EFFECT OF AQUEOUS EXTRACT OF SOLANUM TUBEROSUM (ST), IPOMOEA BATATAS (IB) AND DIOSCOREA ALATA L. (DA) ON FOOD INTAKE, FASTING BLOOD GLUCOSE PROFILE AND BODY WEIGHT IN MALE WISTAR RATS

A DISSERTATION

SUBMITTED TO THE UNIVERSITY OF NIGERIA, NSUKKA
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR AWARD OF THE DEGREE OF MASTERS OF SCIENCE (M.Sc.) IN HUMAN PHYSIOLOGY

BY

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FEBRUARY, 2012
ATTESTATION PAGE

We hereby attest that this work titled “Effect of Aqueous Extract of Solanum tuberosum, Ipomoea batatas and Dioscorea alata L. On Food Intake, Fasting Blood Glucose and Body Weight in Male Wistar Rats” was carried out under our supervision.

.......................... ..........................
Dr U.S.B Anyaehie       Dr E.E. Iyare
(Supervisor)            (Co-Supervisor)
CERTIFICATION PAGE

I, Olubobokun Titilope H., a postgraduate student in the Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus and with Reg. No. PG/MSC/09/54097 certifies that this work titled “Effect of Aqueous Extract of Solanum tuberosum, Ipomoea batatas and Dioscorea alata L. On Food Intake, Fasting Blood Glucose and Body Weight in Male Wistar Rats” was carried out by me. The work in this dissertation is original and has not been submitted in part or full for any other diploma or degree of this or any other University.

........................................

Olubobokun Titilope H.
DEDICATION

I dedicate this work to the Almighty God- the giver of life, Favor and journey mercies.

To my dear parents Mr and Mrs Olubobokun and my love Olatunbosun Oladapo, for their patience, love and understanding.
ACKNOWLEDGEMENT

I really appreciate my Supervisors, Dr U.S.B Anyaehie for his time, guidance, energy as well as constructive criticism and also greatly indebted to Dr E.E. Iyare for his immense and sustained interest in my success and for the wonderful role played in this project work.

I also appreciate all my lecturers; Mr D.C Nwachukwu, Dr U.I Nwagha, Dr. I.A. Orizu and Mr Nweke for their tutoring and mentoring in the course of my study in this department.

I also want to appreciate all my course mates who made my stay in this department worthwhile (Aizenabor Glory, Udekweweze Damian, Ilokanuno Chinedu and Chime Paschal ) and the Staff of the Department of Physiology, University of Nigeria, Enugu Campus.

My regards also goes to the wonderful friends: Okoro Uchechi for being a wonderful roommate, Ezeh Chigozie and Briggs Nengi for helping me during the course of my practical works. Ugwuegbe Ugochukwu, Chukwu Peter for their love and encouragement, Gbenga and Kehinde for accommodating me, and all members of PGSF, UNEC for their support and prayers. Thanks all for making my stay here a wonderful and memorable one.

My regards also goes to my parents and siblings, for their financial, spiritual and moral support. Thanks all for being a wonderful part of my life.

I must also appreciate the Olatunbosuns for their great love and assistance showered on me.

Finally, I sincerely appreciate Olatunbosun Oladapo Emmanuel for his love, financial support and encouragement all through the course of this study and at all times. You always give me a great reason to go on. Thanks for being there for me.
To God be all the Glory; for He made all things possible.

**ABSTRACT**

Increased awareness of association between chronic disease and excess body weight has motivated consumers to seek weight loss and management aids that are safe and effective. Potato, a common starchy tuber in our environment is believed to contain substances that can help maintain body weight without side effects. Water yam is also believed to possess both antioxidant and hypoglycemic constituent which might help maintain blood glucose level which might result in weight loss. This present study was carried out to determine the effect of the aqueous extract of *Solanum tuberosum* (ST), *Ipomoea batatas* (IB) and *Dioscorea alata L.* (DA) on food intake, fasting blood glucose and body weight in male wistar rats. 50 acclimatized male wistar rats weighing 170g-180g were used for this study. The extract was prepared by homogenizing the tubers and then centrifuged, heated to inactivate the enzymes, centrifuged again, after which it was evaporated to dryness and the residue reconstituted in 0.9% Nacl (1g in 10ml of normal saline). The animals were randomly assigned into 10 groups of 5 rats each. Group 1 served as the control and were fed with 0.3ml of normal saline, Group 2-4 were fed with ST extract, group 5-7 were fed with IB extract and group 8-10 were fed with DA extract at 100, 200 and 300mg/kg body weight (bwt) respectively. In the ST group, there was no significant difference between food intake and blood glucose level of the group fed with 100 and 200mg/kg bwt, but there was an increase in weight gain in both groups (p<0.05). However, at 300mg/kg bwt, there was a significant decrease in food intake and glucose level (p<0.05) which probably might have led to the weight loss observed. Similar observations were obtained for the ST and DA groups respectively. IB extract was observed to significantly reduce food intake at 200 and 300mg/kg bwt and a decrease in blood glucose level (p<0.05) which probably resulted in the
weight loss observed in this group. Results showed that in the extract-treated groups, the food intake, blood glucose level and body weight were significantly reduced at p<0.05 when compared with the control group. These findings suggest that the consumption of potato and water yam can cause reduction in food intake probably by: increasing satiety (feeling of fullness) and reduction in weight gain probably by using up the body’s reserve of fat as a result of the low blood glucose induced by them. It is recommended that consumption of potato and water yam could be incorporated as an ingestible diet in reducing excessive body weight gain so as to control overweight and obesity.
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CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

Excessive body weight gain could ultimately lead to overweight or obesity. Obesity specifically refers to an excessive amount of body weight that may come from muscles, bone, adipose (fat) tissue, and water or excessive accumulation of body fat (Flegal, 2010). Obesity in its gross manifestation poses a real threat to health. The etiology of obesity is multifactorial but can be abridged to accommodate two dominant categories: physiological and environmental elements. The physiological aspects of obesity include body metabolism, hormones and the neurological components of appetite regulation (Karon, 2007). Environmental causes include the abundance of high calorie foods in the Western diet, as well as the prevalence of increasingly sedentary lifestyles due to technological advances (Karon, 2007).

The worldwide prevalence of obesity has reached epidemic proportions (James et al, 2001), making the research for effective solutions to reduce obesity a public health priority. Obesity and overweight are associated with the development of life-threatening chronic conditions and an increased risk for cardiovascular disease, metabolic syndrome, hypertension, stroke, diabetes, osteoarthritis, sleep apnea, depression, gallbladder disease, type 2 diabetes, and certain types of cancer (Lenz and Hamilton, 2004; Must et al, 1999). Therefore viable and sustainable solutions for effective weight loss and prevention of weight gain are urgently needed.

Increased awareness of association between chronic disease and excess body weight has motivated consumers to seek weight loss and management aids that are safe and effective
without side effects because the safety of weight management products is one of the top concerns among consumers. (Jefferson, 2000). Pharmalogical agents and over the counter supplements designed to suppress hunger or decrease appetite, block fat absorption, or reduce stomach volume have had limited success and are often accompanied by numerous side effects such as dizziness, increased blood pressure or heart rate, chest pain, heart attack, stroke and seizure (Pittler and Ernst, 2004). Certain appetite suppressants, thermogenic formulas, fat blockers and other weight loss products contain ingredients known to cause adverse effects or interact adversely with certain medications (Dyck, 2000). Therefore, there is a large and continuously growing market for other dietary regimen for appetite control.

One part of the solution could be for individuals to increase consumption of complex carbohydrates because they have nutritional attributes thought to benefit weight control, including slowly digestible carbohydrates, high fibre and protein contents, and moderate energy density. Generally, selection of a diet high in fibre, low in energy density and glycemic load, and moderate in protein is thought to be particularly important for weight control (Abete et al, 2010). An essential component of weight management is appetite control and one way of controlling appetite is to maintain a feeling of satiety (Holt et al, 1995). Enhanced satiety with feelings of fullness for longer periods can result in less food consumed at meals and reduced snacking between meals, thereby promoting weight loss.

