EFFECTS OF *Senna siamea* ON THE HISTOLOGY AND HISTOCHEMISTRY OF THE COLON IN OPOIOD-INDUCED CONSTIPATION IN WISTAR RATS

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SEPTEMBER, 2015.

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MSc/MED/756/2011-2012

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,

AHMADU BELLO UNIVERSITY ZARIA, NIGERIA.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN HUMAN ANATOMY

SEPTEMBER, 2015.

DECLARATION

I declare that the work in this thesis, entitled "Effects of *Senna siamea* on the Histology and Histochemistry of the Colon in Opoiod-Induced Constipation in Wistar Rats" has been carried-out by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria under the supervision of Prof. S.S Adebisi and Dr (Mrs) J.N. Alawa.

The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Peter Ayo OMOTOYINBO ---------- ----------

Signature **Date**

CERTIFICATION

This thesis, entitled "Effects of *senna siamea* on the Histology and Histochemistry of the Colon in Opioid-Induced Constipation in Wistar Rats" by Peter Ayo Omotoyinbo meets the regulation governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation

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DEDICATION

This thesis is dedicated first to GOD ALMIGHTY who sustained me through my stay here in ABU, Zaria. Secondly, to my darling Mother (Mrs. Duro Omotoyinbo) for her relentless support both financially and morally. Thirdly, to my granny (Mrs. Oyekemi Famojuro) and finally to my brothers, 2nd Lieutenant E.A Omotoyinbo and Engineer M.D Omotoyinbo, may the LORD bless and keep you all. Amen.

ACKNOWLEDGEMENTS

My sincere appreciation goes to my major supervisor Prof. S.S. Adebisi, who is also the Head of Human Anatomy Department, Ahmadu Bello University, Zaria, thank you Sir for taking your time to review this thesis. My prayer is that God should continue to strengthen you in all your endeavours. Amen! My passion for this work was instigated by Dr. (Mrs.) J.N. Alawa. Ma, you are indeed a role model to emulate as far as academics and excellence is concerned. I have not relented in praying for you. I am highly indebted to Barrister Ola, Mr. Peter Akpulu, Mr Abel Nwosorene, Dr. A.A Buraimoh, Professor S.A. Ojo, for his fatherly guidance, Dr. J.A Timbuak, Dr. S.A. Musa and many of my friends who directly or indirectly participated in conducting the research. I say may the Lord in His abundance bless you all. Amen. To my caring and ever-concerned mother (Mrs. Duro Omotoyinbo), I love you even more! I cannot forget the support and encouragement given to me by Joro R.M. Joshua, Stella O. Amuzor, Engineer &Mrs. Teryima Igbax, Sunday Godwin Obaje, Dr. Joshua, Itoro George, Emmanuel Oguche, Daniel Richard, Monday Nnwako, Abraham Akubo and many others. To the Agbede's family, I am very grateful for your relentless support through prayers. To my lovely auntie Iyabo Adeleke, I cannot forget your effort in a haste, God bless your home.

I am highly indebted to Navy Captain K.O. Ojo of the Nigerian Defence Academy**,** Kaduna for his moral and financial support, may the Lord continue to keep you and your family Sir.

To my lovely and ever-supportive brothers, 2nd Lieutenant E.A. Omotoyinbo and Engineer M.D. Omotoyinbo, you are the best brothers in the world and I'm proud of you guys! I am also grateful to the former Head of Department of Human Anatomy Department, ABU, Zaria, Dr.S.B.Danborno. I duly acknowledge and appreciate your kind gestures in the course of this research.

Most importantly, I return all the praise to the most-high God in heaven for His protection, mercy, love and compassion over me and my family. May His glorious name be praised forever-more. Amen!

