CHAPTER 4

EFFECT OF *ALOE FEROX* MILL. AQUEOUS LEAF EXTRACT ON BIOCHEMICAL 
PARAMETERS IN LOPERAMIDE-INDUCED CONSTIPATED RATS

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Effect of Aloe ferox Mill. aqueous leaf extract on biochemical parameters in loperamide-induced constipated rats

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Running title: Toxic effect of A. ferox on constipated rats

Abstract

Aloe ferox Mill. is a widely used medicinal plant in South Africa for the treatment of many ailments including constipation. The present study evaluated the toxicological effect of aqueous leaf extract of the herb at 50, 100 and 200 mg/kg body weight for 7 days on the haematological parameters as well as liver and kidney function indices in loperamide–induced constipated rats. The extract did not cause any significant (p>0.05) effect on the kidney and liver–body weight ratio as well as the kidney function indices including serum levels of creatinine, uric acid, urea, calcium and potassium ions at all the dosages investigated. Whereas the serum levels of total protein, albumin, bilirubin and gamma glutamyl transferase (GGT) were not affected, the elevated activities of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) in the untreated constipated animals were normalized following treatment with extract. The data obtained with respect to the haematological analysis indicated that the extract at different doses had no significant (p>0.05) effect on the haematological parameters with the exception of lymphocyte count which was increased in the untreated constipated rats. This was however attenuated after administering the herb. The available evidence in this study suggests that A. ferox may be safe as an oral remedy for
constipation. Generally, the effect of the extract compared favourably well with senokot, a recommended drug for the treatment of constipation.

Keywords: Aloe ferox, marker enzymes, haematological parameters, function indices

Introduction

Constipation is a common health problem in which the affected person experiences uncomfortable or infrequent bowel movements (Wald, 2006). Some of the most common causes of constipation include medication, lack of exercise, lack of liquid and fiber in the diet, irritable bowel syndrome, problems with intestinal functions and changes in habits or life style such as traveling, pregnancy and old age (Longstreth et al., 2006). The menace has a substantial impact on morbidity and quality of life (Drossman et al., 1993), which may be characterized by unexplained abdominal pain, discomfort and bloating in association with altered bowel habits (Thompson et al., 1999). The use of chemotherapeutic drugs such as senna, correctol, exlax, senokot and gaviscon is very common as a means of treating constipation. However, the treatment should not last for more than seven days to avoid addiction (Meiring and Joubert, 1998).

Herbal remedies are commonly employed in developing countries for the treatment of various diseases, including constipation. The rationale for utilization of medicinal plants rested largely on the belief that they are safe and free of side effects (Leonardo et al., 2000). However, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies. Therefore, thorough scientific investigations of medicinal plants are imperative in order to validate their folkloric usage (Zhu et al., 2002).
Aloes have been used therapeutically since ancient times (Morton, 1961; Crosswhite, 1984). Majority of the scientifically based research has however, been done exclusively on two Aloe species namely Aloe vera and Aloe arborescens. Aloe gel from Aloes is sold commercially worldwide as an ingredient to a wide range of health care, cosmetic and therapeutic products (Rajasekaren et al., 2005). This commercial activity and the widespread use of Aloe in traditional medicine, has led to the upsurge of both clinical and biochemical research focusing on the toxicological and safety risk assessment of these plants.

Aloe ferox Mill. (Aspodelaceae), popularly known as Ikhala (Xhosa) and Cape aloe (English), is widely distributed throughout South Africa (Shackleton and Gambiza, 2007). It is an arborescent perennial shrub with a single stem of 2–3 m in height. The plant is crowned by a large rosette of numerous leaves which are glaucous and oval-lanceolate. The leaf is used as infusion or decoction against diarrhea and tooth abscesses (Githens, 1979), sexually transmitted infections (Kambizi et al., 2007), wound healing (Grierson and Afolayan, 1999), arthritis and rheumatism (Hocking, 1997; Van Wyk et al., 1997), conjunctivitis and eye ailment (Crouch et al., 2006), and as a laxative and insect repellant (Watt and Breyer-Brandwijk, 1962).

Our recent investigation revealed that oral administration of loperamide in rats inhibited intestinal water secretion and colonic peristalsis which extended the fecal evacuation time and delayed intestinal luminal transit. These are indications that the drug is capable of inducing constipation in rats. Further investigation also revealed the laxative effect of aqueous leaf extract of A. ferox in loperamide-induced constipated rats (Wintola et al., 2010a). However, to the best of our knowledge as at the time of carrying out this study, there appears to be dearth of information on the toxic effects of the extract during the treatment of constipation. Therefore, the
aim of this study was to provide information on the safety/toxicity risk associated with the use of
the plant in the treatment of constipation.

**Materials and methods**

**Chemicals**

Loperamide hydrochloride was a product of Sigma Chemical Co., St. Louis, MO, USA while Senokot was a product of Reckitt Benckiser Pharmaceutical (Pty) Ltd, South Africa. The assay kits used for biochemical assays were products of Randox Laboratories Limited, Ardmore, Co Antrim, United Kingdom. All other chemicals and reagents used were of analytical grade.