The potato is a starchy tuberous crop which is common in our environment, it is best known for its carbohydrate content (approximately 26 grams in a medium potato). The predominant form of this carbohydrate is starch (Cummings et al, 1996) and this starch is said to be resistant to digestion in the intestine. Protease inhibitor 2 derived from potato (PI2) is claimed to reduce
appetite and food intake, stimulate the satiety hormone cholecystokinin (CCK) and lower postprandial glucose peaks when taken before a meal. The presence of PI2 in the intestine could result in increase in the level of CCK (Liddle, 1997) naturally released in response to a meal (Badman and Flier 2005). Following consumption of a meal, CCK, a well-characterized gut peptide hormone is secreted into the bloodstream by endocrine cells (Crawley and Corwin, 1994; Liddle, 2000). This hormone then acts on various tissues including the gastrointestinal tract, where it stimulates enzyme secretion and delays gastric emptying, creating a feeling of fullness (Beglinger, 1994). CCK also acts on the brain leading to feelings of satiety (Crawley and Corwin, 1994). Therefore, this plant may lead to the development of a dietary regimen which could serve as an ingestible diet in reducing excessive body weight gain so as to control overweight and obesity.

1.1 JUSTIFICATION FOR THE STUDY

Overweight and obesity are global epidemic and are associated with the development of life-threatening chronic conditions, which have made consumers to seek for weight loss supplements. Current weight reducing agents and over the counter supplements designed to suppress hunger and control obesity have had limited success and are often accompanied by numerous side effects (Pittler and Ernst, 2004). Therefore, there is a large and continuously growing market for other ingestible approaches for appetite control. PI2 derived from potato is claimed to reduce appetite and food intake, stimulate the satiety hormone CCK and lower postprandial glucose peaks when taken before a meal. Therefore the purpose of this study is to determine if potato could be incorporated as one of the ingestible approaches to reducing excess weight gain and obesity in our environment. Water yam
has been observed to possess hypoglycemic and antioxidant potential, most notably carotenoids and anthocyanins like potato (Brown et al., 2004), but there is paucity of data on its effect on food intake and body weight changes, therefore this study will investigate this and add to knowledge.

1.2 AIM OF THE STUDY

The aim of this study is to determine the effect of the aqueous extract of *Solanum tuberosum*, *Ipomoea batatas* and *Dioscorea alata L.* on food intake, fasting blood glucose and body weight in male wistar rats and if it could be incorporated as an ingestible approach in reducing excessive body weight gain which may be useful in controlling overweight and obesity.

1.3 OBJECTIVE OF THE STUDY

To determine the effect *Solanum tuberosum*, *Ipomoea batatas* and *Dioscorea alata L.* on:

1. Food intake
2. Fasting blood glucose
3. Body weight
1.4 OPERATIONAL DEFINITION OF TERMS

1. Food intake: The amount of feed eaten daily by each group. It is calculated by subtracting the final from the initial.

2. Body weight: The mean body weight of each group.

3. Body weight changes: The changes observed in the body weight every week. It is calculated by subtracting the body weight at the beginning of the week from the body weight at the end of the week.

4. Fasting blood glucose level: The mean glucose level recorded after an overnight fasting.

5. Potato: refers to both type of potato used in this study, i.e. irish and sweet potato.
CHAPTER TWO

LITERATURE REVIEW

2.0 OVERWEIGHT AND OBESITY

Obesity is a chronic condition defined by excess body fat which results from insufficient caloric expenditure and excessive caloric intake or defined as a body mass index of 30 kg/m$^2$ (Grundy, 1998). Certain people have a genetic predisposition to obesity; however environment and lifestyle influence overeating and the lack of physical activity to a greater extent than genetics.

Obesity is a multifactorial disorder associated with a host of comorbidities including hypertension, type 2 diabetes, dyslipidemia, coronary heart disease, stroke, cancer (e. g, endometrial, breast, and colon), osteoarthritis, sleep apnea, and respiratory problems. The mechanisms contributing to obesity are complex and involve the interplay of behavioral components with hormonal, genetic, and metabolic processes (Westerterp-Plantenga et al; 2005 Tremblay F et al, 2007). Obesity is largely viewed as a lifestyle-dependent condition with two primary causes: excessive energy intake and insufficient physical activity. Although both factors must be considered in any individually tailored intervention. The western diet, characterized by elevated intakes of red meat, saturated fat and refined carbohydrates and low intakes of fibre and calcium, is associated with increased risk for insulin resistance and obesity (Van Dam et al, 2002). The obesity epidemic also closely corresponds to the consumption of diets with a high glycemic load (simple carbohydrates such as sugars, white bread, soft drinks, e.t.c) though the role of simple carbohydrates in weight gain and obesity is not completely clear; however evidence suggests that they are closely related (Brand Miller et al, 2002; Jenkins et al, 2002).
The body’s ability to maintain energy and nutrient balance is dependent upon a complex regulatory system that allows the body to achieve and maintain a steady state of energy and nutrient balance. Sustained increases in energy intake can lead to increased body weight and an accompanying increase in energy expenditure. Body weight will stabilize and energy balance will be achieved when energy expenditure is increased to the level of energy intake. Conversely, a decrease in energy intake will disrupt energy balance and produce a loss of body weight accompanied by a reduction in energy expenditure. Body weight will stabilize when energy expenditure declines to the level of energy intake.

Weight loss occurs when an individual is in a state of negative thermodynamic flux, when the body is exerting more energy (in work and metabolism) than it is consuming (i.e. from food or other nutritional supplements), it will use stored reserves from fat or muscle, gradually leading to weight loss. Therapeutic weight loss, in individuals who are overweight or obese, can decrease the likelihood of developing diseases such as diabetes (Butler, 2001), heart disease, high blood pressure (Lean, 2000), osteoarthritis, and certain types of cancer. Methods of weight loss which include use of drugs and supplements that decrease appetite, block fat absorption, or reduce stomach volume tend to be accompanied by numerous side effects such as dizziness, increased blood pressure or heart rate, chest pain, heart attack, stroke and seizure (Pittler and Ernst, 2004). Abstinence from food is not a good approach to weight loss; it leads to “hunger” which is the biological drive that forces one to search for food. It determines when one eats and how much to eat (Blundell et al. 1996), sometimes leading to eating more than one used to or need. It was thought that energy restriction might be the most effective way for individuals with insulin resistance, obesity and/or non-insulin dependent diabetes mellitus to improve their glucose control and plasma lipid profile and lose weight (Kelley et al, 1993). However, weight loss by
this method also reduces satiety and increases appetite, which makes adherence to an energy-restricted diet difficult (Cummings et al, 2002).

Attention to diet in particular can be beneficial in reducing the impact of diabetes and other health risks of an overweight or obese individual. Altering the macronutrient composition of the diet may be a more effective way to increase the rate of fat and weight loss and increase insulin sensitivity (Crovetti et al, 1998).

2.1 COMPLEX CARBOHYDRATES

Carbohydrates are the main source of energy in most human diets. Chemically, carbohydrates include a range of components such as polyhydroxy, aldehydes, ketones, alcohols and acids, as well as their derivatives and polymers, e.g. starch and other polysaccharides. The chemical classification of carbohydrates is usually based on molecular size and monomeric composition, three principal groups being sugars (1–2 monomers), oligosaccharides (3–9 monomers) and polysaccharides (10 or more monomers) (FAO/WHO, 1998). Due to the chemical diversity of carbohydrates, specific methods for analysis of various carbohydrates in foods have become routinely available.

Nutritionally, it is important to differentiate between two broad categories of carbohydrates:

1. Simple carbohydrates: those digested and absorbed in the human small intestine, providing carbohydrates to body cells and those passing to the large intestine, forming substrate for the colonic microflora (Asp, 1996; Englyst and Englyst, 2005).