ABSTRACT

Senna siamea is a medicinal plant native to Asia. It is widely distributed in different parts of Nigeria, where it is employed in traditional ethno-medicinal and ethno-veterinary practices for a variety of ailments. In this study, the effect of *S. siamea* on smooth muscle contractility of the ileum in an organ-bath was demonstrated as well as evaluating the histology and histochemistry of the colon in opioid-induced constipation in Wistar rats. Thirty (30) female Wistar rats with mean weight of 126.7g were used in this work. They were randomly grouped into five groups (I-V) of six rats each and treated as follows; Group I received Normal saline, Group II received Loperamide(3mg/kg), Group III received *S. siamea*(300mg/kg) and Loperamide(3mg/kg), Group IV received Loperamide and *S. siamea*, Group V received Bisacodyl 5mg/kg (Standard laxative). Constipation was induced in Group II for 6-days using loperamide at 3mg/kg. There was a significant improvement in the nature of stool (size and texture) of animals treated with extract (*S. siamea*) after induction with loperamide. Results obtained at the in vitro phase (contractility experiment) of this work showed that *S. siamea* possess laxative effect by relaxing the rapid contraction of the ileum of wistar rats. In this study, *S. siamea* was observed to relax the contractility of ileum in organ-bath (tyrode solution) which was similar to what was observed when loperamide was administered. *Senna siamea* was effective in treating opiate-induced constipation at the in-vitro and in-vivo phase as it ameliorate the constipative effects of loperamide. In addition, *Senna siamea* improved evacuation of stool by reducing the muscle tone of the Gastro-intestinal tract. There was no statistical significant difference in stool weight, organ-body weight ratio, and change in body weight of experimental animals at $P \leq 0.05$. Histological analysis using Heamatoxylin and Eosin stain revealed several enlarged and numerous goblet cells around the crypt of Lieuberkuen in the loperamide treated group (loperamide 3mg/kg) while goblet cells of extract treated groups(*S.siamea* 300mg/kg) were fewer and reduced in size which could be attributed to the laxative effect of the extract on the mucosa of the GIT. Goblet cells of bisacodyl (standard laxative) 5mg/kg treated group appeared fewer and enlarged. PAS was used to specifically stain neutral mucin. Mucous cells in loperamide treated group stained PAS positive with numerous and enlarged goblet cells. This is in sharp contrast with results obtained in extract treated group as fewer stained mucous cells were observed. Based on our observations, we therefore conclude that *Senna siamea* had laxative effect on the colon of constipated wistar rats by suppressing goblet cell production of mucous, improvement of faecal evacuation as well as its relaxing effect on the ileum.

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CHAPTER ONE

INTRODUCTION

Senna siamea (*Cassia siamea*) is a non-nitrogen fixing leguminous tree in the family Leguminosae and sub-family Caedalpinoidea. There are over 400 known species of Cassia. *Senna siamea* is native to south and south-east Asia from Thailand and Myanmar (Brandis 1906, Gamble 1922) to Malaysia, India, Sri Lanka and Bangladesh (Khan and Alan, 1996). It has been cultivated world-wide and is naturalized in many locations (Gutteridge, 1997). It is commonly called Bombay blackwood, *cassod tree*, *kassod tree*, pheasant wood, pheasant-wood, *Siamese cassia*, *Siamese senna*, Thai *cassia*, Thai copper pod, Thailand shower. In Nigeria, *Senna siamea* is widely distributed in the southwest and some areas in the North. It is commonly referred to as 'ewe cassia' in the southwest where it is believed to cure fever and has also serve some other medicinal purposes (especially the leaves) (Ogunkunle 2006). In the North, it is known as "Labadiya" and commonly planted as shelter belts. The chemical composition of *Senna siamea* (cassia leaves) has been determined from recent studies and contains saponins, anthraquinones, phytobatannins, alkaloids and crude proteins (Smith, 2009).

Senna siamea (*S.siamea*) is an ingredient found in several commercial laxative products and a US Food and Drug Administration (FDA) - approved non-prescription drug. The leaves and the fruit (pods) of *Senna* are used as stimulant laxatives, which function by anthraquinone cathartic action, and are generally well tolerated in the adult population, but when this is taken at much higher than recommended doses or when used chronically (laxative abuse), adverse effects may occur as reported in other substance such as ethanol (Adebisi, 2003). Constipation refers to bowel movements that are infrequent or hard to pass (Chatoor and Emmanuel, 2009) and a common cause of painful defecation. Opioids stimulate the absorption of fluids, mainly by delayed transit,

increasing contact time for absorption, and by stimulating mucosal sensory receptors that facilitate further fluid absorption (De Luca and Coupar, 1996)

1.1 STATEMENT OF THE RESEARCH PROBLEM

Senna siamea has been widely used as a laxative but its effect on the colon in treating opiate-induced constipation has not been fully established.Constipation occurs as an adverse drug reaction to opioid treatment for pain relief, especially among opioid-addicts and regular users. The prevalence of constipation is 2-fold higher among Africans of lower socio-economic status and in nursing home residents (Higgins *et al*., 2004).

