EFFECT OF INDOMETHACIN ON SELECTED PROTEIN DIGESTING ENZYMES IN THE NORWAY RAT, *RATTUS NORVEGICUS*

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DECLARATION

Candidate

This is my original work and has not been presented for the award of a degree in any university or any other award.

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DEDICATION

I dedicate this entire work to my family. My queen Claire, and my princesses Hazel, Harriet and Havana. May this work be a source of inspiration to you for your academic achievements and excellence.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
ANTU	α-naphthyl thiourea
Bioassay	Biological assay
®	Registered trade mark
HCl	Hydrochloric acid
LCMV	Lymphocytic choriomeningitis
NACOSTI	National Commission for Science, Technology and Innovation
NSAID	Non-steroidal anti-inflammatory drug
UK	United Kingdom
µg	Microgram
µl	Micro litres
SAS	Statistical Analysis System
S.E.	Standard Error

DEFINITION OF OPERATIONAL TERMS

Denaturation	a process in which proteins or nucleic acids lose their quaternary or tertiary structure which is presents in their native state by application of some external stress or compound.
Enteropathy	pathology of the intestines.
Enteritis	inflammation of the intestines.
Enterocolitis	the inflammation of the digestive tract involving enteritis of then intestine and colitis of the colon.
Gastritis	the inflammation of the stomach.
Pancreatitis	the inflammation of the pancreas.
Rodenticide	a chemical or poison used to kill rodents.
Enzyme	a biological catalyst which is protein in nature and is used to speed up chemical reactions in a living organism.
Enzyme activity	a measure of an enzymes' catalytic ability

ABSTRACT

Rats are rodents of the genus *Rattus* and are commensals. They are rapid breeders and tend to breed all year round. Rats are responsible for the transmission of many diseases, are sources of allergens and harbour fleas and ticks which are parasites that carry diseases. Rats destroy agricultural produce, household items and documents leading to huge financial losses. Biological and physical methods of rat control have proved inefficient, while rodenticides use is associated with high cost, risks and dangerous side effects. Indomethacin has been shown to lead to 100% mortality in the Norway rat experimentally, with accumulation of undigested food in their stomach. This research therefore determined the effects of indomethacin on selected protein digesting enzymes pepsin, trypsin and amino peptidase in rats. One hundred and fifty female rats were randomly divided into six groups of twenty five rats each. Group A was used as control while B, C, D, E and F served as experimental groups and were given 12.5mg/Kg, 25mg/Kg, 50mg/Kg, 100mg/Kg and 150mg/Kg of indomethacin respectively. Five rats in each group were euthanized at intervals of one hour, two, four, eight and twelve hours from the time of indomethacin administration. The rats were dissected and the gut contents removed. The digesta from the stomach, duodenum and ileum were collected, centrifuged, and the supernatants gently pipetted into sterile vials and then frozen in liquid nitrogen. The activity of digestive enzymes in the specimens were determined by continuous spectroscopic enzyme bioassays. Analysis of data was done by one way analysis of variance (ANOVA) while significant difference in the means of experimental groups was determined using post hoc ANOVA test (L.S.D). Regression analysis showed a linear relationship between indomethacin dose and enzyme activity. The results were expressed as mean ± standard error. The results showed that increase in dose of indomethacin administered leads to a decrease in the enzyme activity. Pepsin activity at 12.5mg/Kg dose was 214.2±0.6µg/mol while at a dose of 150mg/Kg the activity was 51.4±0.5µg/mol. Trypsin activity at a dose of 12.5mg/Kg, was 113.5±0.03 µg/mol while at 150mg/Kg dose the activity was 29.0±0.1µg/mol. Amino peptidase activity at 12.5mg/Kg dose was 126.5±0.04µg/mol while at 150mg/Kg dose, the activity was 33.5±0.09µg/mol. The enzyme activity is inversely proportional to the length of time taken after indomethacin administration. Pepsin activity after one hour was 214.2±0.6µg/mol while at twelve hours the activity was 108.8±0.4µg/mol. Trypsin activity after one hour was 113.2±0.03µg/mol while after twelve hours the activity was 55.5±0.05µg/mol. Amino peptidase activity after one hour was 126.5±0.04µg/mol while after twelve hours the activity was 67.8±0.03µg/mol). The mean enzyme activities in all groups were significantly different from each other at $p<0.05$. The results of this study have provided better understanding of the physiological basis of functioning of indomethacin as a rodenticide and its effects in digestion of rats. The findings of this study should be used by government officers to educate the masses on control of rats using indomethacin. The mechanism by which indomethacin decreases protein digestive enzyme activity should be investigated.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Rats are rodents in the genus *Rattus*. The most common commensal rats are the black rats, *Rattus rattus* and the Norway rats, *Rattus norvegicus* (Aplin *et al.,* 2003; Bradford, 2015). Rats are adapted to live in different range of habitats. In urban areas they live around warehouses, residential buildings, and other human settlements (Stephen *et al.,* 2007); in agricultural areas they inhabit crop fields and barns, while in the wild they are found in cliffs, rocks, the ground, and even trees. Rats breed very rapidly and maintain their populations through constant reproduction (Swartz, 2014).

Rats transmit many diseases including Hantavirus pulmonary syndrome, and rat-bite fever, (Slak, 2010). Rats' body hair or waste are poisonous and also cause allergy (Himsworth *et al.,* 2013). Their feeding habits are destructive, and their nesting behaviours can compromise the structure of infested buildings (Stephen *et al.,* 2007; Shiels *et al*., 2014). Rats cause a lot of destruction in agricultural communities by feeding on seeds and grains (Almeida *et al.,* 2013). The rat holes allow entry to a number of other pests into the buildings. If rats gnaw on electrical wires, short-circuit fires will be the result. Rats therefore cause serious financial loss both in the home, work environment and in agricultural areas (Scott and Dallas, 2007). It is therefore imperative that effective control measures are applied against them.

Methods of controlling rodents include biological, physical and chemical means. Biological means is achieved by keeping of other animals that act as predators, for example cats, dogs,

ferrets and birds among other animals, in areas that are infested by rats (Sherman, 2007). The main physical methods of controlling rats include baited traps and rat-proof construction (Alonge, 2003). Chemical method of control which employs the use of rodenticides is the most popular method used. Chemicals used include fluoroactate, warfarin congeners, strychnine, α-naphthyl thiourea (ANTU) and fluoroacetamide (Scott and Dallas, 2007). However, all these methods have proved unsatisfactory in rat control.

Indomethacin, a human drug under the class of non-steroidal anti-inflammatory drug (NSAID), developed to abate inflammatory responses to indolic hormones, serotonin and tryptophan (Brayfield, 2014), has been shown to cause 100% death in rats with minimal side effects to users and other animals (Taiwo and Conteh, 2008). Undigested, blood-stained feed was observed in the stomach of experimental rats after their death as a result of indomethacin administration in doses ranging from 83mg/Kg-250 mg/Kg (Taiwo and Conteh, 2008). This was linked to gastric ulcerations in the stomach wall. However there is no report on the effects of indomethacin on digestion. The presence of undigested food in the stomach could result from the effects of indomethacin on digestive enzymes in the stomach of rats.

1.2 Statement of the problem

Rats are some of the most destructive animals known. Rat feeding habits are destructive while their nesting behaviours can compromise the structure of infested buildings (Stephen *et al.*, 2007; Riofrío-Lazo and Páez-Rosas, 2015). Rat holes permit entry of dangerous pests into buildings. They cause short-circuit fires, if they gnaw on electrical wires, which bring about destruction to property and loss of lives (Shiels *et al*., 2014). Rats contaminate food and surfaces

which leads to spread of diseases (Lupo, 2018). Rats also cause extreme destruction in agricultural communities. They destroy farm equipment, stored grain and field crops every year leading to huge losses in terms of millions of dollars. (Almeida *et al.,* 2013). Their urine and droppings spoil seeds and grains in the stores reducing their sale value leading to food scarcity and insecurity (Almeida *et al.,* 2013; Lupo, 2018). When crops are damaged significantly by rats, the growers' ability to supply agricultural commodities to the market is reduced. This results in the broader economy suffering due to fewer products for processing and sale as a result of decline in production (Desoky, 2014).

Rats transmit many diseases like plague (McBride *et al.,* 2005; Slak, 2010), which can cause death, introduce vectors like fleas and ticks at home (Himsworth *et al.,* 2013) and are a source of allergens to humans. Rats are therefore a health risk to human beings (Himsworth *et al.,* 2014). However, the physical and biological methods of rodent control are inefficient while the chemical method is expensive, risky and associated with negative effects to humans and his animals (Alonge, 2003).Therefore, this research was conducted to determine the effects of indomethacin on selected protein digesting enzymes in rats.

1.3 Justification

Rodents are controlled using biological, physical and chemical methods. Biological method includes keeping of other animals like cats, among others to predate on rats (Alonge, 2003; Baldwin *et al.,* 2019). However, biological control can be fickle, is very slow as the agents take long to feed on rats, and also only reduce their number (Csanyi, 2018; Crampton, 2018). Physically, rats can be controlled using rat-proof structures and baited traps (Alonge, 2003).

However, rats are suspicious, aware and instinctively wary of anything new to their environment, and this includes traps and bait which are used for their physical control. (Baldwin, *et al.,* 2019). The chemical method, which is by use of rodenticides like fluoroacetate among others, is the most popular and widely used method. These rodenticides are associated with dangerous side effects because they are poisonous to man and his other animals and are expensive (Stephen *et al.,* 2007).

Because of these short comings of rat control methods, serious attempts must be made to look into alternatives which are cheaper and with less dangerous side effects to man and his animals, like indomethacin. A study done on rodenticidal effects of indomethacin found that rats and mice given indomethacin in doses ranging from 83mg/kg-250mg/kg had undigested feed with blood stains in their stomachs, while those in the control group had normal digestion. (Taiwo and Conteh, 2008). However, there is no documented study on the effect of indomethacin on digestion. Therefore, this research was intended to determine the effects of indomethacin on selected protein digesting enzymes in the Norwegian rats.

1.4 Research questions

i) What is the effect of varying indomethacin concentration on the activity of protein digesting enzymes (pepsin, trypsin and amino peptidase) in the digestive tract of the Norway rats?

ii) What is the effect of length of time taken after administration of indomethacin on selected digestive enzyme (pepsin, trypsin and amino peptidase) activity in the Norway rats?

1.5 Null hypotheses

i) Varying indomethacin doses has no effects on the activity of pepsin, trypsin, and aminopeptidase in the Norway rats.

ii) Varying the length of time after indomethacin administration has no effect on the activity of pepsin, trypsin, and aminopeptidase in the Norway rats.

1.6 Objectives

1.6.1 General objective

To determine the effects of indomethacin on pepsin, trypsin and amino peptidase in the Norway rat, *Rattus norvegicuss*.

1.6.2 Specific objectives

i) To determine the effects of varying indomethacin concentration on the activity of pepsin, trypsin, and aminopeptidase in the Norway rats.

ii) To determine the effect of time taken after administration of indomethacin on the activity of pepsin, trypsin, and aminopeptidase in the Norway rats.

1.7 Significance of the study

The mode of action of action of indomethacin when administered to rats and its effects on digestion of food in the rats' stomach was a gap in this study. The physiological basis of indomethacin has been established in this research, and will be applied in the control of rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of rats

Rats belong to the Kingdom Animalia, sub-Kingdom Bilaterria, and Infra-Kingdom Deuterostomia. They belong to the phylum Chordata, sub-phylum Vertebrata, and Infra-phylum Gnathostomata. Rats also belong to the Super-class Tetrapoda, class Mammalia, sub-class Theria, and Infraclass Eutheria. They are members of the order Rodentia, and sub-order Myomorpha. Their Super-family is Muroidea; family Muridae; and subfamily Murinae, while their genus is *Rattus* (Aplin *et al*., 2003; Bradford, 2015).

The black rats, *Rattus rattus* and the Norway rats, *Rattus norvegicus* are the most common rat species (Bradford, 2015). Typically, the weight of adult male rats is between 450 to 650 grams, while that of adult females' ranges between 350 to 450 grams. They measure about 9 to 11 inches in length excluding the tail measurement. However, female rats are usually smaller than their male counterparts. Rats vary greatly in their color. Some of the colors they exhibit include lighter and darker shades of white, grey, blacktan and brown (Aplin *et al*., 2003).