**Plant material**

Fresh, mature whole leaves of *Aloe ferox* were collected in Ntselamanzi Area of Nkonkobe Municipality in the Eastern Cape Province of South Africa. The plant was authenticated by Prof DS Grierson of Department of Botany, University of Fort Hare and a voucher specimen (Wintola Med.2009/01) was deposited at the Giffen Herbarium of the University.

**Preparation of aqueous extract**

The leaves of *A. ferox* were thoroughly washed with distilled water, cut into thin pieces and dried in the oven at 40°C for 24 h. The dried leaves were grinded into powder and 100 g of the material was extracted by shaking for 24 h in 1 L of distilled water on an orbital shaker (SO1 orbital shaker, Stuart Scientific, Stone UK). The extract obtained was filtered through Whatman No 1 (70 mm) filter paper and then Freeze dried (Vir Tis benchtop k, Vir Tis Company,
Gardiner, NY) to give a yield of 24.4 g. This was reconstituted in distilled water to give the required doses of 50, 100 and 200 mg/kg body weight for the experiment.

**Animals used**

Male albino rats (*Rattus norvegicus*) of Wistar strain with a mean weight of 140 ± 3.67 g were obtained from the experimental animal house of the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare, Alice. The animals were housed in clean plastic cages placed in a well ventilated house with optimum condition (temperature 23 ± 1°C, Photoperiod; 12hrs natural light and 12hrs dark; humidity; 45-50 %). They were acclimatized to animal house condition for 7 days and allowed free access to commercial pelleted rat chow (Pioneer Food (Pty) Ltd, Huguenot, South Africa) and water. The cleaning of the cages was done on a daily basis. The study was carried out following approval from the Ethical Committee of the University of Fort Hare on the use and care of animals.

**Induction of constipation in the rats**

Constipation was induced in the animals by oral administration of 1 mL loperamide (3 mg/kg body weight in 0.9% sodium chloride for 3 days) (Bustos et al., 1991), while the control rats were administered with the normal saline only. The passage of reduced, hard and dry fecal pellets indicated constipation in the rats.

**Animal grouping and extract administration**

The rats were grouped into six of four animals each. Group 1 (control) and Group 2 (constipated control) were administered with distilled water. Groups 3, 4 and 5 comprised
constipated rats treated with 50, 100, 200 mg/kg body weight/day of A. ferox extract respectively while Group 6 comprised constipated rats administered with senokot (600 µg/kg of body weight/day). The treatment continued for 7 days and the administration was done using metal oropharyngeal cannula.

**Collection of blood sample and isolation of organs**

After 7 days of extract administration, the rats were humanely sacrificed by ether anaesthetization and the neck area was quickly cleared of fur to expose the jugular vein. The vein, after being slightly displaced, was sharply cut with sterile surgical blade and an aliquot (2 mL) of the blood was collected into sample bottles containing EDTA (BD Diagnostics, Preanalytical Systems, Midrand, USA) for haematological analysis. Another 5 mL of the blood was collected and centrifuged at 1282 g x 5 min and the serum was carefully aspirated with a pasteur pipette into sample bottles for the various biochemical assays. The rats were quickly dissected and the whole liver and two kidneys excised, freed of fat, blotted with clean tissue paper and then weighed. The organ to body weight ratio was determined by comparing the weight of each organ with the final body weight of each rat.

**Determination of haematological parameters**

Using the standard method of Alexander and Griffiths (1993), the following analyses were carried out: red blood cell or red cells count (RBC), haemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), white blood cells (WBC) and white blood cell differential counts (Horiba ABX Diagnostics, Pentra 80 Montpellier, France).
Determination of biochemical parameters

The procedures described by Tietz (1995) were used for the determination of serum levels of creatinine, urea, calcium, uric acid, total bilirubin, total protein and albumin. The activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were determined in the serum according to the procedure outline in the Randox and Manufacturer’s assay kits.

Statistical analysis

Data were expressed as mean ± SD of four replicates and were subjected to one way analysis of variance (ANOVA) followed by Duncan multiple range test to determine significant differences in all the parameters. Values were considered statistically significant at p<0.05.

Results

The administration of aqueous leaf extract of A. ferox to constipated rats at all the dosages investigated did not affect the liver-body weight ratio as well as the serum levels of albumin, total protein, bilirubin and GGT (Table 1). The untreated constipated rats exhibited significant increase in the activities of serum ALP, ALT and AST. However, continuous treatment with the extract for 7 days restored the enzyme activities back to normalcy. On the other hand, the herb did not alter all the kidney function indices investigated in this study (Table 2).

In addition, the aqueous leaf extract of the plant had no significant effect on the haematological parameters investigated (Table 3). The only exception manifested in the
lymphocyte concentration which was significantly increased in the untreated constipated rats. The level was however, reverted back to normal following administration of the extract. Generally, the effect of the treatment with the extracts compared favourably well with senokot, a standard constipation drug.