2. Complex Carbohydrates: The nondigestible (“unavailable”) carbohydrates are commonly referred to as “dietary fibre”. Chemically, dietary fibre is also a carbohydrate (EFSA, 2007).
Consumption of both simple and complex carbohydrates raises blood glucose levels, which in turn stimulate insulin release by the pancreas, hypersecretion of insulin leads to a rapid drop in blood glucose level, prompt onset of hunger, and the desire to eat soon after the initial meal. This cycle leads to more frequent meals, overall increase in caloric intake and obesity (Hill et al, 1990), but long term animal models have shown that diets high in simple carbohydrates are more rapidly absorbed into the blood stream than complex carbohydrates causing hyperglycemia and hypersecretion of insulin which promote the growth of fat tissue, visceral fat stores and higher concentrations of lipogenic enzymes and can promote weight gain than do moderate calorie, complex carbohydrate diets (Brand- Miller et al, 2002).

Studies have also shown that diets based on low fat foods that produce a low glycemic response may enhance, minimize postprandial insulin secretion (Slabber et al, 1994). Postprandial blood glucose levels are influenced by the rate of gastric emptying (Lefebvre, 1999). Reducing the rate at which carbohydrates are digested and thus delaying glucose absorption may aid in reducing hyperglycemia. Therefore, controlling gastric emptying by dietary and pharmacological means in order to minimize postprandial glucose represents a new approach to glycemic control.

Complex carbohydrates are said to be made up of dietary fibre and resistant starch.

### 2.1.1 Dietary Fibre

The term “dietary fibre” was originally defined as “that portion of food which is derived from cellulose which is digested very poorly by human beings” (Trowell, 1972). The recognition that polysaccharides added to foods, notably hydrocolloids, could have effects similar to those originating from plant cell walls led to a redefinition of dietary fibre to include “polysaccharides and lignin that are not digested in the human small intestine” (Trowell et al., 1976).
The U.S. Food and Nutrition Board (FNB) defines “total dietary fibre” as the sum of “dietary fibre”, consisting of non-digestible carbohydrates and lignin that are intrinsic and intact in plants, and “functional fibre”, consisting of isolated, non-digestible carbohydrate components with demonstrated beneficial physiological effects in humans (IoM, 2005). The rationale behind this differentiation is that there is epidemiological evidence for beneficial effects of foods naturally high in dietary fibre, such as whole-grain cereals, some fruits and vegetables, and that dietary fibre can be regarded as a marker of such foods.

The interest in defining and quantifying dietary fibre in foods lies in the physiological effects that are associated with their consumption, which include decreased intestinal transit time and increased stool bulk, reducing blood total and/or LDL cholesterol concentrations, and reducing post-prandial blood glucose and/or insulin concentrations, among others (AFSSA 2002; IoM, 2005; Mann et al, 2007).

The components included in dietary fibre are by definition resistant to hydrolysis and absorption in the small intestine, it is important to the health of the digestive system (AACC, 2001). They pass the upper gastro-intestinal tract and enter the colon substantially unmodified. Viscous, water-soluble fibre such as β-glucans and pectin can modify blood glucose response and total and LDL-cholesterol concentrations by interfering with digestion and absorption of glycemic carbohydrates and cholesterol and/or bile acids, respectively (AFSSA, 2002). Inhibitory effects on mineral absorption, i.e. of iron, zinc and calcium, have been attributed to fibre-associated complexing compounds, notably phytic acid in cereals and leguminous seeds. Dietary fibre components are subject to more or less extensive anaerobic fermentation by the colonic microflora. The extent of fermentation is dependent on both substrate and host factors, e.g. molecular structure and physical form of the substrate, bacterial flora and transit time. Less
fermentable types of fibre, such as in lignified outer layers of cereal grain, generally have the most prominent faecal bulking effects due to their ability to bind water in the distal colon. Fermentable fibre also contributes to the faecal bulk through increased microbial mass (IoM, 2005).

A research also found a link between higher fibre diets and reduced cancer incidence. Diets high in complex carbohydrates may play a positive role in cardiovascular disease and diabetes management (GR, 2006). They are digested at a slower rate than simple carbohydrates. This slower digestion offers a more continual and stable flow of energy. They provide energy, fibre, vitamins, and minerals and are low in fat. Complex carbohydrates are a source of energy, short and long term, and they help to stimulate metabolism. When metabolism is sped up, calories are burned better, which can lead to hunger between meals. A diet based on complex carbohydrates with the addition of fruits and vegetables will cause effortless, permanent, healthful weight loss without restricting food or causing hunger.

### 2.1.2 Resistant Starch

Resistant starch is the starch that is ‘resistant’ to enzymatic digestion in the small intestine. Resistant starch is found naturally in foods such as legumes, bananas (especially under-ripe, slightly green bananas), potatoes, and some unprocessed whole grains. Natural resistant starch is insoluble, fermented in the large intestine and a prebiotic fibre (i.e., it may stimulate the growth of beneficial bacteria in the colon). Other types of resistant starch may be soluble or insoluble, and may or may not have prebiotic properties (Higgins, 2004). Resistant starch appears to exert beneficial effects within the colon as well as body wide. Health benefits in the colon include enhanced laxation, extensive fermentation and the production of important short chain fatty acids.
and increased synthesis of a variety of “good” bacteria (Cummings et al., 1996; Nofrarias et al. 2007; Murphy et al. 2008) both of which are believed to protect the colon from harmful microorganisms and even cancer (Hylla et al. 1998). Systemic effects of resistant starch include improvements in glucose tolerance and insulin sensitivity, reductions in blood lipid levels, increases in satiety and potential uses in weight management (Higgins, 2004, Bodinham et al., 2010). Potatoes have been found to possess a fairly great quantity of resistant starch. The amount of resistant starch found in potatoes is highly dependent upon processing and preparation methods. For example, cooking and then cooling potatoes leads to nearly a two-fold increase in resistant starch (Englyst et al. 1992, Murphy et al. 2008). Even processed potatoes (e.g., potato flakes) appear to retain a significant amount of resistant starch with the potential to confer health benefits.

2.2 POTATO

The potato is a starchy, tuberous crop from the perennial Solanum tuberosum of the Solanaceae family (also known as the nightshades). In terms of nutrition, the potato is best known for its carbohydrate content (approximately 26 grams in a medium potato). The predominant form of this carbohydrate is starch (Cummings et al, 1996). A small but significant portion of this starch is resistant to digestion by enzymes in the stomach and small intestine, and so reaches the large intestine essentially intact. This resistant starch is considered to have similar physiological effects and health benefits as fibre: It provides bulk, offers protection against colon cancer (Hylla et al, 1998 ), improves glucose tolerance and insulin sensitivity, lowers plasma cholesterol and triglyceride concentrations, increases satiety, and possibly even reduces fat storage (Raben et al, 1994).
Potato is used in a number of ways. It provides the body with an essential source of fuel and energy, which one need even when dieting. As a rich carbohydrate source, they help to fuel all reactions in the body needed for movement, thinking, digestion and cellular renewal. It also provides vitamins such as Vitamin C which act as antioxidant (Cotton et al., 2004) and Vitamin B6, a substance needed for cellular renewal, a healthy nervous system and a balanced mood.

The Agricultural Research Service in Navarre, America, has identified 60 different kinds of phytochemicals and vitamins in potato skins. Many of these were flavonoids, which help protect against cardiovascular-disease by lowering levels of bad LDL-cholesterol and keeping arteries fat-free. Potato provide one of the most concentrated and affordable sources of potassium significantly more than those foods commonly associated with being high in potassium (e.g., bananas, oranges, mushrooms, etc.) (Drewnowski et al., 2011). Potatoes is also said to contain an assortment of phytochemicals with antioxidant potential, most notably carotenoids and anthocyanins (Brown et al. 2001). Potato have high amount of polyphenolic compounds which have an important role in stabilizing lipid oxidation and are associated with antioxidant activity that the phytochemicals from potato have significant effects in vitro on antioxidant and anticancer activities (Huang, 2004).

