CHAPTER 8

GENERAL DISCUSSION

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Diabetes mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide (WHO, 1992). It is caused by deficiency in insulin secretion or impaired effectiveness of insulin's action or combination of both. The global prevalence of diabetes has risen drastically over the past two decades. Hence, necessitates constant re-assessment of glycaemic control in people with diabetes and appropriate adjustment of therapeutic regimens. This fact is supported based on the side effects such as gastrointestinal upsets and lactic acid intoxications shown by the synthetic anti-diabetic drugs (Li et al., 2004). Phytotherapy is an alternative medicine widely used as an herbal remedies for the management of diabetes. In developing countries about 80% of the populations are now using traditional medicine as their primary health care (Grover and Yadav, 2004). The people of the Eastern Cape province of South Africa have a long history of traditional medicine usage for the treatment of various ailments including diabetes mellitus (Van Wyk et al., 1997).

The uses of medicinal plants have long played a significant role in the lives of the Xhosa people, the primary inhabitants of the study area. Most rural communities of the region are regularly use wild harvested herbal medicines as an initial response to illness (Dold and Cocks, 2002). Although, there is an increasing interest in the use of medicinal plants for the treatment of diseases still it is necessary to provide scientific evidence to justify their ethnomedicinal usages. This view is supported by WHO (2007) who recommended further evaluation of traditional plants use for the treatment of diabetes mellitus.

Ethnobotanical information

Medicinal plants used for the management of diabetes mellitus were discovered during our ethnobotanical survey conducted at the study area. This study was carried out to documents the indigenous knowledge of experienced traditional herbalists considering the current rate of deforestation and the concurrent loss of biodiversity (Kambizi and Afolayan, 2001). The information on the plants used for the treatment of diabetes were obtained through structured questionnaire and interviewed with the traditional healers of the region. The plants were collected from the thick forest and field accompanied by local traditional healers. *Strychnos henningsii* Gilg (*Loganiaceae*) among other plants was repeatedly mentioned and highly recommended by the traditional healers and rural dwellers (Oyedemi et al., 2009). Thus, the choice of *Strychnos henningsii* for further study was based on the ethnomedical information obtained from indigenous Xhosa people of South Africa who have been using the plant for many years for the management of diabetes mellitus.

Antioxidant activity

The role of oxidative stress in the pathogenesis of diabetes mellitus is well established due to increased production of oxygen free radicals and a sharp reduction of antioxidant defense systems (Fridlyand et al., 2005). Prolonged exposure to oxidative stress could lead to oxidative damage which possibly contributes to pancreatic beta cell dysfunction (Song et al., 2007). Antioxidant agents of natural origin have attracted special interest because they can protect human body from oxidative damages caused by free radicals (John, 1991). Investigation into the antioxidant properties of *S. henningsii* both *in vitro* and *in vivo* was conducted to understand its mechanism of actions. The phytochemical analysis of the plant extract revealed the presence of bioactive compounds such as tannins, flavonoids, phenolics,

saponins, steroids and alkaloids. These compounds are known to be biologically active aiding the activities against diabetes through different mechanisms (Price, 1987; Teotia and Singh, 1997; Marles and Farnworth, 1995). These mechanisms include enzymatic degradation and scavenging of free radicals that are generated during or at the onset of diabetes (Penckofer et al., 2002). Further quantitative analysis of the plant extract showed high levels of phenolics contents followed by proanthocyanidins, flavonols and flavonoids. These compounds may account for the strong antioxidant activities observed both *in vitro* and *in vivo* studies. Thus antihyperglycemic effect observed in this study could be attributed to the strong antioxidant capacity demonstrated by this plant. The decreased level of lipid peroxidation in hepatotoxic treated rats induced with CCl_4 at the dose of 500 and 1000 mg/kg body weight suggest an efficient protective mechanism in response to reactive oxygen species that are generated in hepatotoxic rats.

Toxicological studies

The effect of oral administration of aqueous bark extract of *S. henningsii* at 250, 500 and 1000 mg/kg body weight was investigated on some haematological and biochemical parameters in male Wistar rats for 28 days. In this study, the levels of Hb, RBC, haematocrit or PCV, MCHC and LUC were not significantly altered. However, white blood cells and its differentials were appreciably affected at certain doses. The observed results indicate the influence of the extract on the immune system and phagocytic activity of the animals (Ghasi et al., 2000). The biochemical indices of liver and kidney damage monitored in this study are useful markers for assessing the functional capacities of the organs. A selective effect at specific doses was indicated in liver marker indices, the fact which is strengthened by the organ- body weight ratios of the liver while others did not show any evidence of extract

toxicity either by inflammation or cellular constriction (Idell et al., 1985). The alterations observed at some haematological and biochemical parameters at different dosages imply parameter and dose selective toxicity of the plant. The available evidence from this study implies that the extract may not be completely safe as oral remedy.