Plate 2.1 The Norway rat

2.2 Habitats of rats

Rats are found in a wide range of habitats. In cities and towns, they live around warehouses, residential buildings, and other places where humans live (Stephen e*t al.,* 2007). In towns and cities, rats prefer living in dry upper parts of buildings and so are mainly found in wall cavities and ceilings that are falling apart. Rats also inhabit agricultural areas, especially crop fields and barns. Black rats also live in the wild, where they inhabit rocks, cliffs, the ground, and trees. Black rats are also found around fences, ponds, river banks, streams, and reservoirs (Stephen e*t al.*, 2007).

2.3 Reproduction and life cycle of rats

Rats breed very rapidly, with some of the species breeding throughout the year. They maintain their high populations by reproducing constantly. For instance, with suitable conditions, a brown rat is capable of reproducing throughout the year and a female can reproduce up to five times within the same year. They have a gestation period of 21 days, and can produce litters numbering up to 14, although the most common number is seven (Swartz, 2014). They reach sexual maturity at the age of about five weeks. Rat populations grow rapidly since under ideal conditions, their population can grow by a factor of ten in 15 weeks. As a result, the population can grow from 2 to 15 000 in a year. They have a maximum life span of three years, though most do not reach this age due to high mortality rate of about 95% per year (New scientist, 2017).

2.4 Mating behavior

Male rats help in increasing the likelihood of pregnancy by undertaking multiple ejaculations in a row. Multiple ejaculations also enable the males to mate with multiple females when there are many oestrous females available (McCormick *et al*., 2017). Male rats also mate at shorter intervals than females, and females normally switch partners during group mating. Higher mating success is a preserve of dominant males who also provide females with more ejaculate, and therefore females are most likely to be fertilized by the sperm of dominant males (Ventura-Aquino, 2016). During mating, female rats prefer unknown males to those they have mated with before (Coolidge effect), and will immediately resume copulatory behaviour when a novel sexual partner is introduced (Ventura-Aquino, 2016). Female rats will also have preference for mating with males who during their adolescence did not experience any social stress and they can find this out without observing the difference in the males' sexual performance (McCormick *et al*., 2017).

2.5 Feeding habits and diet of rats

Rats constantly search for food and water. They are omnivorous and eat any type food available in their habitat including protein-based foods like poultry, meat, fish, and eggs (Shiels *et al.*, 2013). Rats prefer meat when available although they are omnivores. Brown rats and house rats scavenge through dustbins for food or eat any food left unprotected in the houses. Rats also eat grain or kill small birds, water creatures such as snails, fish and mussels, reptiles, mammals and insects for food. Other rats, such as the Sulawesi white-tailed rat and Hoffman's rat, are vegetarian, feeding on seeds and fruits (Bradford, 2015). The black rats feed on a range of plant and animal foods. However, they are highly selective on what they feed on. For instance, black

rats in the Pacific Islands select two or three types of food in their diet, with one of them normally plant material, accounting for 75–80% of the diet (Shiels *et al.,* 2013).

Of the plant materials, fruits and seeds are the most common plant material in their diet (Grant-Hoffman and Barboza, 2010). Arthropods, especially insects, form an important part of the diet of a majority of black rats, although they make a smaller part of the *R. rattus* diet relative to plant material (Shiels *et al.,* 2013).However, the diet of black rats differs depending on the habitats and seasons. Within a habitat, the diet will depend on the food available during that season (Riofrío-Lazo, and Páez-Rosas, 2015). A rat will only eat around 3 grams of food per day, but contaminates and destroys 10 times more food than what it actually eats by nibbling on packages, leaving behind its droppings, and partially eating the foods. This makes whatever remains unfit for human or pets' consumption (Lupo, 2018).

2.6 Digestive system and digestion in rats

The rat digestive tract starts from the mouth, followed by the pharynx, oesophagus, stomach, small intestine and then the large intestine. It is also constituted of digestive glands which are divided into large and small glands located outside and inside the digestive tract respectively. The large glands are connected to the digestive tract through excretory ducts, into the mouth cavity in case of salivary glands, and into the duodenum in the case of the pancreas and bile ducts (Petrenko, 2012b; Makarova *et al*., 2016).

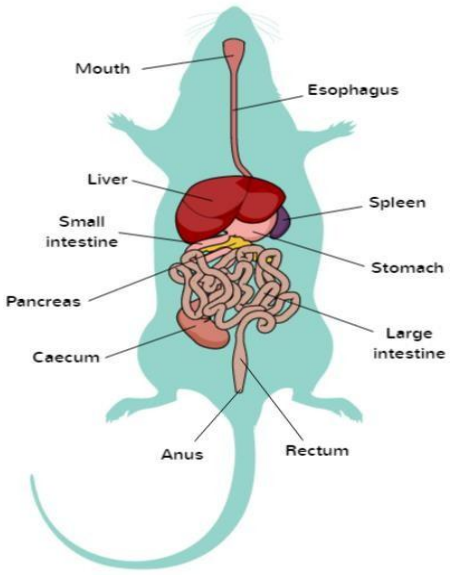


Fig. 2.1: The rat digestive tract

Although rats are omnivorous, cereal products which are solid in nature dominate in their diet. This then requires effective tools for their chewing, grinding and gnawing. As a result, rats have undergone evolution and formed a special dentoalveolar system, consisting of two types of teeth with different structure and functions. Type one is short-crown or short teeth, while type two is long-crown or high teeth. Type one is characterized by non-recoverable physiological attrition during the life, while in type two, there is replenishing of hard tissues in proportion to their wear (Hryn *et al*., 2018).

Pre-digestion processes are done in the oral cavity, where food quality sensing is done by taste perceptions, and bolus formation is achieved through chewing, hydrating and sliming using the salivary fluid. The bolus is then swallowed into the esophagus passing through the pharynx to the stomach through the esophagus. The process of swallowing is aided by the wave-like contraction of the esophageal musculature with voluntary properties only at the beginning of

swallowing, and the correlated change of the mucous membrane configuration. Depending on the food consistency, the food will remain in the stomach undergoing fermentation processes for a period of time between 20 minutes to 6 hours (Hryn *et al*., 2018).

The rodent stomachs have two cavities. In rats, the structure of the stomach is preserved irrespective of the mixed diet which sometimes contains large amounts of cereals and solid food. It is made up of the fore-stomach or esophagus, and the remaining larger part. These two sections are partially separated from each other by a well-defined ridge. Bacterial digestion takes place in the fore stomach while fermentation takes place in the larger part. (Tatarenko, 2016; Petrenko, 2012a; Makarova, 2016). The next section of the digestive tract is the small intestine which is the longest and performs function of consuming nutrients, which are the products of chemical digestion of polysaccharides, fats and proteins. The last phase of this process is absorbing nutrients into the body systems. Digestion in the small intestine takes place in the duodenum, jejunum and ileum. The length of the small intestine in rats is 4-5 times shorter than that of humans.

The digestive process in rats is combined together with bacterial digestion, consisting of cellulose degradation. This process occurs in the cecum, which is located in the distal part of the ileum in the large intestine (Petrenko, 2011). The cecum is considered as the initial part of the large intestine, (Petrenko, 2012c). The wall of the digestive tract has a similar anatomical structure right from the esophagus to the rectum. Its wall is made up of three coaxial membranes consisting of the outer, middle and inner membranes (Hron *et al*., 2017).

Digestion of food in starts in the mouth where salivary enzymes initiate the breakdown of carbohydrates then in the stomach where breakdown of proteins is initiated and finally the small intestines where completion of digestion of carbohydrates, proteins and fats are completed (Bolen, 2019). Initiation of protein digestion takes place in the stomach where pepsin catalyzes the breakdown of proteins into peptides. The next point of protein breakdown is the duodenum where trypsin catalyzes the breakdown of proteins into peptides and peptides into even smaller peptides. The last point of protein digestion is the ileum where peptide breakdown is catalyzed by amino peptidase into amino acids. The amino acids are then absorbed into the body's system (Bowen, 2018).

The proteases include pepsin that catalyzes breakdown of proteins into peptides, trypsin which catalyzes the breakdown of proteins at the basic amino acids into peptides and chymotrypsin that catalyzes the breakdown of proteins at their aromatic amino acids into peptides. Carboxypeptidase is another protease that takes off the terminal amino acid group from a protein, and amino peptidases are enzymes that catalyze the cleavage of amino acids from the amino terminus (N-terminus) of prteins or peptides. The dipeptidases catalyze the conversion of dipeptides into amino acids and tripeptidases catalyzes the hydrolysis of tripeptides, often hydrolyzing the N-terminal amino acids (Bowen, 2018; Bolen, 2019).

2.7 Destructive activities of rats

Rats cause destruction at home , in offices and in agriculture.

2.7.1 At home and in the office

Rats cause structural damage to buildings they inhabit whether in homes, apartments, offices, or anywhere else irrespective of the type of building. They achieve this through, defecation, nest-building, and gnawing (Schmitz, 2017). Rats chew on anything they see as useful in their nest building activities. This could be pieces of paper, wood, books, and even cloths. Rats can gnaw and even burrow into any upholstered furniture so as to create a hidden nest. Insulation is not safe from rats either (Shiels *et al*., 2013). They will tunnel into insulation inside walls and attics, in order to gather soft materials for making their nests or to make a home. Rats can also chew on insulation around wires and this has the potential to cause a fire. Rats even carry out their nest building in large electrical appliances by chewing through insulation and wiring, which can result in the appliance short circuiting, malfunctioning, or enhance the risk of fire (Lupo, 2018).

Rats are more likely to see items and areas that are more hidden away and most undisturbed as a more comfortable and secure home to them (Lupo, 2018). Rats do leave behind faecal droppings and urine trails as they travel around within the home seeking materials for nest making, and searching for food and water. These lead to contamination of the foods and surfaces on which they land, causing the potential spread of disease. The droppings and urine also leave a scent trail for other rats which makes them realize that this is a suitable living area (Schmitz, 2017). If any food item is packaged in a cardboard box or paper wrapping, the food will be eaten while the packaging will be used for nesting. If the food is kept in the pantry or cupboard, the rat will contaminate the food with their hair, urine, and droppings (Schmitz, 2017). They contaminate 10 times more food than what it eats by leaving its droppings, nibbling on

packages, and leaving behind large amounts of food that is partially eaten which is unfit for humans or pets consumption.

Rats gnaw on plastic and wooden containers even when there is no food inside. They end up shredding paper towel, paper, napkins other stored items for their nesting materials. If rats lack points of entry, they will chew on the structure in order to make a hole for entry and this damages the structure (Schmitz, 2017; Lupo, 2018). Damage caused by rodents costs Americans hundreds of millions of dollars every year. Since **v**ery few homeowner's insure damages caused by rats and other rodents, they have had to foot the bills from wires chewed through by rats, surfaces contaminated with rodent urine or faeces, damaged structures, and medical bills related to illnesses caused by rodents (Schmitz, 2017). Rats do not have respect for any item as they gnaw into just any item that is chewable whether stored in the basement, garage, closet or attic. They even destroy irreplaceable substances like precious family heirlooms, documents that are very important and paintings that are valued (Lupo, 2018).

2.7.2 Destructive activities in agriculture

Rodents pose the most serious threat to food security and production. They damage farm equipment, field crops, and stored grain every year amounting to millions of dollars (Almeida *et al.*, 2013). Rodents cause damage to crops at all the stages of their production. They do this in stores, after harvest, they destroy mature grain just before harvest, attack developing grain in the field, digging up seedlings and eating or destroying planted seeds (Segerback, 2009). In addition, they are carriers of more than 60 diseases that can be transmitted to livestock, humans, and other companion animals (Keirn *et al.*, 2011).

Although crops attain their highest value after being harvested, rats reduce their value by contaminating it with their urine and droppings in the store (Sawar, 2016). Since rodents cause significant damage to crops, they reduce the ability of farmers to provide agricultural commodities to the market. When this happens, the broader economy suffers because of reduced production and fewer commodities for processing and sale. If the agricultural sector is the major player in the economy, the multiplier effects of this type of damage may be serious since the agricultural sector provides inputs to nearly all other sectors of the economy (manufacturing, retail trade, and accommodation and food service) (Desoky, 2014).

2.8 Rat- borne diseases

Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) are commensal rodents with a virtually worldwide distribution (Aplin *et al*., 2003). Infestations are a big problem in urban areas due to the fact that rats are the key source of zoonotic pathogens (pathogens transmissible from animals to people) responsible for large numbers in human morbidity and mortality in cities around the world (Himsworth *et al*., 2013). Some of these pathogens are *Rickettsia typhi, Yersinia pestis, Streptobacillus monilliformis*, *Leptospira interrogans* and *Seoul hantavirus*. Wild rats carry several diseases that can be passed on to humans. Rodent droppings and urine are carriers of serious diseases. The Black Death which caused the death of millions in 14th century in Europe is so far the most famous epidemic as a result of close association of rats with people. This was caused by the bacterium *Yersinia pestis* and was described as a plague (Himsworth *et al*., 2014).