### 2.2.1 Constituents Of The Potato

The potato contains vitamins and minerals, as well as an assortment of phytochemicals, such as carotenoids and polyphenols. Chlorogenic acid constitutes up to 90% of the potato tuber polyphenols. Others found in potatoes are 4-O-caffeoylquinic (crypto-chlorogenic acid), 5-O-caffeoylquinic (neo-chlorogenic acid), 3,4-dicaffeoylquinic and 3,5-dicaffeoylquinic acids. (Mendel Friedman, 1997) A medium-size 150 g (5.3 oz) potato with the skin provides 27 mg of
vitamin C (45% of the Daily Value (DV), 620 mg of potassium (18% of DV), 0.2 mg vitamin B₆ (10% of DV) and trace amounts of thiamin, riboflavin, folate, niacin, magnesium, phosphorus, iron, and zinc. The fibre content of a potato with skin (2 g) is equivalent to that of many whole grain breads, pastas, and cereals.

Protease inhibitor 2 derived from potato (PI2) is claimed to reduce appetite and food intake, stimulate the satiety hormone cholecystokinin (CCK) and lower postprandial glucose peaks when taken before a meal. Oral administration of PI2 has been documented to increase CCK levels, reduce hunger, delay gastric emptying and decrease energy intake in humans. (Jenkins et al., 2002; Leeds, 2002; Liddle, 2000; Ludwig, 2002) They have also been reported to inhibit chymotrypsin, trypsin, elastase, oryzin, Pronase E and subtilisin (Antcheva et al., 1996), thereby increasing satiety and probably promote weight loss.

2.2.2 Research Works On Potato (Potato Protease Inhibitors II)

Several researchers have reported on the various effects of Protease inhibitor (PI2) extracted from potato.

Hill et al., (1990) in their study worked on the effect of a protease inhibitor extracted from potatoes (PI2) on food intake in 11 lean subjects. They gave 1.5 g Potato protease inhibitor II in a high-protein soup vehicle (70 kcal) five minutes before being presented with a lunchtime test meal, according to a double-blind, within-subjects design. It was reported that consuming the soup alone led to a non-significant 3% reduction in energy intake but the addition of 1.5 g PI2 to the soup significantly reduced energy intake by a further 17.5%. Their findings suggest that proteinase inhibition may have therapeutic potential for reducing food intake.
Vasselli et al. (1999) and Hu et al. (2004) also reported that oral administration of PI2 was able to significantly decrease hunger ratings in overweight and healthy subjects in their own study. Speigel et al. (1999) in their own study reported that an average 2kg weight loss was observed in overweight women when PI2 was taken daily prior to lunch and dinner for four weeks.

An open label non-randomized study by Dana (2005) on healthy adults who were overweight and obese with initial BMI between 25 and 35kg/m$^2$ showed that taking 15 to 30mg of PI2 before meals, results in statistically significant gradual weight loss and reductions in waist to hip ratios. It was also observed in their study that the weight loss and body changes observed in the study were achieved without side effects that can be associated with over-the-counter weight loss supplements.

Schwartz et al (1994) performed a study to determine whether oral administration of PI2 would delay gastric emptying and modulate postprandial glucose levels in recently diagnosed (within 3 years) type 2 diabetic patients. Each subject that participated in the study came for two visits separated by at least one week, during one visit the patient consumed a glucose/protein solution while the patient consume the same solution at second visit with the addition of 1.5g PI2 thereby serving as their own control. The result shows that the subjects showed a decrease in plasma glucose levels and reduced Gastric Inhibitory Peptide (GIP) levels (which are a reliable indicator of glucose absorption in the small bowel), plasma insulin levels decreased significantly when PI2 was added to the ingested meal.

Spreadbury et al (2003) also conducted a randomized, placebo-controlled, double-blind study to examine the effect of PI2 on postprandial plasma glucose levels. Plasma glucose levels were determined for each subject 30 minutes prior to and every 30 minutes up to 120 minutes after the
meal challenge. A significant decline in postprandial blood glucose was observed in patients treated with 15 and 30mg doses of PI2 but no significant decline occurred in the group taking 7.5mg of PI2 therefore it could be said to be dose-dependent.

In a study by Peters et al (2011), which test for the effect of PI2 derived from potato formulated in a minidrink on appetite, food intake and plasma cholecystokinin levels in humans showed that protease inhibition using PI2 in a minidrink at a dose of 30mg had no functional efficacy on a range of behavioral and physiological appetite and intake control measures, which might disprove the confirmation activity of PI2.

2.3 WATER YAM (*Dioscorea alata* L.)

*Dioscorea alata* L. is a species of yam, a tuberous root vegetable that is bright lavender in color. It is widespread in distribution being grown in tropics and subtropics of Africa, America, Asia and Caribbean (Gooding et al., 1960; Chiedozie et al., 2003). In Indian traditional medicine, the tuber is used as a diuretic, aphrodisiac, anthelmintic and antidiabetic (Kritikar et al., 1956; Rodriguez-Sosa et al., 1992). Researchers have shown that water yam contain most notably carotenoids and anthocyanins like potato (Brown et al., 2004).

In the study carried out by Faiyaz et al (2009), they studied the “Total phenolic content and antioxidant activity of aqueous extract of *Dioscorea alata* L”. Their result showed that it possesses potent antioxidant effect in vitro and phenolic compounds are primarily responsible for the observed antioxidant activity, which could induce weight loss and aid metabolism.

Maithili et al (2011) reported an “antidiabetic activity of ethanolic extract of *Dioscorea alata* L. in glucose loaded and alloxan induced diabetic rats”. Their results indicated that ethanol extract of *Dioscorea alata* L. tubers possesses significant antidiabetic activity in both groups. It has been
reported that flavonoids such as Hydro-Q chromene, gamma-tocopherol-9, alpha-tocopherol, coenzyme Q, 1-feruloylglycerol, cyanidine-3-glucoside, peonidin-3-gentiobioside, alatanins A, B and C have been discovered in the tubers of the plant (Odetola et al, 2006; Cheng et al, 2007).

2.4 GLYCEMIC INDEX

The glycemic index is a numerical ranking system used to measure the rate of digestion and absorption of foods and their resultant effect on blood glucose, GI is a measure of the effects of carbohydrates on blood sugar levels. The GI is “the incremental area under the blood glucose response curve of 50 grams available carbohydrate portion of a test food relative to 50 grams of a reference food (e.g., glucose or white bread) (Jenkins et al. 2002). A food ranking high on the GI produces a large, momentary spike in glucose after it is has been consumed.

The GI is a physiological assessment of a food’s carbohydrate content through its effect on postprandial blood glucose concentrations. Evidence from trials and observational studies suggests that this physiological classification may have relevance to those chronic western diseases associated with over-consumption and inactivity leading to central obesity and insulin resistance. A lower GI suggests slower rates of digestion and absorption of the foods' carbohydrates and may also indicate greater extraction from the liver and periphery of the products of carbohydrate digestion. A lower glycemic response usually equates to a lower insulin demand but not always, and may improve long-term blood glucose control and blood lipids.

There are a number of complexities in the measure and methodological weaknesses inherent in the determination of GI, which severely limits the simple classification of a given food as high, medium or low on the GI, as well as the application of the GI for the purpose of food selection.
(Franz 2006). It must be emphasized that the GI is not an inherent property of a food but rather, the metabolic response of an individual to a food (Pi-Sunyer 2002). Thus, the GI of a carbohydrate-rich food can vary greatly depending on a number of factors.