1.2 JUSTIFICATION

Senna leave consumption reduces the risk of chronic constipation from unhealthy lifestyle, dieting and even in cases of abuse of certain drugs that indirectly induce constipation. Knowledge gained from this study could stimulate the minds of indigenous researchers into the field of medicinal plants and herbal remedies. Getting a natural laxative like *S. siamea* (without drug interaction) will be helpful in ameliorating side-effects of opioid-induced constipation among addicts and regular users.

1.3 STUDY HYPOTHESIS

Aqueous extract of *S. siamea* will induce a laxative effect on opioid-induced constipation in Wistar rats.

1.4 AIM

The study was aimed at evaluating the anti-constipative activity of aqueous extract of *S. siamea* leaves on opioid-induced constipation in colon of Wistar rats.

1.5 OBJECTIVES

The objectives of this present study were to:

i. evaluate the in-vitro effects of *S. siamea* on contraction of smooth muscles of intestine (ileum) before and after exposure to opiates
ii. determine the effects of *S. siamea* on the histology of the large intestine (distal colon) exposed to opiates
iii. histochemically assess the effect of *Senna siamea* on the mucin contents in the distal colon of wistar rats

CHAPTER TWO

LITERATURE REVIEW

2.0 GENERAL DESCRIPTION OF *SENNA SIAMEA*

Senna siamea is a medium-sized evergreen tree, attaining 5 meters height. It rarely exceeds 20m height and 50cm in diameter (Jensen, 1995). It has a dense, evergreen, irregular, spreading crown, a cooked stem, and smooth, grayish bark that is slightly fissured longitudinally. Its young branches have fine hairs. The leaves are pinnately compound with an even leaf arrangement of 7-10 years of ovate-oblong leaflets, 7-8 cm long and 1-2 cm wide. Its flowers are yellow, borne in large terminal panicles that are often 30 cm long. The fruit is a flat pod 15-25 cm long, thickened at both sutures, containing many seeds (Gutteridge, 1997).

Senna induces intestinal peristalsis (laxative property) i.e. stimulated peristalsis begins to replace natural peristalsis, potassium imbalance, and potential damage to the intestinal tract after years of relying on them (Loscutoff, 1998). Potassium imbalance, from long-term use of laxatives, especially at excessive dosage, has been blamed for deaths of apparently, otherwise healthy women (Loscutoff, 1998). Laxatives are always mentioned in discussions of drug interactions because of the concern that they will exaberate potassium losses that may be an otherwise minor side effect of drug therapies (Loscutoff, 1998).

Anthraquinone compounds are famous for their laxative and antifungal properties. Glycosides of anthraquinones, which are hydrolyzed by glucosidase of the intestinal flora to free anthraquinones and are further reduced to anthrones, which are active forms of the laxative effect (Bruneton, 1995). Anthraquinone aglycones are active for the antifungal property (Kupitayanant *et al.*, 2001). Thus, the quantity of total anthraquinone glycosides in the plants indicates the strength of laxative or purgative activities (Aurapa and Wandee, 2009). One of the important sources of

anthraquinones is the *Cassia*/*Senna* plant. The leaves of *S. siamea* were reported to contain anthraquinones, both aglycones and glycosides (Aurapa and Wandee, 2009). Anthraquinones found in the leaves of *S. siamea* are rhein, cassiamin, physcion, chrysophanic acid, and sennosides (Gritsanapan, 1983; Nualkaew, 1999). Anthraquinones stimulate Cl^- secretion and/or inhibit Na+ absorption, resulting in an accumulation of fluid and subsequent increased colonic motility. The increased Cl^- secretion by anthranoid laxatives is due to disruption of epithelial tight junctions, leading to increased permeability of the epithelium and laxative effect (Ewe, 1980; Wanitschke, 1980; Van Gorkom *et al*, 1999). The content of barakol in the fresh and cooked leaves has been reported (Padumanonda and Gritsanapan, 2006).