Rats transmit diseases through exposure to rat-infected feaces and urine, or through direct bites. They can also transmit diseases through intermediate vectors such as fleas, ticks or mites indirectly (Idir *et al.,* 2010). Diseases directly transmitted by rats include Hantavirus Pulmonary Syndrome, which is a viral disease transmitted by the rice rat and spread by getting into direct contact with rat faeces or urine, inhalation of dust contaminated with rat urine or droppings, and when bitten by a rat (Himsworth *et al*., 2013*).* Leptospirosis, which is a bacterial disease, can be transmitted by drinking contaminated water or getting into contact with water that is infected through wading, swimming, or kayaking. Individuals who work outdoors or work with animals are exposed to higher risks of infection by leptospirosis. Rat-bite fever is another rat born disease which is transmissible through contact with a dead rat, a bite, or scratch. Salmonellosis on the other hand is transmitted when one consumes food or water contaminated with rat feces (McBride *et al.,* 2005).

Diseases indirectly transmitted by rats include plague that is carried by rats and transmitted by fleas when taking a blood meal. Domestic rats are normally the most common reservoir of plague. Colorado tick fever is a viral disease transmissible through the bite of a tick which has already taken a blood meal from a bushy-tailed wood rat. Cutaneous leishmaniasis is transmitted to a person through a bite from an infected sand fly that has already fed on a wild wood rat (Himsworth *et al*., 2014.)

2.9 Control of rats

Rats are mainly controlled by use of three methods, namely, physical, biological and chemical methods.

2.9.1 Biological control measures

Biological control methods include keeping of animals to predate on rats. Some of the animals kept for this purpose include birds, cats, ferrets, dogs, and snakes (Alonge, 2003). In rural and suburban environments, outdoor rodents face many natural enemy predators such as cats, raptors, dogs, and coyotes, while in urban areas, biological control is not enough to suppress outdoor rodent populations which easily move into nearby unprotected structures (Baldwin *et al.*, 2019). Biological means of rodents control makes use of rodent-destroying or eating animals.

Pet animals like dogs and cats that are not completely domesticated may kill a rodent too. Birds of prey would also kill rodents but these are not many in urban areas. In case one decides to attract the birds, it will not be an easy task as the birds prefer to hunt on living prey and will not pay attention to baits placed around (Csanyi, 2018). The biological method though still in use in most rural areas of developing countries has many disadvantages in that it can be fickle, is a very slow process since it takes lots of time and patience for these biological agents to reduce rat population, and that these biological agents can only reduce the number of rodents but cannot completely wipe them out (Csanyi, 2018).

2.9.2 Physical/Mechanical control measures

Physical methods of rat control, which include modification of the habitat, exclusion, and sanitation, which are very effective in doing away with rodent problems. The first line in solving rat problems is by sealing all entry holes; cleaning up clutter in buildings and storing items off the floor to allow for regular and proper cleaning and inspection of buildings (Alonge, 2003).

The main integrated pest management (IPM) strategies for rats include keeping exterior trash handling areas clean, exclusion, and removing or trimming any vegetation that obscures the ground.

Baiting of snap traps can be done with attractants like food items and cotton string. Peanut butter or honey can be used to stick other foods to the trigger. Snap traps can also be placed in cardboard or plastic boxes designed to hold snap traps. Snap traps should never be used in classrooms and if they must, they should be placed in tamper-resistant containers or in areas that students cannot access. Alternatively, snap traps may be set at night and removed in the morning before students arrive. These should be labelled with a number and marked on a diagram to ensure all are recovered (Baldwin *et al.,* 2019).

For trapping to be successful, traps should be put in place in advance for several days before they are set and should be allowed to remain in place for at least a week before being moved to a new location because both roof and Norway rats are very wary of new things in their habitat. To be most effective, traps should be set along rat runways with the trigger side of the trap being on the wall side. For successful baiting, food preference for the rat should be known because different rat species prefer different food types. Use of multiple baits should be encouraged as it provides a variety of choices and some of the baits should have cotton balls since pregnant rats will scavenge them for making of their nests (Baldwin *et al.,* 2019).

The physical methods of control are inefficient, because rats by instinct are very wary of things new to their environment, including rat control measures like traps and baits. Rats become very

suspicious about anything that is new in their environment as such traps will initially cause a lot of discomfort with a sense of threat among the rats (Baldwin *et al.,* 2019). Although rodent traps are the most favorite physical method, handling live rodents that have been trapped can leaves one vulnerable to bites and contamination. On the other hand, dead rats harbor fleas that are normally associated with many diseases (Scott and Dallas, 2007). Using traps over a large area is labour intensive and traps also end up trapping non target species (Witmer, 2011). Ultrasound repellants, which scare away rats by emitting sound that is inaudible to the ears of humans but uncomfortable for rodents, can also be used. However, ultrasounds end up being absorbed quickly by physical structures and furniture while at the same time the rats also get used to the sound quickly (Alonge, 2003).

2.9.3 Chemical control measures

Rodenticide toxicity

Rodenticide toxicity is caused by many types of rodent poisons that are categorised into two; anticoagulants, and non-anticoagulants. Anticoagulant rodenticides kill by interfering with the of Vitamin K activation, an important component in the synthesis of blood clotting factors in the liver. Non-anticoagulant rodenticides show varied mechanism of action and include bromethalin, strychnine, cholecalciferol, and zinc phosphide (Means, 2004). When a significant amount of an anticoagulant rodenticide is ingested, it interferes with coagulation of blood and results in spontaneous bleeding. Some of the specific clinical signs include bleeding into body cavities, widespread bruising, and blood in the urine or faeces. If the bleeding is sudden and significant, cardiovascular shock and death can occur. Bleeding occurs internally or externally and may affect any part of the body (Means, 2004). The toxicity symptoms are more variable

for non-anticoagulant rodenticides and depend on the chemical used and dose applied. The clinical signs are; lethargy, respiratory paralysis, rapid onset of seizures, limb weakness, ataxia, vomiting, neurologic signs, anorexia, nausea, diarrhoea, and muscle tremors (Panther Pest Control Team, 2017).

Rodenticides use is associated with high costs and many dangerous side effects (Witmer 2011). Rodenticides are dangerous because they can poison children, pets, or employees when accidentally ingested or when absorbed through the skin and not washed immediately (Stephen *et al.,* 2007). A rodent that has been poisoned may crawl away and die in a secluded area, rot there and its carcass may end up creating hazardous disease-related conditions and may also produce noxious odors that are difficult to get rid of. Rodents that have been poisoned can also contaminate freshwater bodies and supplies or end up in the food chain of wild animals. Rodenticides are also toxic and persist in the tissues of rodents killed (Eisemann *et al*., 2007), kill non- target organisms (Pitt *et al.,* 2015) and rodents have developed wide spread resistance against them (Pelz, 2007)

2.10 Indomethacin

2.10.1 An overview of Indomethacin

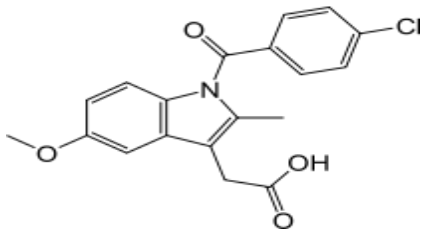
Indomethacin is a human drug under the class of non-steroidal anti-inflammatory drugs (NSAID). Its development was specifically meant to abate inflammatory responses to indolic hormones, such as serotonin and tryptophan (Brayfield, 2014). Its discovery was done in 1963 while its first approval for use in the U.S. was done in 1965 (Jnos and Robin, 2006). Indomethacin is used for treatment of ankylosing spondilitis, rheumatoid arthritis, gout, acute

musculoskeletal disorders, degenerative joint diseases, oedema, and inflammation, after surgical technique and pain related to primary dysmenorrhoea in humans (Hardman *et al*., 2001; Lucas, 2016).

2.10.2 Chemical structure and properties

Indomethacin, is a derivative of a non-steroidal anti-inflammatory indole and is chemically designated as 1-(4-chlorobenzoyl)-5-methoxy-2 methyl-1H-indole-3-acetic acid; with a molecular weight 357.79g/mol and a molecular formula of $C_{19}H_{16}ClNO_4$ (F.D.A., 2016).

Its structural formula is as follows:



Indomethacin USP is sparingly soluble in alcohol but almost insoluble in water, with a pKa of 4.5. It is quite stable in neutral or slightly acidic media but when reacted with a strong alkali it decomposes. Non-steroidal anti-inflammatory drugs (NSAIDs) are normally used for treating inflammation, fever, and pain. Unfortunately, these drugs normally show some side effects, mainly on the gastrointestinal tract like erosions of the gastric mucosa, ulcerations, perforations and bleeding (Brayfield, 2014).

2.10.3 Mechanism of action as human medicine

Indomethacin is a non-selective inhibitor of cyclooxygenases (COX) 1 and 2 (Brune and Hinz, 2004). These enzymes are derived from arachidonic acid and participate in the synthesis of prostaglandin, which are hormone-like molecules found in the body, where they show a variety

of effects, some of which may lead to inflammation, pain, and fever (Lucas, 2016). Because indomethacin is involved in inhibiting both COX-1 and COX-2 (Brune and Hinz, 2004), it is involved in inhibition of the production of prostaglandins in the stomach and intestines, which aid in the maintenance of the gastrointestinal tract's mucous lining.

Indomethacin, just like other non-selective inhibitors of COX can result in formation of peptic ulcers (Lee *et al*., 2016). These ulcers cause serious bleeding and/or perforations that may end up requiring the patient to be hospitalized. The ulcers can also enhance the effects of vasopressin that may lead to; edema, hyperkalemia, hypernatremia and hypertension (Lee *et al*., 2016). Indomethacin is known to have high acute toxicity for both rats and humans. In rats, the toxicity is at the level of 12 mg/kg while for humans the exact data is nonexistent, but some fatal human cases, especially in both children and adolescents have been reported (Taiwo and Conteh, 2008).

2.10.4 Effects of Indomethacin on rats

Indomethacin uncouples oxidative phosphorylation at concentrations that are supratherapeutic and is depressing to the biosynthesis of mucopolysaccharides (Slagle, 2001). These are normally secreted into the stomach and in the mucosae of intestines by goblet cells where they protect the surfaces against substances that are very acidic and very alkaline in nature to the stomach and intestines respectively. Lack of mucopolysaccharides exposes the mucosae to acids and alkali, leading to the formation ulcers, necrosis and erosions (Slagle, 2001).

Indomethacin is reported to be an inhibitor to the synthesis of prostaglandins, with cytoprotective effects on the gastric mucosa. It is actually thought that this is the main

mechanism of indomethacin's gastrotoxic effect. Indomethacin is also an effective tocolytic agent (Giles and Bisits, 2007). However the explanation on the gastrotoxic effects of NSAIDs cannot only be done by looking at their inhibitory effect on the synthesis of prostaglandins, rather, there are reports that majority of NSAIDs damage the gastric mucosa through necrosis and apoptosis of the gastric mucosa cells (Slagle, 2001).

Many other aspirin-like drugs have been shown to inhibit other cellular enzymes. For instance, phosphodiesterase enzyme inhibition and mitochondrial phosphorylation uncoupling are important when considering the effects of NSAIDs when administered at high local or systemic concentrations (Amanullah *et al*., 2018). It has been reported that damage to mitochondria at the site of local high concentrations results in haemorrhage, failure of mucosal integrity, perforation of stomach wall and ulceration (Slagle, 2001). When the concentration of indomethacin in the blood is high, there will be uncoupling of endothelial cell DNA leading to the death of the cell, vascular damage, necrosis, thrombosis and tissue/organ ischaemia. The latter pathway has been shown to be the most plausible cause of organ damage, haemorrhagic necrosis and death in rodents (Taiwo and Conte, 2008).

2.10.5 Effects of Indomethacin on the rat stomach

Indomethacin is a major cause in the decrease of nitric oxide (NO) levels in the gastric secretion which results in decreased synthesis of the mucosal and mucosal barrier content (Guha *et al.*, 2009; Chakraborty *et al.*, 2014). Indomethacin also causes gastritis (Werawatganon *et al.*, 2014; Badii *et al*., 2016) and induces peptic ulcers (Lee *et al.*, 2016). Because the stomach lining contains gastric acid and pepsin secreting glands, gastritis causes a reduction in production of

gastric acid and pepsin enzymes (NIDDK, 2015). Peptic ulcers also tend to have some secondary effects on gastric acid and pepsin (Ramakrishnan *et al.,* 2007).