2.5 FACTORS AFFECTING THE GLYCEMIC INDEX

2.5.1 Variety: Different varieties of a given carbohydrate-rich food (e.g., short-grain vs. long-grain rice, linguini pasta vs. rotini pasta, red potatoes vs. russet potatoes) can produce significantly different GIs. According to a published international table of GI values (Atkinson et al. 2008), the GIs for potato varieties range from a low of 56 for a boiled Pontiac potato from Australia to a high of 111 for a baked U.S. Russet Burbank.

2.5.2 Origin: Ironically, even for presumably the same variety, the GI value can vary widely depending on where it was grown. For example, russet potatoes grown in Australia have a GI ranging from 87-101, placing them in the high category, whereas russets grown in the U.S. and Canada have GIs ranging from 56-77, placing them in the more moderate category (Fernandes, 2005).

2.5.3 Processing: Grinding, rolling, pressing, mashing, and even thoroughly chewing a starch-rich carbohydrate will disrupt the amylase and/or amylopectin molecules, making them more available for hydrolysis and thereby increasing the GI (Collier & O’Dea 1982, Pi-Sunyer 2002). For example, Wolever and colleagues (2003) showed that the GI of a one inch cube of *Solanum tuberosum* could increase by almost 25 percent simply by mashing the cube. Chemically modifying a carbohydrate-rich food can also affect its GI. Decreasing the pH of a starch (e.g., by adding acid) can lower the GI; thus, adding vinegar to potatoes (such as when making potato
salad) will lower the GI of the potato. Similarly, acetylation or the addition of betacyclodestrin has been shown to decrease the GI of potato starch (Raben et al. 1997).

2.5.4 Preparation: Cooking has been shown to exert a differential effect on GI of a carbohydrate-rich food, particularly one that is high in starch. For example, a study by Fernandes et al. (2005) examined the effect of cooking on the GI of potatoes prepared in a variety of different ways including mashed; baked; reheated; boiled; boiled and cooled; and fried. The results indicated that the GI values of potatoes varied significantly depending on both the variety and cooking method used, ranging from intermediate (boiled red potatoes consumed cold: 56) to moderately high (roasted white potatoes: 73; baked russet potatoes: 72). Similarly, Kinnear et al. (2011) investigated the effects of cooking and cooling on the GI of four novel potato varieties and found significant variability in the effects. Specifically, cooking and cooling reduced the GI of two potato varieties by 40-50%, while it produced only 8-10% reduction in the other two varieties.

2.5.5 Between-Subject Variability: Research clearly shows that individuals can vary significantly in their glycemic responses to the same food (Wolever 2003). Nonetheless, in laboratory studies, this source of variation is reduced to the point where it is no longer statistically significant by expressing an individual’s glycemic response to the food of interest relative to that of a reference food (e.g. white bread or glucose).

2.5.6 Within-Subject Variability: Not only do blood glucose responses to similar foods differ between individuals, they can vary significantly in the same person on different occasions. Sometimes, the within-subject variation can sometimes be greater than the between-subject variation. In a study published in the British Journal of Nutrition, Williams (2008) examined the
reliability of the GI among four different foods (white bread, glucose, chickpeas and mashed potatoes) using the intra-class coefficient (ICC), a measure having values between zero and one, with values closer to one indicating a better reliability and values closer to zero indicating poor reliability. The ICC for white bread, glucose, and chickpeas were 0.50, 0.49, and 0.28, respectively, while the ICC for mashed potatoes was significantly lower at 0.02, indicating a very poor repeatability. It bears noting that these studies were all done in a laboratory under highly controlled conditions (i.e., using 50 grams of a single food at the same time of day, etc.). The variation would likely be much greater under less controlled or more “real life” conditions.

2.5.7 Time of Day: The time of day during which glycemic response is measured may impact not only the absolute glycemic response but also the relative glycemic response (Wolever 1996; Gannon et al. 1998). For example, Wolever and Bolognesi (1996) compared the glycemic responses to two different breakfast cereals under two conditions: after a 12-hour fast and at midday, four hours after consuming a standard breakfast. The AUCs at midday were significantly less than those after the 12-hour fast, despite the fact that the subjects consumed the exact same foods. More specifically, the mean AUC response to the high-fibre cereal was 50 percent lower than that of the low-fibre cereal after the 12-hour fast, while this difference shrank to just 10 percent at midday.
CHAPTER THREE

MATERIALS AND METHODS

3.0 PLANT SAMPLE COLLECTION

Fresh Irish Potato (*Solanum tuberosum*), Sweet Potato (*Ipomoea batatas*) and water yam (*Dioscorea alata* L.) weighing between 50-60g were purchased locally from the Ogbette main market, Enugu. The plants were subsequently identified and authenticated by a Botanist of the Botany Department of the University of Nigeria, Nsukka.

3.1 PLANT EXTRACTION

The tubers were chopped into small pieces and homogenized in distilled water for 30sec using a fisher scientific tissue-miser homogenizer. Homogenate was filtered through muslin cloth into centrifuge tubes and then centrifuged at 120rpm for 20 mins. Supernatant was filtered through muslin cloth into test tubes. After capping, extracts in test tubes were boiled five minutes in a water bath to inactivate the enzymes. After cooling with tap water, the solution was again filtered through muslin cloth into centrifuge tubes and centrifuged at 120rpm for 15 mins. For use, the residue was evaporated to dryness. The dried extract was reconstituted in freshly prepared normal saline (1g of extract in 10ml of normal saline) for administration to test animals. Extract was stored in capped tubes and refrigerated until when required for use. Plant extraction was carried out according by the method of Al-salkhan *et al* (1995) but with slight modifications.

3.2 ACUTE ORAL TOXICITY STUDY

Acute oral toxicity test was carried out according to the OECD (Organization for Economic Co-operation and Development) guidelines No. 423 (Lorke, 1983). Six male wistar rats weighing 180g were used. Rats were kept for overnight fasting prior to extract administration. Two rats
each received a single oral dose (2000mg/kg, body weight) of \textit{ST}, \textit{IB} and \textit{DA} extract respectively. After the administration of extract, food was withheld for further 3-4 hours. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention during the first 4 hours) and daily for 5 days. There were no mortality or any toxic reactions found at the maximum tested dose of 2000mg/kg. Therefore, all extract were administered at 100, 200 and 300mg/kg body weight.

3.3 EXPERIMENTAL ANIMALS

Fifty (50) Male Wistar rats were used for this study. The animals are inbred healthy male rats gotten from the University of Nigeria, Enugu campus Animal House. The animals were kept in a conducive, healthy environment for the period of the experiment in clean steel-gauzed cages. They were fed on standardized animal pellets (suppex starter fed\textsuperscript{R}) and tap water ad libitum for two weeks for acclimatization to standard laboratory conditions before the experiment. Before the commencement of the experiment, the rats weighed averagely between 170 and 180g

3.4 ANIMAL GROUPING AND TREATMENT

After the two weeks acclimatization period, the rats were divided into ten groups of five rats each. They were fed as follows:

Group 1 serves as the normal control which received 0.3ml of 0.9% sodium chloride

Group 2 received \textit{ST} extract at 100mg/kg of body weight

Group 3 received \textit{ST} extract at 200mg/kg of body weight

Group 4 received \textit{ST} extract at 300mg/kg of body weight

Group 5 received \textit{IB} extract at 100mg/kg of body weight

Group 6 received \textit{IB} extract at 200mg/kg of body weight
Group 7 received *IB* extract at 300mg/kg of body weight

Group 8 received *DA* extract at 100mg/kg of body weight

Group 9 received *DA* extract at 200mg/kg of body weight

Group 10 received *DA* extract at 300mg/kg of body weight

All the treatments were carried out for a period of 21 days. The different groups were fed the extract orally before feeding. The animals were fed with standard rat feed and tap water ad libitum.