Anthraquinones act directly on the intestinal wall (in the colon regions). They are degraded in the colon to produce more active metabolites, mainly anthrones (de Witte, 1993). Anthraquinone laxatives increase fluid electrolyte accumulation in the distal ileum and colon (change in absorption and secretion of water; retention of potassium) through unknown actions, possibly via an irritation of the intestinal mucosa and endothelial cells (Subhuti, 2002). There may also be a direct stimulation of peristaltic activity. The bianthrones, especially sennosides, as found in rhubarb and *senna*, appear to be more active as laxatives than the simple anthraquinones. In single-dose treatment of constipation, the effects of the anthraquinones are noted in about 6-8 hours, the time it takes for them to reach the colon (Subhuti, 2002). Use of anthraquinones should probably be limited in dosage and duration to avoid any potential adverse health consequences related to melanosis coli. A limiting daily dosage corresponding to 20-30 mg of anthraquinones from *Senna* leaf has been recommended in the herbal literature, based on European suggestions for safe use (Blumenthal, 2000). Over-the-counter stimulant laxative drug products are deemed

safe and effective, when administered in amounts of 12-50 mg of sennosides per dose, once or twice daily (Padumanonda and Gritsanapan, 2006).

2.1 COMPOSITION OF *SENNA SIAMEA*

Senna contains anthraquinones, including dianthrone glycosides (1.5% to 3%), *sennosides* A and B (rhein dianthrones), and *sennosides* C and D (rhein aloe-emodin heterodianthrones) (Newall *et al*., 1996, Bisset, 1994). Numerous minor *sennosides* have been identified, and all appear to contribute to the laxative effect. The plant also contains free anthraquinones in small amounts including rhein, aloe-emodin, chrysophanol, and their glycosides (Newall *et al*., 1996, Bisset, 1994). *Senna* pods also contain the same rhein dianthrone glycosides as the leaves (Newall *et al*., 1996, Bisset, 1994).

Carbohydrates in the plant include 2% polysaccharides and approximately 10% mucilage consisting of galactose, arabinose, rhamnose, and galacturonic acid (Newall *et al*., 1996, Bisset, 1994). Other carbohydrates include mannose, fructose, glucose, pinitol, and sucrose (Newall *et al*., 1996)

Flavonols present include isorhamnetin and kaempferol. Glycosides 6-hydroxymusizin and tinnevellin are also found. Other constituents in *Senna* include chrysophanic acid, salicylic acid, saponin, resin, mannitol, sodium potassium tartrate, and trace amounts of volatile oil (Newall *et al.*, 1996, Duke, 1985)

2.1.1 PROXIMATE COMPOSITION OF *SENNA SIAMEA*

Proximate analysis of a food is the nutritional composition of that food. It is the estimation of the nutritive value of human food in its chemical form (Smith, 2009). The proximate analysis of *Senna siamea* contain protein (4.01%), crude fibre (12.36%), moisture content (46.01%), ash

content (12.93%) crude fat (12.02%) and carbohydrate content (7.67%)(Smith, 2009). The protein content is relatively low but it can contribute to the formation of hormones which controls a variety of body functions such as growth, repair and maintenance of body protein (Mau *et al.,* 1999). The moisture, Ash, crude fibre and crude fat content are relatively high (Michael and David, 2002). The proximate compositions of *Senna siamea* show a fairly good nutrient constitution when compared to other common vegetables such as *Amaranthus hybridus* (Nwaogu *et al.,* 2006).

2.1.2 ELEMENTAL AND CHEMICAL COMPOSITION OF *SENNA SIAMEA*

The need for supplementary diet rich in mineral content is necessary for a singular ration, to avoid metal deficiency syndrome like rickets and clarification of bones, as a result of calcium deficiency (Nwaogu *et al.,* 2006). Distorted enzymatic activity and poor electrolyte balance of the blood fluid are related to inadequate Na, k, mg and Zn, as they are the most required elements of living cells. The leaves of *Senna siamea* have fairly adequate concentrations of sodium, potassium, calcium, magnesium and iron in comparison with those reported for *A. hybridus* leaf extract (Nwaogu *et al.,* 2006)

Saponin, alkaloids, anthraquinones and phylobatannins are the major phytochemical identified in *S. siamea* leave extract (Smith, 2009).These phytochemical exhibit diverse pharmacological and biochemical actions when ingested by animals (Amadi *et al.,* 2006). Saponin reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intra-luminal physicochemical interactions. Hence, it has been reported to have hypocholesterolemic effect (Price *et al.,* 1987) and thus may aid in lessening the metabolic burden that would have been placed in the liver. Alkaloids are often toxic to man and may have dramatic physiological activities hence their wide use in medicine (Shelton, 2000).