Indomethacin is an agent of denaturation to many enzymes including digestive enzymes (Kilpatrick and Bunk, 2009). In fact, at moderate temperatures, indomethacin accelerates the presence of denatured proteins in the cell (Roussou, 2000). Rats treated with indomethacin showed an increase in gastric volume, decreases the pH of gastric secretions, and increases gastric secretions' free total acidity (Katary and Salahuddin, 2017). Changes in the gastric secretions' pH results in a decline in the enzymes' velocities as enzymes have an optimal pH at which their velocity is maximal (Robinson, 2015).

2.10.6 Effects of indomethacin on the small intestine of rats

Indomethacin interacts with the pancreas to induce pancreatitis (Memis *et al*., 2005; Mahjoub *et al*., 2006). Pancreatitis leads to a reduction in the secretion of pancreatic enzymes since the digestive enzymes' secreting cells are destroyed (Bansal, 2017). Indomethacin also causes chronic pancreatitis which results in pancreatic insufficiency, which is the decrease in the quantity of enzymes present in the digestive fluid (Bansal, 2017). Indomethacin blocks the calcium ion channels preventing its absorption while at the same time reduces the amount of calcium in the duodenum since it is antagonistic to calcium (Kaneko *et al*., 2016). Non-steroidal anti-inflammatory drugs (NSAIDs) induce small intestinal injury (Wallace 2013; Higuchi *et al.*, 2009), cause severe gastrointestinal injuries like inflammation, ulcers, erosions and altered permeability (Scheiman, 2003; Bjarnason and Takeuchi, 2009).

Non-steroidal anti-inflammatory drugs (NSAIDs) cause enteropathy (Jacob *et al.,* 2001), and indomethacin particularly causes intestinal proliferative enteropathy (McOrist and Gebhart, 2006). When there is an increase in the intestines' mucosal permeability due to indomethacin administration, there will be enhanced actions of luminal factors such as bile acids (Somasundaram *et al.,* 2000). Indomethacin if mixed with bile acids can form toxic micelles within the bile fluid that contribute to indomethacin-induced gastrointestinal injury (Zhou *et al.,* 2010; Dial *et al.,* 2015).

Indomethacin has been experimentally found to be a good rat killer, since adult common or Norway rats and house mouse (*Mus musculus*) were observed to die in 2-4 days after consumption of doses between 86 to 250 mg/kg body mass (Taiwo and Conteh, 2008). Widespread necrosis in many organs especially the stomach, intestines, liver, kidneys, heart and brain though thrombosis and ischaemia was found to be the main pathogenic pathway for rats killed by indomethacin (Taiwo and Conteh, 2008). When rats were given indomethacin in doses ranging from 83mg/kg-166mg/kg their stomachs filled with undigested feed stained by blood (Taiwo and Conteh, 2008). However, there is no documented study on the effect of indomethacin on digestive enzymes in the rat stomach.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study location

This research was carried out at the Department of Biochemistry Laboratories in Egerton University Main Campus Njoro, Kenya. The location of the university is in Njoro, approximately 25 kilometres (16 mi), Southwest of Nakuru town. Egerton university's main campus coordinates are: 0°22'11.0"S, 35°55'58.0"E with latitude of 0.369734and longitude of 35.932779) **(**Google, 2014).

3.2 Experimental animals

The experimental animals were purposively sampled to be the female white laboratory rats which are the albino form of the Norway rats, *Rattus norvegicus.*

Plate 3.1 The albino Norway rat

The same sex was preferred because rats normally show sex differences in feeding behavior and females are less aggressive (Fukushima *et al.,* 2015). This made handling of the rats during indomethacin administration much easier. Norway albino rats were chosen by quota sampling,

which samples the closest relation of the experimental organism in case the real organism is not used. The Norway albino rats are the closest in relation to the wild Norway rats. The Department of Zoological Sciences in Kenyatta University provided the experimental rats from their animal house, where the rats had been born and bred. A total of one hundred and fifty (150) female common albino rats were randomly picked from the total rat population in the animal house, fed on commercial rat pellets and given clean drinking water *ad libitum*.

The one hundred and fifty (150) rats were then randomly divided into six equal groups of twenty five (25) rats each labeled A, B, C, D, E, and F. Each group of twenty five (25) rats was kept in a separate meshed cage measuring 45cm length by 45cm width and 26cm height similar to the cages in the animal house in which the rats were being reared. The rats were further randomly divided into five subgroups of five rats each from the original groups A, B, C, D, E, and F during euthanization to give new subgroups 1, 2, 3, 4, and 5 and 6 in conformity with previous experiments that used between 4-6 rats per group (Taiwo and conteh, 2008; Mayo *et al.*, 2016). The rats were given commercial feed bought from Unga Limited in form of rat pallets and clean drinking water for one week before the experiment began without any limitations. All the rats were subjected to the same conditions, that is, average temperature of 28^{o}C, relative humidity of 62% and 12 hours darkness and 12 hours light cycles.

3.3 Indomethacin preparation and administration

One capsule of indocid contains 25mg of indomethacin. One hundred capsules of Indocid® capsules (Cipla pharmaceuticals, Mumbai, India) were bought from a pharmacy and dissolved in 100 millilitres (mL) of distilled water to make a final solution with 25 mg indomethacin/mL

of water. A homogenous mixture was achieved by constantly stirring the mixture, since indomethacin is only sparingly soluble in water. Since 12.5 mg/Kg is the least dose that has been reported to kill rats (Omogbai *et al*., 1999), rats in the experimental groups B, C, D, E, and F were each accurately weighed and given calculated volumes of indomethacin solution equivalent to 12.5 mg/kg, 25 mg/kg, 50 mg/kg, 100 mg/kg and 150 mg/kg body mass respectively. However, rats in group A, the control group, were given plain distilled water (Taiwo and Conte, 2008). The administration of Indomethacin was done by a 1mL syringe by volume fitted with a 1mm bore metal cannula for direct delivery of the drug into the oesophagus so as to avoid the possibility of spitting out the drug by the rats. All the animals underwent fasting for twelve (12) hours overnight before the drug was administered to ensure that there was no food in their digestive system because food decreases the rate of absorption and the extent of gastrointestinal irritation by indomethacin (U.S F.D.A., 2016). Feeding and watering of the animals was resumed as soon as indomethacin administered (Taiwo and Conte, 2008).

3.4 Sample collection

Five rats from each group was randomly chosen and euthanized after one hour, two hours four hours, eight hours and 12 hours from the time of indomethacin administration because indomethacin absorption is fast and peaks in the plasma at 1.02±0.49 (Rytting *et al*., 2014), with a half-life of 4.5 hours (U.S F.D.A., 2016). The timings were also chosen to ensure that no rat died before it was euthanized and sample extracted (German and Bittong, 2009; Mayo *et al*., 2016). Euthanization was achieved by placing the rats in a glass jar with cotton wool soaked in diethyl ether. The euthanized rats were dissected for careful removal of the digestive tract. The guts were dissected, placed on a sterilized and chilled (~4°C) dissecting board and then

unraveled. The stomachs were removed, and the small intestines divided into the duodenum and the ileum. The gut contents were gently squeezed from each of the two intestinal regions with a pair of forceps and the blunt side of a razor blade into sterile vials.

After removal, the gut contents were homogenized on ice (Skea *et al.*, 2005), as described by German and Bittong (2009). Defrosting of the intestinal fluid and pelleted gut contents from the stomach was done followed by the dilution of times 10 volumes in 0.05 M Tris–HCl at pH 7.5, and gentle homogenization by the use of a Polytron homogenizer (Brinkmann Instruments, Westbury, NY), at a setting of 1,100 rpm for 30 seconds (German and Bittong, 2009). The homogenized gut contents were transferred into centrifuge vials and were centrifuged at 10,000×*g* for 5 min (Skea *et al*., 2005; German and Bittong, 2009) in an Eppendorf 5415R desktop centrifuge (Brinkmann Instruments, Westbury, NY) powered by a 12 V power. After undergoing centrifugation, the supernatants from the stomach, duodenum and ileum were gently pipetted into separate sterile centrifuge vials and frozen in liquid nitrogen at -80^{o} until analysis (German and Bittong, 2009).

3.5 Laboratory analysis of samples

The determination of activity of pepsin, trypsin and aminopeptidase in the samples was done by continuous spectroscopic enzyme bioassays specifically the spectrophotometric method (Atkins and Julio, 2006). Factors that affect enzyme activity such as temperature, salt concentration, enzyme and substrate concentration, activators, inhibitors and pH were all controlled (as detailed in individual enzyme assay procedures below), during analysis to increase validity and reliability of results (Daniel *et al.,* 2010). All digestive enzyme activity assays were carried out at 25°C, in line with the temperature measurements (24–26°C) of the

Río Marañon, by the use of BioRad Benchmark Plus micro plate spectrophotometer in triplicates (Wienken, 2010), and Falcon flat-bottom 96-well micro plates (Fisher Scientific, Germany).

Measurement of pH values listed for buffers in the experiment were done at room temperature (22°C). All reactions were run at saturating substrate concentrations as determined for each enzyme with gut (stomach, duodenum and ileum) contents from the six groups. Measurements for enzyme activity for pepsin was done on stomach content, for trypsin on duodenal contents and amino peptidase activity measurements was done in the extract from the ileum of individual rats and recorded while blanks for each enzyme made up of its substrate and homogenate only (in their specific buffers) were conducted at the same time to account for endogenous substrate and/or product in the tissue homogenates and substrate solutions (Skea *et al.,* 2005; German *et al.*, 2004).

3.5.1 Pepsin activity assay

The determination of proteolytic activity of pepsin was carried out using haemoglobin from bovin blood (Merc, Germany) supplied by Sigma Aldrich, as a substrate according to the method of Nalinanon *et al.* (2010) as described by Oliveira *et al. (*2015). Initiation of the reaction was achieved by the addition of 200 µL solution of enzyme into the assay mixture containing 200 µL of 2% haemoglobin, 200 µL of distilled water and 625 µL of the reaction buffer (Tris–HCl at a pH 7.5). To ensure that the amount of enzyme was not excessive for available substrate in the assay system, dilution of times ten (10) was done (Nalinanon *et al.,* 2010). The reaction was conducted at pH 2.0 and 40°C for 20 min.

The termination of enzymatic reaction was achieved by an addition of 200 µL of 50% (w/v) trichloroacetic acid (TCA). Precipitation of unhydrolysed protein substrate was allowed to take place for one hour at 4°C, and then centrifugation at 15,000 x g for 10 minutes was done. Measurement of oligopeptide content in the supernatant was done at 280 nm. One unit of activity was defined as the amount of product causing an increase of 1.0 in absorbance at 280 nm per min. A blank was run using the same method; except that the enzyme was added into the reaction mixture after the addition of 50% (w/v) TCA (Oliveira *et al.,* 2015). The results were indicated as µg/mol. This process was repeated for all the rats in all the remaining groups.

3.5.2 Trypsin activity assay

Assay of trypsin activity was carried out in the intestinal fluid from the duodenum section using a modified version of the method designed by Erlanger *et al.* (1961), as described by Gawlicka *et al.,* (2000) and German and Bittong, (2009). The substrate, 2mM Nα-benzoyl-l-arginine-p-nitroanilide hydrochloride (BAPNA), was dissolved in 100mM Tris–HCl buffer (pH 7.5) by heating to 95°C (German *et al*. 2004). A volume of 95µl BAPNA was combined with 5µl of homogenate in a microplate, and the increase in absorbance was read continuously at 410 nm for 15 min German and Bittong (2009) and the process repeated for all the rats in all the groups.

3.5.3 Amino peptidase activity

Measurement of amino peptidase activity was done in the intestinal fluid from the ileum section according to Roncari and Zuber (1969), as described by German *et al.* (2004) and German and Bittong (2009). A volume of 90µl of 2.04mM l-alanine-*p*-nitroanilide HCl was dissolved in

200mM sodium phosphate buffer (pH 7.5) then combined with 10µl of homogenate in a microplate. The increase in absorbance was read continuously at 410 nm for 15 min and activity determined using a p-nitroaniline standard curve. Amino peptidase activity was expressed in U (1 µmol *p*-nitroaniline liberated per minute) per gram of wet weight of pelleted gut contents (German and Bittong, 2009). This procedure was repeated for each rat in all the groups.

