C2C12 myocytes on the four isolated compounds and revealed that lupeol (**3**) and α-amyrin-3O-ß-(5-hydroxy) ferulic acid (**1**) were inactive in lowering blood glucose levels, whereas betulin (**2**) was slightly active, and epicatechin (**4**) was active in lowering blood glucose levels. The alpha-glucosidase assay on α-amyrin-3O-ß-(5-hydroxy) ferulic acid (**1**), betulin (**2**), lupeol (**3**), and epicatechin (**4**) indicated that (**1**) inhibited alpha-glucosidase.

In conclusion, the results obtained from the various assays executed on the four plant extracts screened validated the use of all four plant extracts for the treatment of diabetes by traditional healers and herbalists. This conclusion was drawn as all the different plant extracts had the potential to lower blood glucose levels or to inhibit alpha-glucosidase. The assays, however, also revealed the cytotoxicity of the *S. pinnata* extracts to 3T3-L1 preadipocytes and of *E. transvaalense* to Chang liver cells, indicating that these plants should be used with caution in the treatment of diabetes. The application of different assays revealed that different plant extracts may function in different ways to lower blood glucose levels as indicated by the *P. divaricata* extract. No activity was detected in the C2C12 myocytes, 3T3-L1 preadipocytes and Chang liver cell assays, but it inhibited alpha-glucosidase and alph-amylase to some extent.

The fractionation of the crude acetone plant extract of *E. undulata* lead to the isolation, for the first time, of four compounds from *E. undulata,* namely; a new α-amyrin-3O-ß-(5-hydroxy) ferulic acid, betulin, lupeol and epicatechin. The consequent hypoglaecamic assays disclosed that the isolated epicathechin has the potential to lower blood glucose levels and the newly isolated α-amyrin-3O-ß-(5-hydroxy) ferulic acid has the potential to inhibit alpha-glucosidase. The present study is, according to our knowledge, the first report on the alpha-glucosidase inhibitory activity and glucose utilization in C2C12 myocytes of the crude acetone extract of *E. undulata* and its purified compounds.

Positive results were obtained from this study. It is however, recommended that further assays be performed on the four plant species evaluated for their hypoglycaemic activity and toxicity. This should be done by using an aqueous extracts as used by traditional healers and herbalists. It is also of great concern that in this study none of the

naphthoquinones, nor diospyrin or 7 methyl-juglone were isolated from the root bark of *E. undulata*. It is possible that the seasonally dependent interrelationship between the amount of 7-methyljuglone and lupeol reported by Khan (1985) in respect of *E. natalensis* may also be true for *E. undulata*. It is therefore necessary that this interrelationship be investigated as juglone is toxic (Thiboldeau *et al.* 1994; Ganapaty *et al.* 2004 and Kong *et al.* 2008)

It is also recommended that the extract of *E. undulata* should be tested *in vivo* in a rat or mouse model for its hypoglycaemic activity as the activity of some of the plant extracts may differ *in vitro* and *in vivo*. The purified compounds should be tested for their inhibition of the enzyme alpha-amylase and not just for alpha-glucosidase. Due to time constraints and insufficient purified compounds the above mentioned test could not be performed.

CHAPTER 6

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6.1 Congress attended

Deutschländer, M.S., Lall, N., Van de Venter, and S. Roux. 2006. Detection of anti-diabetic activity in South African plant extracts. IPUF. Botswana. July 2006.

Deutschlander, M.S., Lall, N. and Van de Venter. 2010. Isolation and identification of a novel anti-diabetic compound from *Euclea undulata* Thunb. SAAB. University of the North West, North West Province, South Africa. January 2010.

6.2 Publications from this thesis

Deutschländer, M.S., Lall, N. and Van de Venter, M. 2009. Plant species used in the treatment of diabetes by South African traditional healers: an inventory. *Pharmaceutical Biology.* 47 (4): 348 - 365

Deutschländer, M.S., Van de Venter, M. Roux, S, Louw, J. and Lall, N. 2009. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. *Journal of Ethnopharmacology*. 124 (3): 619-624