**Discussion**

The use of herbal medicine is very popular in the developing countries of the world (Kim, 2005). Despite the widespread use, few scientific studies have been undertaken to ascertain the safety or toxicity risks of these herbal remedies. Previous investigation has revealed that oral administration of *A. ferox* produced laxative effect in loperamide-induced constipated rats (Wintola et al., 2010a). The present study has further shown that the extract may be relatively safe as an oral remedy.

According to Moore and Dalley (1999), an increase in organ-body weight ratio is an indication of inflammation while a decrease may be due to cell constriction. The non significant difference in the organ-body weight ratio observed in the treated constipated animals compared to the control implied that the extract did not cause any cellular constriction and/or inflammation in both liver and kidney (Schmidt et al., 2007). This finding compares favourably well with Amresh et al. (2008) in which the administration of *Cissampelos pareira* did not produce any effect on organ-body weight ratio investigated.

Albumin forms part of the total concentration of the blood serum protein and both are manufactured in the liver. Bilirubin, on the other hand, is a major product that results from the breakdown and destruction of old red blood cells. It is an important product with diagnostic values that is removed from the body by the liver (Chowdhury et al., 1989). Therefore, these
metabolites serve as good indicators to assess the functional capacity of the liver. The absence of significant effect on these indices in the constipated rats following treatment with *A. ferox* extract may be an indication that the normal functioning of the liver was not affected.

Measurement of serum enzyme activities is a valuable tool in clinical diagnosis because it provides information on the effect and nature of pathological damage to tissues. ALP is a marker enzyme often employed to assess the integrity of plasma membrane and endoplasmic reticulum (Shahjahan et al., 2004); while GGT is a membrane-localized enzyme that plays a major role in glutathione metabolism in the liver (Kaplan and Pesce, 1996). Damage to structural integrity of the liver is reflected by increase in the activity of ALP in the serum probably as a result of leakage from altered cell membrane structure (Yakubu et al., 2003). Therefore, the increase in serum activity of this enzyme in the untreated constipated rats observed in this study may be an indication of damage to the plasma membrane, leading to a compromise of membranal integrity. However, the non significant difference in GGT activity suggests that glutathione metabolism may not be affected by the extract.

The transaminases (AST and ALT) are well known enzymes used as biomarkers to predict possible toxicity to the liver (Rahman et al., 2001). The enzymes occupy a central position in the metabolism of amino acids by retaining amino groups during degradation of amino acids to form new ones. The elevation in the serum activities of these transaminases as observed in the untreated constipated rats suggested possible damage to the hepatocytes arising from change in membrane permeability (Latha et al., 1998). This may have a consequential effect on the metabolism and regulation of amino acids in the liver. Oral administration of aqueous extract of *A. ferox* lowered the elevated activities of the enzymes in the constipated
animals comparable to the control. This indicated the non toxic nature and protective action of the extract in reversing liver due to constipaton induction.

Renal function indices such as serum electrolytes, urea, creatinine and uric acid can be employed to assess the functional capacity of the kidney (Sunmonu and Oloyede, 2006). Urea is the end product of protein metabolism and its concentration is influenced by the rate of excretion. Creatinine is the waste product of muscle metabolism while uric acid is the major catabolic product of purine metabolism (Tietz, 1986). The serum levels of these metabolites have been used as important indices for the evaluation of toxic effects of chemicals on the kidney (Davis and Bredt, 1994). In this study, treatment of the constipated rats with the extract did not alter these kidney function indices. Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids. They comprise the most single important factor in the transfer and movement of electrolytes between the extracellular and intracellular compartment (Zilva et al., 1991). The absence of significant effect of A. ferox on calcium and potassium ions may be an indication that these ion dependent processes were not adversely affected. Similar observation was reported by Odeyemi et al. (2009) while studying the toxicological effect of Mentha longifolia oil on Wistar rats. This may be an indication that the herb did not alter normal body homeostasis which may be indicative of nephroprotective effect of the herb.

Assessment of hematological parameters is used to determine the extent of deleterious effect of a foreign compound including plant extracts in the blood. It can also be used to explain blood- relating functions of plant extracts or its products (Yakubu et al., 2007). In this study, the absence of significant alteration in the haematological parameters of constipated rats compared with the control may be an indication that the extract has no adverse effect on the blood system. The untreated constipated animals exhibited a rise in the lymphocyte counts which are the main
effector cells of the immune system. The administration of *A. ferox* extract was however effective in reverting the level back to near normal. This may be an indication that the herb did not affect the haematological parameters at the doses investigated.

**Conclusion**

It can therefore be concluded from this study that treatment of constipated animals with the aqueous extract of *A. ferox* did not produce any significant toxicological effect on the haematology as well as liver and kidney function indices. The effect of the extract also compared favourably well with senokot, a recommended drug for the treatment of constipation. Therefore, the herb may be relatively safe as an oral remedy for constipation.

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References