3.6 Statistical analysis

Enzyme activity data was analyzed using "SAS for Windows, 13.0" software. Statistical analysis of enzyme activity data was done by one way analysis of variance (ANOVA) to determine whether the two bordering means differed significantly in experimental groups when indomethacin concentrations were varied and also when time intervals were varied after indomethacin administration. Post hoc ANOVA tests were also done particularly the least significant difference (LSD) between the means of enzyme activities to determine whether all the treatments were different from one another. Values were interpreted to be statistically significant at $p<0.05$. Regression analysis was also done to determine the relationship between concentration and enzyme activities of pepsin, trypsin and amino peptidase.

3.7 Research permits and other logistical considerations

Graduate School of Kenyatta University approved the research proposal (Appendix I) and also provided a letter of introduction to the National Commission for Science, Technology and Innovation (NACOSTI) (Appendix II). Authority to conduct research was sought and granted by the National Commission for Science, Technology and Innovation granted authority to carry out research (Appendix III), as well as a research permit (Appendix IV).

CHAPTER FOUR

RESULTS

4. 1 Effect of varying indomethacin concentration on enzyme activity

4.1.1 Pepsin activity in the stomach

When the mean enzyme activity for pepsin was calculated for each group, the control group had the highest mean enzyme activity (239.2±1 µg/mol). In the experimental groups, the group that recorded the highest enzyme activity (214.2±0.6 µg/mol) was that which was given the lowest dose of 12.5mg/Kg, while the group given the highest indomethacin dose of 150mg/kg had the least enzyme activity (5.6±0.4 µg/mol) (Table 4.1). The mean enzyme activity of pepsin in the stomach decreased with increase in the dose of indomethacin administered (Table 4.1). All the mean pepsin activity values in each group were found to differ significantly from each other at $p<0.05$ when different indomethacin concentrations were administered (Table 4.1).

Table 4.1: Mean values (± S.E) for pepsin activity at different concentrations (mg/Kg)

Experimental groups	Pepsin enzyme activity (µg/mol)				
	Sub-Group1 (1h) n=5	**Sub-Group2 (2hs) n=5**	**Sub-Group3 (4hs) n=5**	**Sub-Group4 (8hs) n=5**	**Sub-Group5 (12hs) n=5**
Group A	238.8±0.4^{a}	239.2±1^{a}	239.2±1^{a}	238.4±0.5^{a}	238.6±1.2^{a}
Group B	214.2±0.6^{b}	184.8±0.8^{b}	167.4±0.6^{b}	119.8±0.4^{b}	108.8±0.4^{b}
Group C	170.8±0.6^{c}	162.2±0.4^{c}	140.6±0.5^{c}	105.0±0.4^{c}	92.2±0.4^{c}
Group D	129.8±0.7^{d}	119.8±0.4^{d}	93.0±0.8^{d}	43.0±0.5^{d}	33.0±0.4^{d}
Group E	71.4±0.5^{e}	60.0±0.7^{e}	45.2±0.4^{e}	22.0±0.5**e**	15.8±0.4^{e}
Group F	51.4±0.5^{f}	42.4±0.4^{f}	19.6±0.3^{f}	9.0±0.3^{f}	5.6±0.4^{f}

N.B Means followed by same letters are not significantly different from each other at $p<0.05$

Regression analysis done on the data showed that there was a negative linear relationship between indomethacin concentration and pepsin activity. When indomethacin concentration is increased, the pepsin activity was decreased in all the experimental groups (Fig. 4.1).

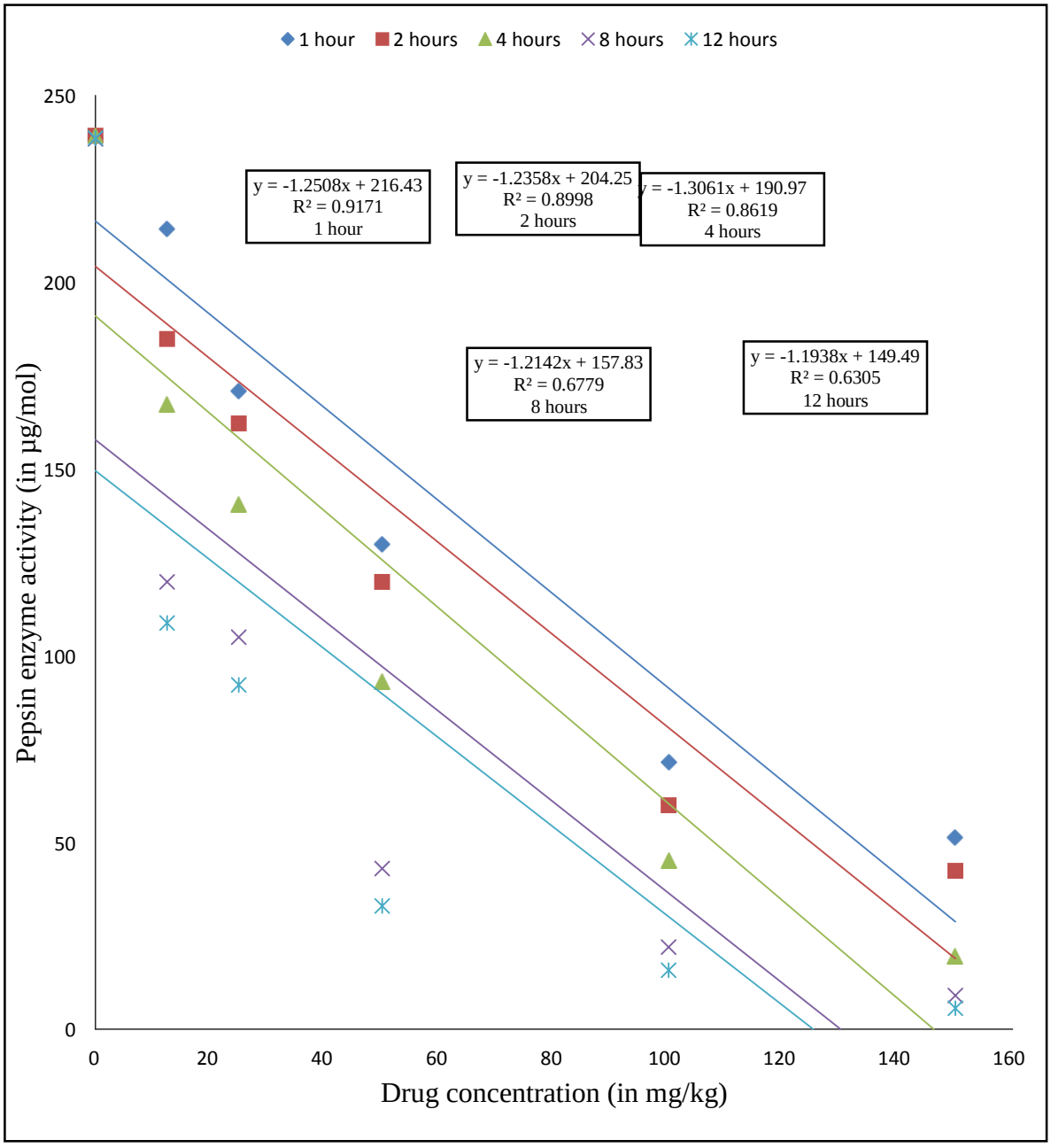


Fig. 4.1: Regression curve for effect of varying indomethacin concentration on pepsin activity in Norway rats

4.1.2 Trypsin activity in the duodenum

The mean trypsin activity for the control groups, given only distilled water, was found to be the highest (126.8±0.06 µg/mol). In the experimental groups, the group that was given the highest dose of indomethacin at 150mg/kg recorded the least mean trypsin activity at (3±0.1 µg/mol), while the group that was given the lowest dose of 12.5mg/Kg recorded the highest trypsin activity(113.5±0.03 µg/mol) (Table 4.2). In all the experimental groups, the results showed that, an increase in indomethacin dose administered leads to a decrease in the mean activity of trypsin (Table 4.2). At $p<0.05$, the mean trypsin activity values were significantly different from each other when different drug concentrations were administered (Table 4.2).

Table 4.2: Mean values (± S.E) for trypsin activities at different concentrations (mg/Kg)

Experimental Groups	Trypsin activity (µg/mol) n=5				
	Sub-Group1 (1hr) n=5	**Sub-Group2 (2hrs) n=5**	**Sub-Group3 (4hrs) n=5**	**Sub-Group4 (8hrs) n=5**	**Sub-Group5 (12hrs) n=5**
Group A	126±0.09^{a}	126±0.05^{a}	126.8±0.06^{a}	125±0.05^{a}	125.9±0.05^{a}
Group B	113.5±0.03^{b}	95.6±0.04^{b}	86±0.04^{b}	62.6±0.07^{b}	55.5±0.05^{b}
Group C	90.8±0.06^{c}	82.3±0.06^{c}	68.8±0.05^{c}	54.6±0.07^{c}	45.3±0.05^{c}
Group D	67.9±0.08^{d}	56.6±0.09^{d}	47.4±0.08^{d}	34.6±0.08^{d}	26.8±0.08^{d}
Group E	36.8±0.10^{e}	30.6±0.08^{e}	22.8±0.09^{e}	15.6±0.08^{e}	12.4±0.09^{e}
Group F	29.0±0.1^{f}	23±0.074^{f}	15±0.05^{f}	8.8±0.1^{f}	6.3±0.1^{f}

N.B: Means followed by same letters are not significantly different from each other at $p<0.05$

When regression analysis was done on the data, the results showed a negative linear relationship between indomethacin concentration and trypsin activity. An increase in indomethacin concentration resulted in a negative linear decrease in trypsin activity in all the experimental groups (Fig. 4.2).

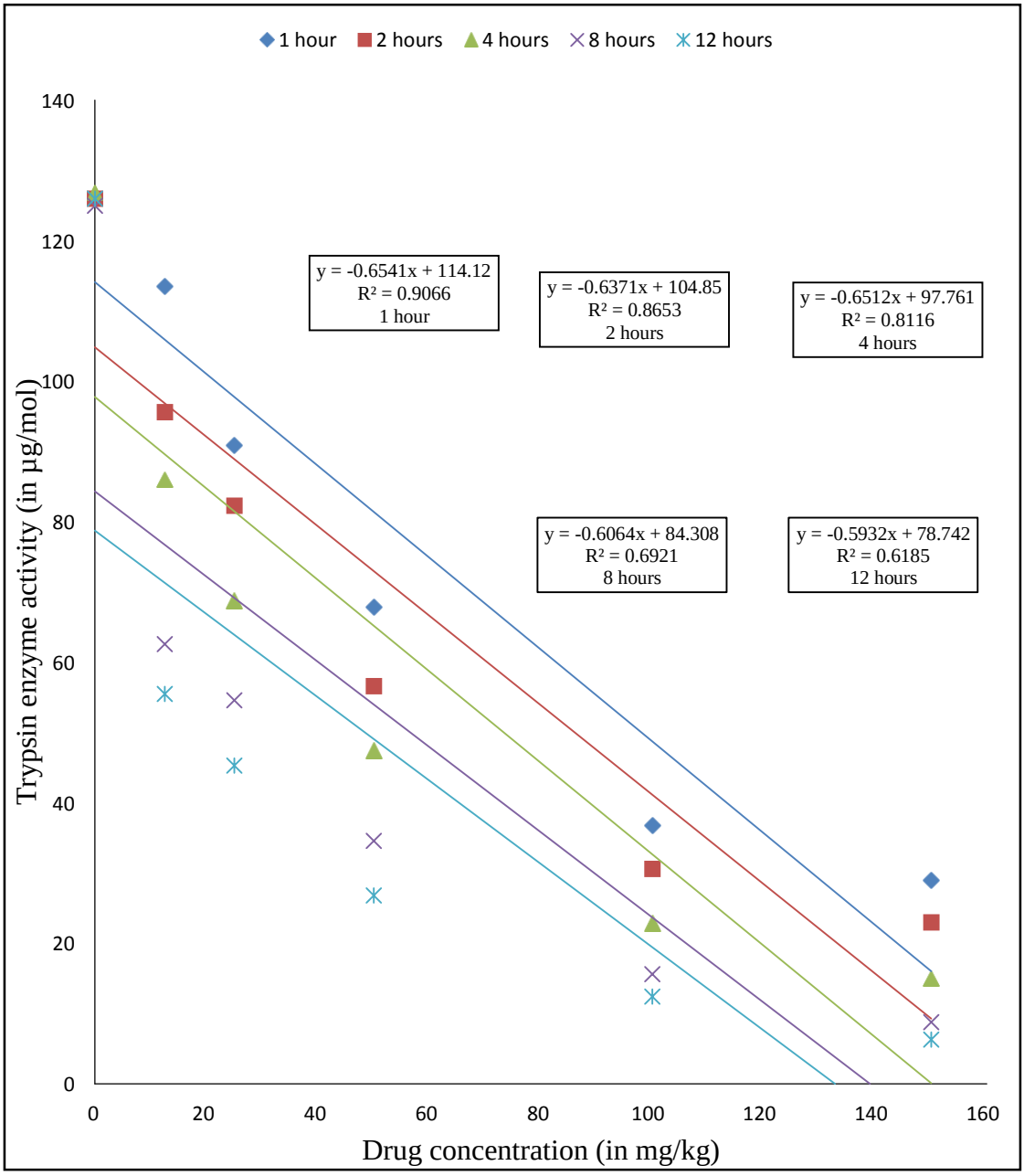


Fig. 4.2: Regression curve for effect of indomethacin concentration on trypsin activity in Norway rats

4.3 Amino peptidase activity in the ileum

The highest mean amino peptidase activity (141.9±0.05 µg/mol**)** was recorded in the control group. However, in the experimental groups the least amino peptidase activity (7.6±0.10 µg/ml) was recorded in the group that was given the highest indomethacin dose of 150mg/kg while the highest enzyme activity (141.5±0.04 µg/mol) was recorded for the group given the least dose of indomethacin of 12.5mg/Kg. The results therefore show that the mean amino peptidase activity decreased with increase in the indomethacin dose given in all the groups (Table 4.3). The mean values for aminopeptidase activity were significantly different in groups given different indomethacin concentrations at p=0.05 (Table 4.3).