Antidiabetic and clinical significance

Diabetes mellitus is a complex syndrome involving decreased sensitivity of target tissues to insulin or deterioration of insulin secretion or both (Alberti and Zimmet, 1998). The antidiabetic and antilipidemic property of *S. henningsii* extract at the doses of 125, 250 and 500 mg/kg body weight were investigated in diabetic rats induced with streptozotocin - nicotinamide for 15 days. These chemicals are well known for the induction of diabetes mellitus in rats that resemble non obese type 2 diabetes (Tomonori et al., 2006). The plasma glucose level was very high with polyuria, weight loss and polyphagia to confirm diabetic state. The plant extract was able to reduce blood glucose levels either by potentiate insulin secretion from the existing beta cells or by its release from the bound form. Similarly, various complications associated with this disease were ameliorated after extract administration as shown in this study. Furthermore, the plant extract exhibited strong antilipidemic effect which might implies the presence of bioactive compounds that inhibit or activate some enzymes involves in lipid metabolism (Babu et al., 2007; Swantson-Flatt et al., 1990). In addition, the result obtained from this study on the clinical parameters provides evidence that administration of *S. henningsii* extract could reverse anemic condition as well as alleviating some disturbed markers of the liver. The elevated kidney markers were drastically reduced at certain doses while there was no significant improvement on the white blood cells and some

of its related indices. The study also revealed the ability of the extract to utilize glucose properly which is reflected on the body weight gain of the animals.

Probable mechanisms of action

The results obtained from this study has established that *S. henningsii* extract possessed strong glucose utilization on the 3T3-L1 adipocytes cell lines with a respond of 278.63% of the control at 12.5µg/ml. Meanwhile the extracts lacked efficacy on Chang liver cell lines with a respond of 103.54%. The extract displayed a promising effect on the liver cell lines when combined with metformin an antidiabetic standard drug. This showed a probable synergistic potential of the extract if combined with other plants of medicinal values. It may also justify the rationale behind the therapeutic benefits attributed to plant mixtures by the herbalists. Moreover, *S. henningsii* is a potent antidiabetic plant, hence the people using its bark for the management of diabetes, have possibly been relieved through the ability to stimulate glucose metabolism in the body. The cytotoxicity result revealed non toxic effects of the extract on both cell lines. The observation which contradicts the report of Deutschlander et al. (2009) who observed increased glucose utilization might be due to toxic effect on fat cells. In addition, the results obtained on α-glucosidase and amylase showed appreciable and weak inhibitory activities respectively. Based on previous phytochemical studies, it can be conclude that S. *henningsii* should be further investigated to identify the compounds responsible for its glucose metabolism especially on the adipocytes tissue

Protein glycation is one of the consequences of elevated blood glucose in diabetic patients (Ulrich and Cerami, 2001). The reaction of glucose and other reducing sugars with protein reversibly produces Amadori products and over a long period produced glycation end products (Singh et al., 2001). These reactions are greatly accelerated and are important in the

pathogenesis of diabetic complications (Kiho et al., 2000). The *in vitro* glycation was studied with bovine serum albumin as the model protein. In this study, the plant extracts significantly suppressed protein glycation at concentration of 1 mg/ml. The presence of high phenolics contents and the antioxidant potential as shown in this study could be potent inhibitors of both glycation reaction and the subsequent end products. The plant extract was effective at physiological concentrations and exhibited dose-response relationships. Oxidative stress is known to increase lipid accumulation which promotes generation of reactive oxygen species (Mika et al., 2008). The study revealed inhibitory activity of *S. henningsii* extract on hydrogen peroxide while there was no significant effect on lipid accumulation on 3T3-L1 cell lines using Oil Red O staining method. The scavenging activity against hydrogen peroxide might be responsible for the protection of lipid peroxidation a contributing factor to protein glycation. These results underline the importance of *S. henningsii* extract in diabetes and justify the possibility of therapeutic use of this plant for the prevention of diabetic complications by the traditional healers.

Conclusion

So far, this study has justified the ethnomedicinal usage of *Strychnos henningsii* for the management of diabetes mellitus. The antidiabetic activity of the aqueous bark extract of *S. henningsii* may be attributed to the presence of antioxidant compounds such as tannins, saponins, flavonoids, flavonols and other phytochemical compounds. These compounds have contributed to the antioxidants and free radical scavenging activity of this plant as a means of delaying, lessening and preventing the pathogenesis or secondary complications of diabetes mellitus. The beneficial effect of this plant on the red blood cells and its differentials together with liver and kidney functional indices of diabetic animals has given credence to the

ethnotherapeutic usage of this plant as an antidiabetic plant. Moreso, the significant *in vitro* glucose utilization on the 3T3-L1 adipocytes cell lines as well as protein glycation inhibitory activity also support the antidiabetic potential of *S. henningsii*. The toxicological evaluation of the plant extract did not have a remarkable adverse side effect on both haematological and biochemical parameters except at certain dosages.

Recommendation

The use of animals as a tool is an effective model that allowed determination of relative metabolic factors that play a significant role in disrupting the glycemic homeostasis of man. However, it will be of great importance if analysis of some enzymes, hormones, insulin signaling pathway and pancreatic beta cell mass and insulin secretion was investigated. Further studies on the exact mechanisms of action for the hypoglycemic effect of this plant need to be evaluated to understand its therapeutic target. Similarly, isolation and characterization of compounds responsible for the antidiabetic potentials of *Strychnos henningsii* extract would be of great importance.

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