### 3.5 MEASUREMENT OF PARAMETERS

Fasting blood glucose was determined after an overnight fasting and body weight was measured before the commencement of extract administration and further reading were taken every 7 days. Thereafter, food intake was determined everyday by giving 100g of feed to all groups and the remaining quantity is been measured the following day to determine the quantity eaten by each group (Flint *et al.* 2000; Holt *et al.* 1995, 2001). Fasting blood glucose level and changes in body weight were measured on days 0 (initial reading), 7, 14 and 21. Fasting blood glucose level was measured using a glucometer (Life Scan Inc. milano, Italy), and the body weight was measured with a spring balance.

### 3.6 STATISTICAL ANALYSIS

The data was analyzed using student’s t-test and p<0.05 was considered statistically significant. Data obtained from biochemical studies were analyzed for comparison between means for treated groups and control groups for statistical difference. The results were expressed as Mean ± Standard Error of Mean (SEM).
CHAPTER FOUR

RESULTS

4.1: The Effect Of ST Extract On Food Intake

Table 4.1 shows the effect of ST on the food intake of the various groups at the different concentrations. The result shows that the food intake of the test groups was significantly lower than those of the control group. There was an inverse relationship between the extract doses and food intake. The food intake decreased as the extract dose increased. At 100mg/kg, the food intake of the group was not significantly different from the control group but at 200mg/kg it became significantly different and more significantly reduced at 300mg/kg.

Table 4.1: The Effect Of ST Extract On Food Intake

<table>
<thead>
<tr>
<th>GROUPS/WEEKS</th>
<th>Food intake (grams) in week 1</th>
<th>Food intake (grams) in week 2</th>
<th>Food intake (grams) in week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.57±2.10</td>
<td>89.86±1.65</td>
<td>92.14±1.84</td>
</tr>
<tr>
<td>ST (100mg/kg)</td>
<td>84.86±1.91</td>
<td>85.14±1.82</td>
<td>87.00±0.82*</td>
</tr>
<tr>
<td>ST (200mg/kg)</td>
<td>82.29±2.08*</td>
<td>78.43±2.94*</td>
<td>78.14±1.79*</td>
</tr>
<tr>
<td>ST (300mg/kg)</td>
<td>79.00±2.81*</td>
<td>72.00±2.71*</td>
<td>73.71±1.94*</td>
</tr>
</tbody>
</table>

Significantly different from control at p<0.05=*
4.2: The effect of IB extract on food intake

Table 4.2 shows the effect of IB extract, on the food intake of the various groups at the different concentrations. The result shows that the food intake of the test groups was significantly lower than those of the control group at all doses of the extract. At 300mg/kg, the food intake became more significantly lower than all the test groups. They could be said to be dose-dependent, (i.e.) There was an inverse relationship between the extract doses and food intake. The food intake decreased as the extract dose increased.

Table 4.2: The effect of IB extract on food intake

<table>
<thead>
<tr>
<th>GROUPS/WEEKS</th>
<th>Food intake (grams) in week 1</th>
<th>Food intake (grams) in week 2</th>
<th>Food intake (grams) in week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.57±2.10</td>
<td>89.86±1.65</td>
<td>92.14±1.84</td>
</tr>
<tr>
<td>IB (100mg/kg)</td>
<td>79.71±2.18*</td>
<td>74.86±2.69*</td>
<td>74.86±2.25*</td>
</tr>
<tr>
<td>IB (200mg/kg)</td>
<td>78.43±1.80*</td>
<td>71.43±1.46*</td>
<td>70.43±1.04*</td>
</tr>
<tr>
<td>IB (300mg/kg)</td>
<td>77.86±2.14*</td>
<td>70.86±2.53*</td>
<td>69.29±1.70*</td>
</tr>
</tbody>
</table>

Significantly different from control at p<0.05=*

38
4.3: The effect of DA extract on food intake

Table 4.3 shows the effect of DA extract on the food intake of the various groups at the different concentrations. The result shows that the food intake of the test groups was significantly lower than those of the control group. At 100mg/kg, the food intake was not significantly different from the control group but at 200mg /kg it became different and more reduced at 300mg/kg. There was an inverse relationship between the extract doses and food intake. The food intake decreased as the extract dose increased.

Table 4.3: The effect of DA extract on food intake

<table>
<thead>
<tr>
<th>GROUPS/WEEKS</th>
<th>Food intake (grams) in week 1</th>
<th>Food intake (grams) in week 2</th>
<th>Food intake (grams) in week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.57±2.10</td>
<td>89.86±1.65</td>
<td>92.14±1.84</td>
</tr>
<tr>
<td>DA (100mg/kg)</td>
<td>87.86±1.91</td>
<td>86.14±1.82</td>
<td>87.00±0.82*</td>
</tr>
<tr>
<td>DA (200mg/kg)</td>
<td>77.86±3.24*</td>
<td>74.29±2.54*</td>
<td>79.29±1.30*</td>
</tr>
<tr>
<td>DA (300mg/kg)</td>
<td>76.42±2.37*</td>
<td>73.57±4.19*</td>
<td>75.71±2.97*</td>
</tr>
</tbody>
</table>

Significantly different from control at p<0.05=*
4.4: The effect of ST extract on fasting blood glucose level

Table 4.4 shows the effect of ST extract on the fasting blood glucose profile level. Fasting blood glucose for rats ranges from 50 to 109 mg/dL. Therefore, the fasting blood glucose level of all the different study groups all still fall within the normal range. The initial glucose level was not significantly different from the control but over the weeks, the glucose level of test groups became lower, having more significance at 300mg/kg.

Table 4.4: The effect of ST extract on fasting blood glucose level

<table>
<thead>
<tr>
<th>GROUPS/WEEK</th>
<th>FASTING BLOOD GLUCOSE LEVEL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial glucose level</td>
</tr>
<tr>
<td>Control</td>
<td>61.00±3.38</td>
</tr>
<tr>
<td>ST (100mg/kg)</td>
<td>60.60±3.40</td>
</tr>
<tr>
<td>ST (200mg/kg)</td>
<td>62.60±4.25</td>
</tr>
<tr>
<td>ST (300mg/kg)</td>
<td>71.60±1.21</td>
</tr>
</tbody>
</table>

Significantly different from control at p<0.05--*
4.5: The effect of IB extract on fasting blood glucose level

Table 4.5 shows the effect of IB extract on the fasting blood glucose level. Therefore, the fasting blood glucose level of all the different study groups still fall within the normal range. The glucose level of test groups became lower significantly at 200mg/kg but having more significance at 300mg/kg over the weeks.

Table 4.5: The effect of IB extract on fasting blood glucose level

<table>
<thead>
<tr>
<th>GROUPS/WEEK</th>
<th>FASTING BLOOD GLUCOSE LEVEL (mg/dl)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial glucose level</td>
<td>After 1 week</td>
<td>After 2 weeks</td>
<td>After 3 weeks</td>
</tr>
<tr>
<td>Control</td>
<td>61.00±3.38</td>
<td>79.60±2.53</td>
<td>89.20±4.86</td>
<td>97.40±5.94</td>
</tr>
<tr>
<td>IB (100mg/kg)</td>
<td>64.20±2.15</td>
<td>66.20±4.66</td>
<td>66.80±5.43</td>
<td>71.80±4.47*</td>
</tr>
<tr>
<td>IB (200mg/kg)</td>
<td>70.40±1.63</td>
<td>67.40±2.87*</td>
<td>60.20±1.39*</td>
<td>54.40±2.42*</td>
</tr>
<tr>
<td>IB (300mg/kg)</td>
<td>66.40±3.64</td>
<td>57.00±2.17*</td>
<td>53.60±2.06*</td>
<td>51.20±1.32*</td>
</tr>
</tbody>
</table>

Significantly different from control at p<0.05--*
4.6: The effect of DA extract on fasting blood glucose level

Table 4.6 shows the effect of DA extract on the fasting blood glucose level. The extract caused a reduction in the fasting blood glucose level of test groups in a dose dependent manner, i.e., the fasting blood glucose level reduced more as the extract dose increased. At 300mg/kg bwt, it became more significantly lower and causing slight hypoglycemia in the test group.