Table 4.3: Mean values and ± S.E values for amino peptidase enzyme activities at different concentrations (mg/Kg)

Experimental Groups	Amino peptidase enzyme activity (µg/mol) n=5				
	Sub-Group1 (1h) n=5	**Sub-Group2 (2hs) n=5**	**Sub-Group3 (4hs) n=5**	**Sub-Group4 (8hs) n=5**	**Sub-Group5 (12hs) n=5**
Group A	141.5±0.04^{a}	140.0±0.04^{a}	141.9±0.05^{a}	141.4±0.0^{a}	140.6±0.03^{a}
Group B	126.5±0.04^{b}	106.6±0.04^{b}	97.5±0.05^{b}	86.8±0.06^{b}	67.8±0.03^{b}
Group C	101.2±0.03^{c}	85.5±0.05^{c}	77.4±0.03^{c}	70.7±0.08^{c}	56.2±0.04^{c}
Group D	76.2±0.03^{d}	59.5±0.06^{d}	50.7±0.08^{d}	41.0±0.10^{d}	28.6±0.10^{d}
Group E	41.7±0.04^{e}	34.4±0.09^{e}	26.6±0.10^{e}	19.6±0.10^{e}	13.6±0.10^{e}
Group F	33.5±0.09^{f}	26.5±0.10^{f}	17.6±0.10^{f}	9.5±0.1^{f}	7.6±0.10^{f}

N.B Means followed by same letters are not significantly different from each other at p<0.05

A negative linear relationship was found to exist between indomethacin concentration and amino peptidase activity after the data was taken through regression analysis. A decrease in concentration of indomethacin resulted in a linear increase in the activity of amino peptidase (Fig. 4.3).

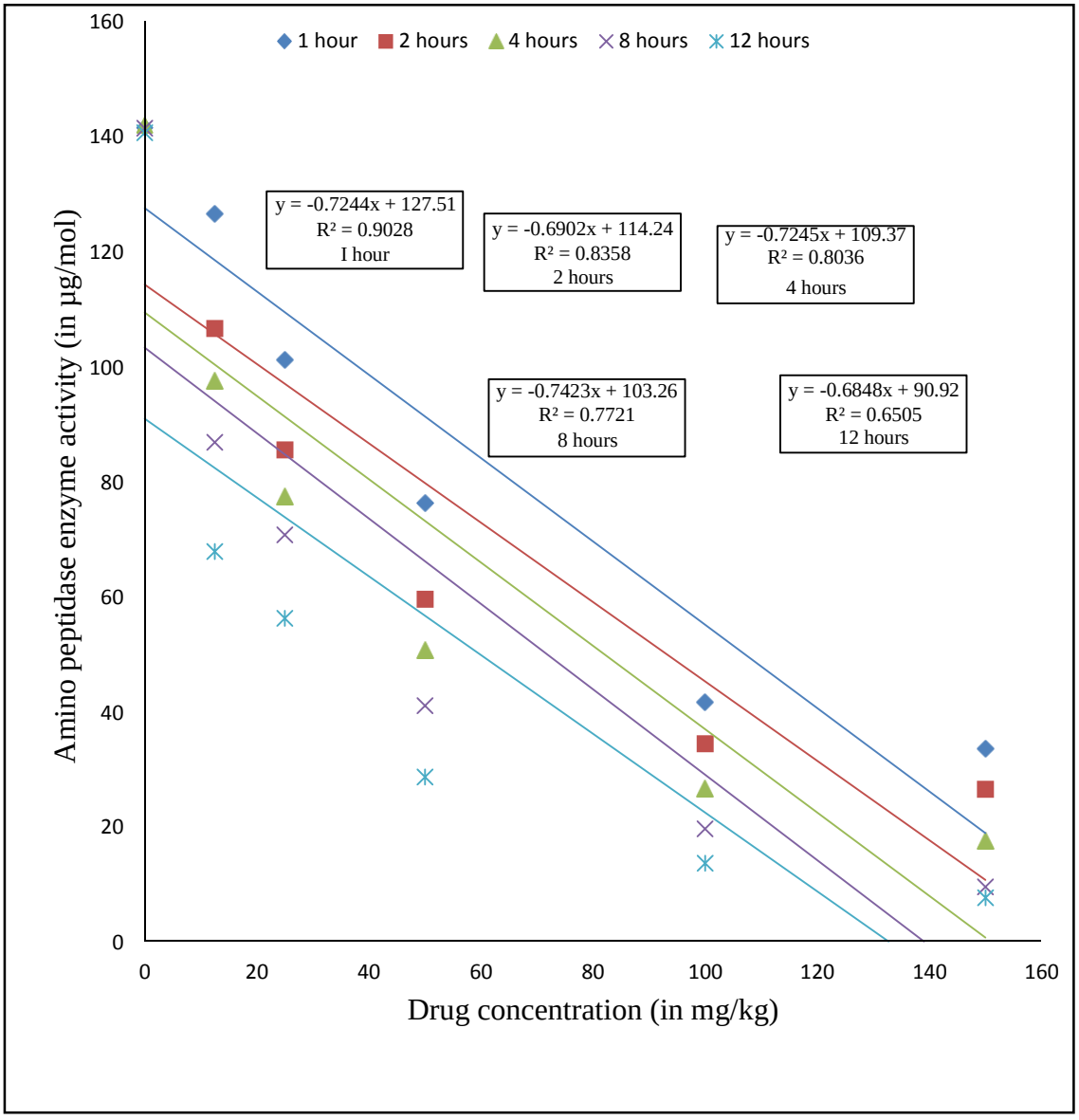


Fig. 4.3: Regression curve for effect of indomethacin concentration on amino peptidase activity in the Norway rat.

4.2 Effect of time taken after drug administration on enzyme activity in the rats

4.2.1 Pepsin activity in the stomach

The results show that pepsin activity decreased when more time was allowed before euthanization after indomethacin administration in the experimental groups. the highest pepsin activity was recorded after one hour (239.2±1.0 µg/mol) while the least enzyme activity was recorded after 12 hours (5.6±0.4 µg/mol), However, the mean pepsin activity remained high in the control groups irrespective of the time taken after drug administration. Increase in time taken after indomethacin administration led to a decrease in pepsin activity (Fig. 4.4). The mean enzyme activity at different time intervals at the same drug concentrations for the experimental groups was significantly different from each other at $p<0.05$. However, that of the control group showed no significant difference at different time intervals.

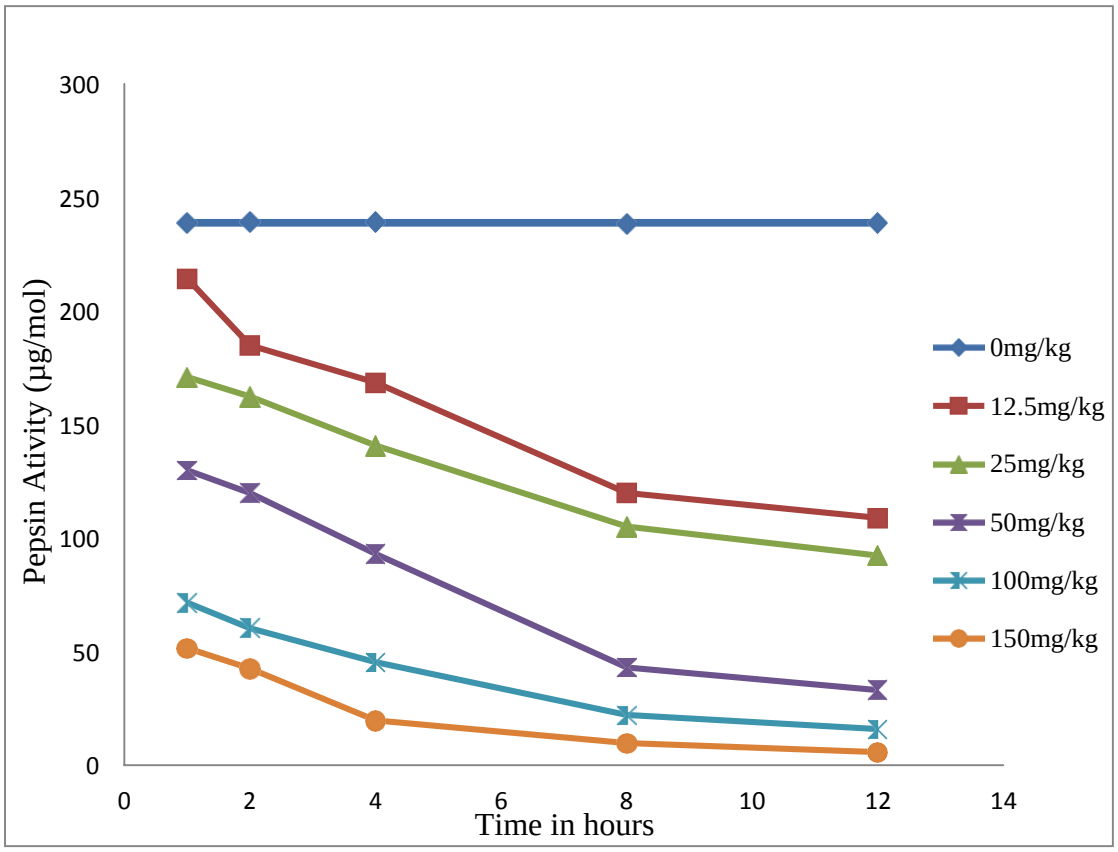


Fig. 4.4: Effect of time taken after indomethacin administration on pepsin activity

4.2.2 Trypsin activity in the duodenum

The mean trypsin activity remained high in the control group irrespective of the time taken after indomethacin administration with activity range of 239.2±1.0-238.4±0.5 µg/mol. There was, however, a decrease in trypsin activity as time went by for all the experimental groups with the highest activity was reported after one hour (113.2±0.03 µg/mol), while the least trypsin activity reported after 12 hours (6.3±0.10 µg/mol) (Fig. 4.5). From the results trypsin activity decreases as more time is taken after indomethacin administration. The mean enzyme activity at different time intervals was found to be significantly different from each other at $p<0.05$ in the experimental groups. However, that of the control group showed no significant differences at different time intervals (Fig. 4.5).

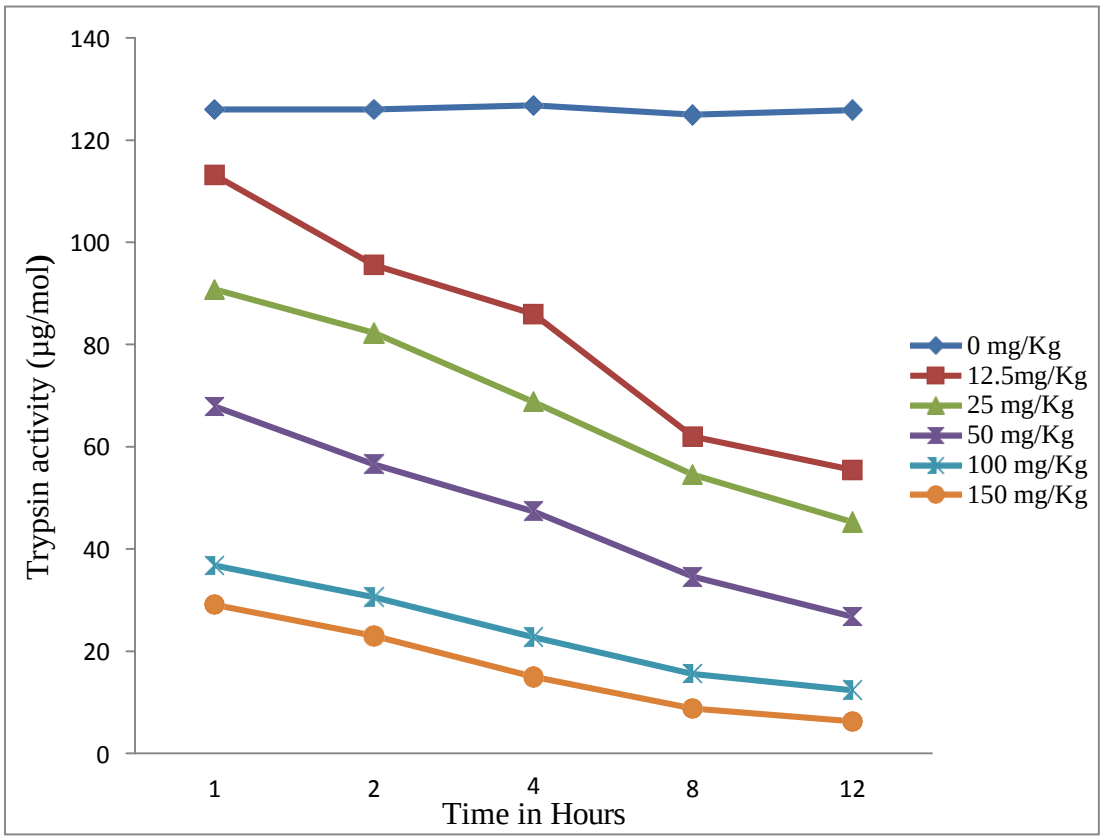


Fig. 4.5: Effect of time taken after indomethacin administration on trypsin activity

4.2.3 Amino peptidase activity in the ileum

The control groups recorded highest mean amino peptidase activity irrespective of the time taken before being euthanized after drug administration with a range of 141.9±0.04-140.0±0.04 µg/mol. There was a decrease on amino peptidase activity as time went by for the experimental groups; rats euthanized after one hour interval had the highest mean amino peptidase activity (126.5±0.04 µg/mol) while those euthanized after twelve hours recorded the least enzyme activity (7.6±0.2 µg/mol) (Fig. 4.6). The results show that at $p<0.05$, the experimental groups showed significant difference in their mean values at different time intervals except group six that showed no significant difference in their means at eight hours and twelve hours. There was no significant difference in the mean enzyme activities of rats in the control group (Fig. 4.6).

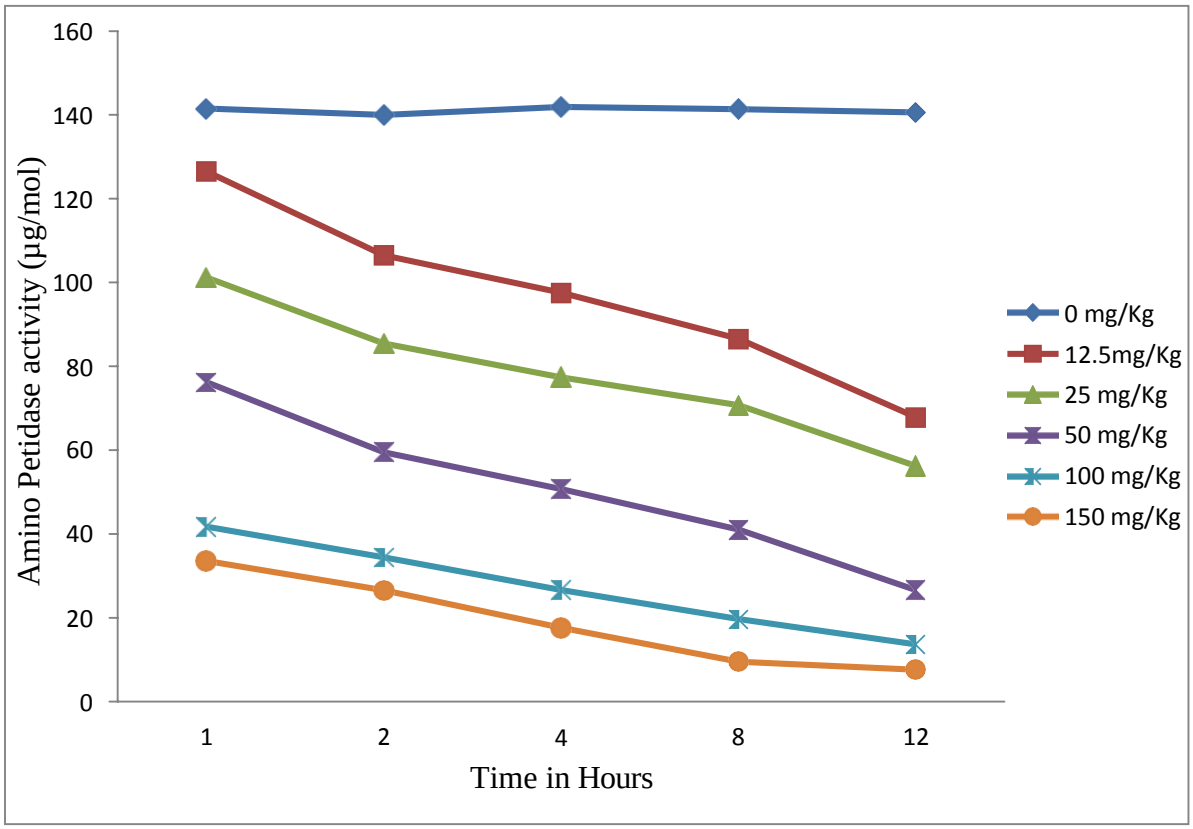


Fig. 4.6: Effect of time taken after indomethacin administration on amino peptidase activity

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMENDATIONS

5.1 Discussion

5.1.1 Dose dependent effects of indomethacin on protein digesting enzymes

5.1.1.1 Dose dependent effects of indomethacin on pepsin activity in the stomach

The results of this study showed that indomethacin affects the activity of pepsin by decreasing its activity. The higher the dose of indomethacin administered, the lower the activity of the enzyme. This could be because indomethacin is known to cause gastro-toxic effects (Polat *et al*., 2010). Indomethacin causes gastritis (Werawatganon *et al.,* 2014; Badii *et al.,* 2016) and induces peptic ulcers (Lee *et al.,* 2016). Gastritis leads to a decrease in the production of gastric acid and pepsin because the stomach lining contains gastric acid and pepsin secreting glands (NIDDK, 2015). Peptic ulcers reduces secretion of gastric acid and pepsin (Ramakrishnan *et al.*, 2007). The gastric acid is involved in activation of pepsin from the inactive pepsinogen so decreased amounts result in less pepsin available in the gastric juice leading to a total decline in the amount of pepsin. Since the activity of enzymes depend on the amount of enzyme available (Mayo *et al.,* 2016), the activity of pepsin will be decreased. Increase in the dosage of indomethacin leads to a more decreased gastric acid and pepsin production because indomethacin action is dose dependent (Oluwabunmi and Abiola, 2015) and thus a corresponding decrease in the enzyme activity.

Indomethacin is a known denaturation agent of many enzymes including digestive enzymes (Kilpatrick and Bunk, 2009). At the same time, indomethacin at moderate temperatures has also

been shown to accelerate the presence of denatured proteins in the cell (Roussou, 2000). It has also been observed that rats treated with indomethacin showed increase in gastric volume, free total acidity of gastric secretion and changes in pH of the gastric secretion by decreasing their pH (Katary and Salahuddin, 2017). Since enzymes have an optimal pH at which their velocity is maximal, decrease in the pH of the gastric secretion will lead to a decline in their velocities (Robinson, 2015). Furthermore, the decrease in gastric secretion pH leads to further denaturation of the pepsin as changing pH causes change in the protein or enzyme conformation denaturation (Dwevedi *et al.*, 2010). Moreover, it has been found that when enzymes are denatured their activity is also lowered (Kishore, 2012), as denaturing of enzymes means less is available for action on the substrate. By lowering their levels, their activity is also lowered as the concentration of enzyme affects the rate of its digestion directly and therefore its activity (Nikitina *et al.*, 2010). As the dose of indomethacin is increased, the denaturing effect is also increased leading to decreased activity of the pepsin enzyme as indomethacin effects depend on the dose administered (Oluwabunmi and Abiola, 2015).

The findings of this study are supported by the observation that winster rats treated with indomethacin, in an experiment to determine the gastroprotective effects of the leaves of S*pondias mombin and Ficus exasperate*, showed decreased pepsin activity (Sabiu *et al*., 2015). The higher the dose administered the higher the effects of indomethacin as indomethacin effects are dose dependent (Oluwabunmi and Abiola, 2015; Taiwo and Conteh, 2008) and the lower the pepsin activity, since the concentration of the enzyme affects the rate of its digestion directly and therefore its activity (Nikitina *et al*., 2010); Enzyme concentration and activity are also directly proportional (Mayo *et al*., 2016).

5.1.1.2 Dose dependent effects of indomethacin on trypsin activity in the duodenum

This study found out that indomethacin affects trypsin by lowering its activity. The activity of trypsin also reduces with increase in the dose of indomethacin administered to the rats. This could be due to the denaturizing effect of indomethacin on trypsin enzyme (Kilpatrick and Bunk, 2009). The reduction in trypsin activity could have resulted from pancreatitis, caused by indomethacin (Mahjoub *et al.*, 2006; Memis *et al.*, 2005), which leads to reduced secretion of pancreatic enzymes (Bansal, 2017). This results in decreased amount of trypsin, leading to reduction in its activity as the concentration of trypsin affects the rate at which it digests directly and therefore its activity (Nikitina *et al.*, 2010). Indomethacin also causes chronic pancreatitis (Pezzilli *et al*., 2010) which leads to a decrease in the amount of digestive enzymes present in the digestive fluid (Bansal, 2017). This causes a decrease in the amount of trypsin and thus a decrease in the enzyme activity as enzyme concentration and activity is directly proportional (Mayo *et al.*, 2016)

Enterohepatic cycling redelivers into the duodenum significant concentrations of indomethacin (20% to 65% of the initial dose) (Boelsterli and Ramirez-Alcantara, 2011) leading to much higher levels of indomethacin in the duodenum. The higher levels of indomethacin will cause more reduction in calcium ion levels, denature more trypsin and cause more severe pancreatitis leading to lower levels of trypsin and thus decreased enzyme activity (Mayo *et al.*, 2016). When the dose of indomethacin is increased there will be more effects on the enzyme leading to a further decrease in trypsin activity as indomethacin effects depend on the dose given (Oluwabunmi and Abiola, 2015).

Indomethacin blocks the calcium ion channels (Kaneko *et al*., 2016) and reduces its amount in the duodenum since it is antagonistic to calcium. Since calcium ions stabilize the structure of trypsin (Gilliland and Teplyakov, 2006) and activates trypsin from trypsinogen (Reraty *et al.,* 2000; Papaleo *et al*., 2005), low amounts of calcium will lead to fewer and less stable trypsin resulting in lower trypsin activities. When the concentration of indomethacin is increased, these effects become more pronounced and hence the decrease in trypsin enzyme activity with increase in indomethacin dosage since the action of indomethacin depends on the dosage given (Oluwabunmi and Abiola, 2015; Mayo *et al.,* 2016). The results of this study agree with the findings that in an experiment to determine the rodenticidal effects of indomethacin, Norway rats and mice given indomethacin showed increased intensity in anorexia, lesions, vascular congestion, hemorrhage, gastric ulceration, and glandular necrosis as the doses were increased from 86mg/Kg to 250mg/kg (Taiwo and Conteh, 2008).