### Table 4.6: The effect of DA extract on fasting blood glucose level

<table>
<thead>
<tr>
<th>GROUPS/WEEK</th>
<th>FASTING BLOOD GLUCOSE LEVEL (mg/dl)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial glucose level</td>
<td>After 1 week</td>
<td>After 2 weeks</td>
<td>After 3 weeks</td>
</tr>
<tr>
<td>Control</td>
<td>61.00±3.38</td>
<td>79.60±2.53</td>
<td>89.20±4.86</td>
<td>97.40±5.94</td>
</tr>
<tr>
<td>DA (100mg/kg)</td>
<td>65.20±2.91</td>
<td>67.20±1.65*</td>
<td>63.80±2.18*</td>
<td>61.00±1.72*</td>
</tr>
<tr>
<td>DA (200mg/kg)</td>
<td>65.20±2.75</td>
<td>68.80±1.84*</td>
<td>61.00±2.58*</td>
<td>58.40±1.55*</td>
</tr>
<tr>
<td>DA (300mg/kg)</td>
<td>67.80±3.98</td>
<td>65.60±5.85*</td>
<td>55.40±4.95*</td>
<td>49.80±2.58*</td>
</tr>
</tbody>
</table>

Significantly different from control at p<0.05--*
4.7: The effect of ST extract on body weight changes

Table 4.7 shows the mean body weight and weight changes of the various treatment groups given ST extract and those of the baseline control. The initial body weight of all the groups was not significantly different but over the weeks, the body weight of the test groups became significantly lower than the control group. There was more significant weight loss at 300mg/kg.

**Table 4.7: The effect of ST extract on body weight changes**

<table>
<thead>
<tr>
<th>GROUPS/WEEKS</th>
<th>BODY WEIGHT(grams)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial weight</td>
<td>Week 1</td>
<td>Week 2</td>
<td>Week 3</td>
</tr>
<tr>
<td>Control</td>
<td>180.00±0.63</td>
<td>195.00±2.74</td>
<td>220.00±1.58</td>
<td>255.00±1.48</td>
</tr>
<tr>
<td>ST (100mg/kg)</td>
<td>179.20±0.37</td>
<td>190.00±2.55</td>
<td>215.80±0.86</td>
<td>235.00±2.92*</td>
</tr>
<tr>
<td>ST (200mg/kg)</td>
<td>180.20±0.49</td>
<td>186.80±0.50*</td>
<td>198.00±1.18*</td>
<td>210.60±1.29*</td>
</tr>
<tr>
<td>ST (300mg/kg)</td>
<td>180.00±0.63</td>
<td>170.80±0.49*</td>
<td>175.00±0.44*</td>
<td>178.80±0.37*</td>
</tr>
</tbody>
</table>

Significantly different from control at p<0.05--*
4.8: The effect of IB extract on body weight changes

Table 4.8 shows the mean body weight and weight changes of the various treatment groups given IB extract and those of the baseline control. The initial body weight of all the groups was not significantly different but over the weeks, the body weight of the test groups became significantly lower than the control group. There was more significant weight loss at 200mg /kg and 300mg/kg probably because of the reduced food intake at both concentrations.

Table 4.8: The effect of IB extract on body weight changes

<table>
<thead>
<tr>
<th>GROUPS/WEEKS</th>
<th>BODY WEIGHT(grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial weight</td>
</tr>
<tr>
<td>Control</td>
<td>180.00±0.63</td>
</tr>
<tr>
<td>IB (100mg/kg)</td>
<td>179.00±0.63</td>
</tr>
<tr>
<td>IB (200mg/kg)</td>
<td>180.00±0.32</td>
</tr>
<tr>
<td>IB (300mg/kg)</td>
<td>180.20±0.49</td>
</tr>
</tbody>
</table>

Significantly different from control at p<0.05--*
**4.9: The effect of DA extract on body weight changes**

Table 4.9 shows the mean body weight and weight changes of the various treatment groups given DA extract and those of the baseline control. The initial body weight of all the groups was not significantly different but over the weeks, the body weight of the test groups became significantly lower than the control group. At 100 and 200mg/kg, there was an increase in body weight, but at 300mg/kg, there was a decrease in food intake and slight hypoglycemia which could probably lead to the weight loss observed at this concentration.

**Table 4.9: The effect of DA extract on body weight changes**

<table>
<thead>
<tr>
<th>GROUPS/WEEKS</th>
<th>BODY WEIGHT (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial weight</td>
</tr>
<tr>
<td>Control</td>
<td>180.00±0.63</td>
</tr>
<tr>
<td>DA (100mg/kg)</td>
<td>179.00±0.44</td>
</tr>
<tr>
<td>DA (200mg/kg)</td>
<td>180.00±0.55</td>
</tr>
<tr>
<td>DA (300mg/kg)</td>
<td>180.00±0.63</td>
</tr>
</tbody>
</table>

Significantly different from control at p<0.05--*
CHAPTER FIVE

5.0 OVERVIEW

Significance of appetite control in weight management cannot be overemphasized; appetite control plays a vital role between energy consumption and energy expenditure (Arora, 2006). Sustained increases in energy intake can lead to increased body weight and an accompanying increase in energy expenditure. Body weight will stabilize and energy balance will be achieved when energy expenditure is increased to the level of energy intake. Conversely, a decrease in energy intake will disrupt energy balance and produce a loss of body weight accompanied by a reduction in energy expenditure. Body weight will stabilize when energy expenditure declines to the level of energy intake.

In the present study, it was observed that in the extract-treated groups that there was a reduction in energy intake which probably resulted in the weight loss observed. The food intake, blood glucose level and body weight were significantly reduced at p<0.05 when compared with the control group in a dose dependent manner. The weight loss observed in the extract-treated groups might be due to reduced food intake probably by increasing satiety and the reduction in the fasting blood glucose level because a reduced glucose level may help the body to use stored reserves from fat or muscle, gradually leading to weight loss.

5.1: The effect of extract on food intake

Table 4.1 shows that ST was able to cause a significant reduction in food intake in the ST treated groups than the baseline control. At 100mg/kg bwt, the food intake of the ST group was not significantly different from those of the control group, but at 300mg/kg bwt, it caused a
significant reduction in food intake in the group. These findings are consistent with the report of Little (2005) who observed that the oral administration of a proteinase inhibitor 2 (PI2) has been shown to stimulate CCK release and reduce caloric intake. Hu et al (2004) has also observed that oral administration of PI2 was able to significantly decrease hunger in overweight and healthy subjects in their study. Therefore the reduction in food intake observed might be due to presence of PI2 in the extract which might probably reduce hunger in the rats thereby decreasing food intake.

Table 4.2 showed that IB was able to cause a significant reduction in food intake at 200mg/kg and more at 300mg/kg, therefore they could be said to be dose dependent because food intake decreased as extract doses increased. This is in support of the report by Hill et al (1990) that consumption of PI2 before a meal has been shown to reduce energy intake in healthy subjects. IB was also observed to cause a more reduction in food intake when compared with ST in this study. Food and Nutrition Board (2002) reported that potato contains dietary fibre which has been shown to increase satiety, which may help with weight loss and it is believed that Ipomoea batatas has a higher fibre content when compared with Solanum tuberosum (Nelson, 2009). Therefore this could be responsible for the more reduced food intake observed in the IB treated group than the ST treated group.

Table 4.3 shows that DA was also able to significantly decrease food intake when compared with the control group. At 100mg/kg, there was no significant difference in the food intake of the control and the test group at this dose, however, more significant decrease in food intake was observed at 300mg/kg. The mechanism for the reduction in food intake is probably unknown.
Therefore the reduction in food intake observed in the ST and IB group in this study may be due to PI2 believed to be present in potato or the presence of dietary fibre in potato, thereby reducing food intake probably by increasing satiety. The decrease in food intake might also be due to resistant starch believed to be present in potato whose systemic effects include improvements in glucose tolerance and insulin sensitivity, reductions in blood lipid levels, increase in satiety which could be used in weight management (Higgins, 2004; Bodinham et al. 2010).

5.2: The effect of extract on fasting blood glucose level

All rats used for these study have a normal fasting blood glucose level because as with all fasted mammals, the blood glucose level decreases significantly over time since no sugar is consumed. Fasting blood glucose for rats ranges from 50 to 109 mg/dl (Linda, 1995) and the fasting blood glucose level of rats used for the study ranges from 50 to 97 mg/dl, therefore the fasting blood glucose level of all the different study groups all still fall within the normal range. Initially, the glucose level of all the extract-treated group were not significantly different from the control group but at the end of the study, the glucose level of the various treatment groups became significantly lower than the control group, though they all still fall within the normal range for a fasted rat.

In table 4.4, SA was observed to cause a more significant reduction at 300mg/kg which might be due to the reduced food intake observed at this dose which probably causes reduced plasma glucose. This is consistent with the result of Schwartz et al (1994) which shows that their subjects showed a significant decrease in plasma glucose levels and plasma insulin levels when PI2 was added to the ingested meal by subjects. The reduced glucose level in this group might be due to presence of PI2 in the extract.
In table 4.5, at 100mg/kg, IB did not result in a significant reduction in the fasting blood glucose level, therefore might be said to be less effective at a lower dose but at 200mg/kg and 300mg/kg, IB was observed to cause a more significant reduction in glucose level. The significant reduction in the fasting blood glucose by IB supports the study by Spreadbury et al (2003) whose report shows that supplementation with PI2 has been shown to modify the glycemic response to a meal. Their study showed a significant decline in postprandial blood glucose in patients treated with 15 and 30mg doses of PI2 but no significant decline occurred in the group taking 7.5mg of PI2 therefore it was said to be dose-dependent which might be the reason for the dose-dependent decrease in the fasting blood glucose level observed in the present study.

The IB was also observed to cause a more significant reduction in the fasting blood glucose level than ST in this study. This supports the ratings by Nelson (2009) that Ipomoea batatas has lesser calories than Solanum tuberosum (103- Ipomoea batatas, 128- Solanum tuberosum,) and also a lower glycemic index (54- Ipomoea batatas; 85- Solanum tuberosum). It has also been reported that viscous, water-soluble fibre such as β-glucans and pectin found in potato can modify or reduce blood glucose response by interfering with digestion and absorption of glycemic carbohydrates. (AFSSA, 2002). Thus, the reduce blood glucose level might be due to the water-soluble fibre believed to be present in potato.

In Table 4.6, DA was observed to cause a significant reduction in glucose level when compared with the control. It caused a decrease in fasting blood glucose level in a dose dependent manner i.e the fasting blood glucose level decreased more as the extract doses increased. It has been reported that flavonoids such as Hydro-Q chromene, gamma-tocopherol-9, alpha-tocopherol, coenzyme Q, 1-feruloylglycerol, dioscorin, cyanidine-3-glucoside, (+)-catechin, procyanidine, cyanidine, peonidin3-gentiobioside, alatanins A, B and C has been discovered in the tubers of
the plant (Odetola et al., 2006; Cheng et al., 2007). Any of these substances could have induced the observed effect. At 100 and 200mg/kg bwt, there was normal reduction of blood glucose but it was also discovered that at higher concentration of 300mg/kg, it was observed to cause a more reduced fasting blood glucose level at mean ± SEM (49.80±2.58) by the end of the third week. This supports the study by Maithili et al., (2011) which reported that in glucose loaded normal rats, the treatment with the extract of DA had shown a highly significant reduction ($P < 0.001$) in blood glucose levels at the doses of 100 and 200 mg/kg, respectively and did not produce hypoglycemic activity at both the dose levels in normal, fasted rats.

5.3: The effect of extract on body weight changes

The results show a significant reduction in weight gain in the extract-treated groups when compared with control group. At the initial reading, the mean body weight of the test groups were not significantly different from those of the control group but at the end of the study the mean body weight of the test groups became significantly lower than the control group.

In Table 4.7, at 100mg/kg of ST, there was no significant change between the weight gain of the test group and the control group, the weight of the test group was increasing almost at the same rate with the control group but still lower. At 200mg/kg, ST still caused weight gain but significantly lower than the control and 100mg/kg. The ST was able to cause a significant weight loss at 300mg/kg, which might be due to reduced food intake in this group.

In table 4.8, at 100mg/kg, IB caused weight gain but significantly lower than the control. The extract caused a significant weight loss at 200mg/kg and even more weight loss was observed at 300mg/kg therefore, they could be said to be dose-dependent. The weight loss might be due to the reduction in food intake that was observed in these groups because it was discovered that
more weight loss occurred at the test groups which had lesser food intake. This is consistent with the work of Speigel et al (1999) who also reported an average 2kg weight loss in overweight women when PI2 was taken daily prior to lunch and dinner for four weeks. IB was also observed to cause a significant weight loss than both ST and DA extract probably because of the reduced food intake observed in the IB group.

This study also agreed with the work by Dana (2005) who reported that PI2 is effective for weight loss and improved body measurements when taken before a meal by reducing appetite ratings and reduces between meals snacking in human subjects. It is believed that PI2 has been shown to induce CCK which then leads to satiety and well documented weight loss (Arora, 2006). This assumption is that a food that increases short term satiety decreases the amount of energy ingested subsequently and thus could potentially help in weight management in the long run (Slavin and Green, 2007).

In table 4.9, DA also caused a significant reduction in weight in a dose dependent manner, when compared with the control group. At 100 and 200mg/kg, there was an increase in food intake and glucose level, probably leading to a corresponding increase in body weight in this group, but at 300mg/kg, there was a decrease in food intake and a slightly lower glucose level which could probably lead to the significant weight loss observed in this group.

In this study, it was observed in the extract-treated group that there was reduction in food intake probably by increasing satiety, decreasing hunger or increasing a feeling of fullness in the rats which probably help to lower and control blood glucose level, which may help the body use up stored reserves from fat or muscle, gradually leading to the weight loss observed.
5.4 CONCLUSION

In conclusion, the aqueous extract of *Solanum tuberosum* and *Ipomoea batatas* were found to reduce food intake probably through appetite regulating mechanism, lowering fasting blood glucose level which may help use up the body’s fat reserve, thereby reducing body weight gain. *Dioscorea alata L.* also showed the same effects but the probable mechanism for this is unknown. Therefore, it is recommended that consumption of potato and water yam could be incorporated as an ingestible diet in reducing excessive body weight gain so as to control overweight and obesity.

5.5 CONTRIBUTIONS TO KNOWLEDGE

1. In the present study, it was observed that the potato and water yam were able to reduce food intake probably by increasing satiety (feeling of fullness). The reduced food intake may be due to the effects of PI2, dietary fibre and/or resistant starch believed to be present in potato which has been reported to increase satiety.

2. It was also observed that there was a reduction in the fasting blood glucose level in the extract-treated groups. The glucose lowering effect of potato might be due to the presence of dietary fibre or PI2 present in it. Water yam glucose lowering effect may be due to the presence of flavonoid compounds such as: Hydro-Q chromene, gamma-tocopherol-9, alpha-tocopherol e.t.c. Any of which could have induced the observed effect.

3. The reduction in weight gain in the extract-treated group may be due to the reduced food intake because a decrease in energy intake normally disrupts energy balance and produce a loss in body weight. It may also have been due to the lowering of blood glucose induced by the extract thereby using up the body’s stored reserve of fat.
5.6 RECOMMENDATION

Further work should be done in regards to the observed effects on food intake and body weight of *Dioscorea alata L.* in this study and its probable mechanism of action.
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