5.1.1.3 Dose dependent effects of indomethacin on amino peptidase activity in the ileum

In this study, the results show that the activity of amino peptidase is reduced by indomethacin. As the dosage of indomethacin is increased the activity of amino peptidase is reduced. This could be due to the fact that indomethacin, just like other non-steroidal anti-inflammatory drugs (NSAIDs) has a toxic effect on the small intestines (Scarpignnato, 2008; Peron, 2013). Indomethacin increases the risk of developing necrotizing enterocolitis with intestinal perforation (Sood *et al.,* 2011; Fujii *et al.,* 2002). Enterocolitis causes increased intestinal permeability and a decrease in the expression of amino peptidases (Fadi *et al.,* 2016), which leads to a decrease in the amino peptidase enzyme activity. When the dose of indomethacin is increased, more severe enterocolitis will result leading to even much lower amino peptidase

activity. This is because indomethacin actions are dose dependent (Kurata *et al*., 2015). Enterocolitis is also manifested by decreased intestinal fluid secretion (Barada *et al.*, 2001). Because amino peptidase is part of the intestinal fluid, this implies lower amounts of amino peptidase enzyme leading to lower enzyme activity since the enzyme activity is directly proportional to enzyme concentration (Mayo *et al*., 2016).

Indomethacin denatures amino peptidase (Kilpatrick and Bunk, 2009), and also accelerates the presence of denatured proteins in the cell (Roussou, 2000). This leads to less enzyme available for catalysis leading to reduction in enzyme activity as the amount of enzyme present is directly proportional to enzyme activity (Mayo *et al*., 2016). Since indomethacin actions are dose dependent (Kurata *et al.*, 2015), increase in the dose of indomethacin will lead to a corresponding decrease in the enzyme activity.

Indomethacin causes intestinal proliferative enteropathy (McOrist and Gebhart, 2006), which leads to lowering of enzyme activity of amino peptidase and that this decrease in amino peptidase activity due to enteropathy is dose dependent (Bolsius, 2009). Indomethacin effect on the small intestine has also been found to be dose dependent (Kurata *et al.*, 2015). Therefore, the higher the dose of indomethacin, the more severe the enteropathy; the more severe the enteropathy the more the decreased enzyme activity and hence the lower the enzyme activity of amino peptidase as observed in this research. This then clearly explains why the enzyme activity of amino peptidase decreased as the dose of indomethacin increased in this experiment. These observations are in agreement with studies which found that pigs administered with

indomethacin showed decreased amino peptidase activity and the decrease was dependent on the dose administered (Bolsius, 2009).

5.1.2 Effects of time taken after administration of indomethacin on protein digesting enzymes

5.1.2.1 Pepsin activity

From the results of the study, pepsin activity decreased with increase in time taken before euthanization of rats after indomethacin administration. This is because indomethacin actions are time dependent (Riew *et al.,* 2003; Owoyele *et al.,* 2016). Due to this fact, the more time it takes before euthanization, the more intense is its action on the stomach and the more severe the impact. More time taken after indomethacin administration leads to more gastrototoxic effects (Polat *et al*., 2010), intense ulceration, peptic ulcers (Lee *et al*., 2016), more perforations (Taiwo and Conteh, 2008), severe bleeding and an increase in the ulcer index of the stomach (Katary and Salahuddin, 2017). These then lead to more severe gastritis (Werawatganon *et al.,* 2014) and peptic ulcers (Lee *et al.,* 2016), leading to reduced production of gastric acid and pepsin (Dey *et al.,* 2009) and thus a decrease in pepsin enzyme activity.

Indomethacin also causes enzyme denaturation (Kilpatrick and Bunk, 2009). As such, more time taken before euthanization after its administration also means higher rates of denaturation of pepsin, and further reduction in the pepsin activity. All the above factors combined lead to a decrease in the amount of enzyme and thus a lower pepsin activity since enzyme activity and concentration are directly proportional (Mayo *et al*., 2016). These results are in line with the observation that indomethacin's effects are dependent on time (Taiwo and Conteh, 2008), when

he observed that when Norway rats and mice were given indomethacin, they showed anorexia, dehydration and weakness, and these signs got worse progressively with time. He further noted that dehydration was only seen after 24 hours of observation after indomethacin administration and not immediately or earlier.

5.1.2.2 Trypsin activity

Trypsin activity decreases with increase in time taken before euthanization after indomethacin administration, according to the findings of this study. This is because indomethacin effects are time dependent (Riew *et al.,* 2003; Taiwo and Conteh, 2008; Owoyele *et al.,* 2016). This being the case, increase in time taken after indomethacin administration will lead to more severe effects in the duodenum and thus a decrease in the activity of trypsin. Indomethacin denatures trypsin (Kilpatrick and Bunk, 2009). If more time is allowed after its administration, more denaturation takes place, less trypsin is left and hence the decrease in trypsin activity. Similarly, indomethacin causes pancreatitis (Memis *et al.,* 2005; Mahjoub *et al.,* 2006). If more time is allowed after the drug administration, more severe pancreatitis will result leading to reduced secretion of pancreatic enzymes (Bansal, 2017), resulting in lower amounts of trypsin and thus the reduced enzyme activity. More time after the drug administration also means more bile secretion into the duodenum Bile secretions contain significant amounts of indomethacin due to enterohepatic cycling of the drug (Boelsterli and Ramirez-Alcantara, 2011) which increases the overall concentration of the drug leading to more severe effects as indomethacin effects are dose dependent (Oluwabunmi and Abiola, 2015). This leads to decreased trypsin activity.

Indomethacin also blocks the calcium ion channels and also reduces the amount of calcium in the duodenum due to its antagonistic nature to calcium (Kaneko *et al.,* 2002). If more time is allowed after drug administration, less calcium will be absorbed while at the same time the amount will be further reduced. Because calcium ions are needed for stabilization of trypsin structure (Gilliland and Teplyakov, 2006) and activation trypsin from trypsinogen (Reraty *et al.*, 2000; Papaleo *et al.,* 2005), the amount of trypsin will be reduced and thus its activity (Mayo *et al.,* 2016). Therefore, as more time elapses after indomethacin administration, its effects become more serious and the trypsin activity becomes lower. The findings of this study are supported by the observation that indomethacin effects are spontaneous but not instantaneous, since it was found out that the drug significantly affected the tissue lactate/pyruvate ratio at 3 h but not at 1 h after oral dosing (Jacob *et al.,* 2001). This was in a study to find out the effects of indomethacin on energy metabolism in rat and human jejunal tissue *in vitro.*

5.1.2.3 Amino peptidase activity

Based on the findings of this study amino peptidase activity decreases with increase in the length time taken before rats are euthanized after indomethacin administration. These results agree with the findings that indomethacin actions are time dependent (Owoyele *et al.,* 2016; Riew *et al.,* 2003). These findings were further confirmed by the observation that indomethacin effects are gradual and not immediate (Jacob *et al.,* 2002).

Indomethacin also causes necrotizing enterocolitis (Fujii *et al*., 2002; Sood *et al.*, 2011), which causes decreased intestinal fluid secretion (Barada *et al.,* 2001), increased intestinal permeability and a decrease in the expression amino peptidases (Fadi *et al.,* 2016), leading to a decrease in amino peptidase activity. When more time is allowed the necrotizing enterocolitis

will be more severe, leading to more reduction in the amino peptidase enzyme activity. Indomethacin also causes intestinal proliferative enteropathy (McOrist and Gebhart, 2006; Lim and Chun, 2013), which leads to lowering of enzyme activity of amino peptidase (Bolsius, 2009). More time after the drug administration means more severe enteropathy, leading to further lowering of amino peptidase activity.

Indomethacin increases mucosal permeability of the intestines which can lead to action of luminal factors like bile acids (Somasundaram *et al.,* 2000). These bile acids if mixed with the drug form toxic mixed micelles within the bile fluid that contribute to indomethacin-induced gastrointestinal injury (Zhou *et al.,* 2010; Dial *et al.,* 2015). This then leads to a higher degree of enterocolitis and enteropathy. When more time is allowed there is more intestinal permeability, higher quantities of mixed micelles are formed, more severe gastric injury results, higher degree enterocolitis and enteropathy also results which leads to decreased amino peptidase activity.

Indomethacin also denatures amino peptidase enzyme (Kilpatrick and Bunk, 2009). If more time is allowed after its administration, more denaturation takes place, lower quantities of amino peptidase enzymes are left and hence the decrease in amino peptidase enzyme activity. The findings of this study are in line with the observations that indomethacin effects are time dependent as the first rat died after 28 hours when given a dose of 83mg/Kg indomethacin and not immediately. This was found in an experiment to determine the rodenticidal action of indomethacin in Norway rats and mice (Taiwo and Conteh, 2008).

5.3 Conclusions

(i) Indomethacin lowers activity of pepsin, trypsin, and amino peptidase. The effect was found to be dose dependent. Increase in indomethacin doses led to decrease in the activity of the three enzymes studied (pepsin, trypsin and amino peptidase).

(ii) Indomethacin action on protein digesting enzymes is time dependent. When more time was allowed after administration of indomethacin, there was a decrease in the activity of pepsin, trypsin and amino peptidase.

5.4 Recommendations

(i) Higher doses of at least 100mg/Kg should be administered as they inactivate the enzymes (pepsin, trypsin and amino peptidase) and cause death.

(ii) Since indomethacin's action is gradual, its bait can be taken to nest by the mother rats where humans cannot reach due to the hidden nature of the rats nest, killing more rats.

5.5 Suggestions for further studies

i) Determine the effects indomethacin on other digestive enzymes like carbohydrases and lipases.

ii) Determine the mechanism by which indomethacin reduces the protein digesting enzymes activity. That is, whether it affects enzyme production, or it is an enzyme inhibitor.

iii) Determine the effects of indomethacin on other rodents such as moles which are a threat to food security.

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APPENDICES

Appendix I

Approval of the research proposal letter from Graduate School, Kenyatta University

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 17th August 2016

TO: Onyingo Joseph Opondo
C/o Zoological Sciences Department.

REF: I56/27463/13

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting of 10th August 2016, approved your Research Proposal for the M.Sc. Degree Entitled, "Effect of Indomethacin on Selected Protein Digesting Enzymes in Rats".

You may now proceed with data collection, subject to clearance with the Director General, National Commission for Science, Technology and Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking forms per semester. The form has been developed to replace the progress report forms. The supervision Tracking Forms are available at the University's website under Graduate School webpage downloads.

Thank you.

JACKSON LUVUSI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Zoological Sciences

Supervisors:

1. Dr. Syprine Otieno
   C/o Department of Zoological Sciences
   Kenyatta University
2. Dr. Richard Oduor
   C/o Department of Biochemistry & Biotechnology
   Kenyatta University
3. Dr. Geoffrey M. Karau
   Head of Testing Services
   Kenya Bureau of Standards
   C/o Department of Zoological Sciences
   Kenyatta University

JL/nn

Appendix II

Introduction Letter to NACOSTI from Graduate School, Kenyatta University.

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: I56/27463/2013 DATE: 17th August 2016

Director General,
National Commission for Science, Technology
& Innovation
P.O Box 36023-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR ONYINGO JOSEPH OPONDO– REG. NO. I56/27463/2013.

I write to introduce Mr. Onyingo Joseph Opondo who is a Postgraduate Student of this University. He is registered for M.Sc degree programme in the **Department of Zoological Sciences.**

Mr. Onyingo intends to conduct research for a M.Sc. Proposal entitled, **"Effect of Indomethacin on Selected Protein Digesting Enzymes in Rats".**

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

JL/nn

Appendix III

Letter of authority to carry out research from NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/91772/28193** Date: **22nd February, 2019**

Joseph Opondo Onyingo
Kenyatta University
43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Effect of indomethacin on selected protein digesting enzymes in rats."*** I am pleased to inform you that you have been authorized to undertake research in **Nakuru County** for the period ending **21st February, 2020.**

You are advised to report to **the County Commissioner and the County Director of Education, Nakuru County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nakuru County.

The County Director of Education
Nakuru County.

Appendix IV

Research permit from NACOSTI

THIS IS TO CERTIFY THAT:
MR. JOSEPH OPONDO ONYINGO
of KENYATTA UNIVERSITY , 0-20100
NAKURU,has been permitted to conduct
research in *Nakuru County*

on the topic: *EFFECT OF INDOMETHACIN ON SELECTED PROTEIN DIGESTING ENZYMES IN RATS*

for the period ending:
21st February,2020

Permit No : NACOSTI/P/19/91772/28193
Date Of Issue : 21st February,2019
Fee Recieved :Ksh 1000

.....
Applicant's Signature

.....
Director General
National Commission for Science, Technology & Innovation