Table 1: Proximate composition of *Senna siamea*

Parameters	(%) Percentage
Crude protein	4.01±0.05
Crude fibre	12.36±0.03
Moisture content	46.01±0.22
Ash content	17.93±0.04
Crude fat	12.02±0.05
Carbohydrate	7.67±0.03

(Smith, 2009)

Table 2: Elemental Composition of *Senna siamea*

Parameters	Parts per million(PPM)
Iron (Fe)	112.00±0.05
Magnesium (mg)	876.00±0.04
Manganese (Mn)	35.10±0.10
Potassium (k)	812.00±0.05
Calcium (ca)	932.00±0.22
Sodium (Na)	612.00±0.02
Copper (Cu)	0.84±0.15
Cadmium (Cd)	ND
Lead (Pb)	0.34±0.04
Phosphorus (p)	10.84±0.20
Vanadium	ND

ND- indicates not detected

(Smith, 2009)



Close-up of leaflets (Photo:Sheldon Navie)



Large flower cluster (Photo:Sheldon Navie)



Flowers and flower buds(Photo:Sheldon Navie)



Close-up of flower (Photo:Sheldon Navie)

Figure 1: *Senna* plant and leaves

Factsheet. Weeds of australia, biosecurity queensland edition,2011

2.2 USES OF *Senna siamea*

2.2.1 AGRICULTURE, ETHNO-MEDICINE AND ETHNO-VETERINARY MEDICINE OF *SENNA SIAMEA*

Wood: *Senna* wood is used for furniture, poles, small timber, and fuel wood. It is hard, with specific gravity of 0.6-0.8. The sapwood is whitish, and the heartwood is dark-brown to nearly black, with stripes of dark brown to nearly black, with stripes of dark and light (Gamble, 1922). The fuel wood and charcoal are highly regarded (calorific value of 4,500-4,600kcal/kg), but the wood produces a lot of smoke (Forestry/Fuelwood research, 1994).

Senna siamea is used in water cropping systems, wind breaks, and shelter belts. It is also used as a shade tree in cocoa, coffee, and tea plantations. The tree produces an extensive root system in the upper layer of the soil and, in outer cropping systems, can aggressively compete for nutrients and water. The leaves and the seeds can be eaten by ruminants (Sahni, 1981), but toxic to non-ruminants and water such as pigs and poultry. The young leaves and flowers are used in curry dishes. The species is also used for the production of honey and tannins.

Senna siamea is effective in managing constipation, in association with a number of causes including surgery, childbirth and the use of narcotic pain relievers (Hill, 1992). It is used locally as anti-malaria drugs, especially when decocted (the leaves, bark) (Lose *et al*., 2000). In traditional medicine, the fruit is used for the treatment of fever, skin disease, constipation, diabetes, hypertension, and insomnia (Kinhorn and Balquadrin, 1992). Recent studies have also revealed the antioxidant activity of *Senna siamea* flowers. The alcoholic extract of *Senna siamea* flowers have potent antioxidant activity against free radicals, prevent oxidative damage to major bio-molecules and afford significant protection against oxidative damage in the liver.

Studies on the chronic toxicity of *Senna siamea* leaves in rats revealed that long-term consumption of the leave could produce dose-dependent hepatotoxic effect in rats even at therapeutic dose. Hence, if *Senna siamea* leaf is to be used as a sleep aid for a long period of time, liver function test should be performed periodically and the drug should be stopped immediately the signs of drug-induced hepatitis occur (Rattana Jarasro *et al*., 2003). There is some scientific evidence to support the use of *Senna* in the adult population for the treatment of chronic constipation or constipation induced by childbirth or pharmaceutical drugs. Approximately 80% of terminal cancer patients who are taking opioids for pain relief require laxatives, and *senna* has been shown to be as equally efficacious and safe as lactulose for these patients.

Senna may be one option for adjunct therapy for patients on drugs (opioids, tricyclic antidepressants, phenothiazines) that cause constipation as an adverse effect. There is some scientific evidence to support the use of a single high dose of sennosides in the preparation of the colon and rectum for diagnostic procedures, such as colonoscopies. However, there is some controversy regarding this use, with other studies suggesting that alternatives (for example, sodium phosphate, castor oil) may be superior choices. Although studies have reported the use of *Senna* in paediatric population, there is some negative scientific evidence against the use of *Senna* in children.

Leaves and pods of *S.siamea* are browsed by ruminants, but highly toxic to pigs and possibly to other monogastrics. It is suitable for shelter belt plantations, but not as shade tree in agroforestry because of root competition. The heartwood has laxative properties and is used for a variety of ailments of blood forming organs, genito-urinary tract, and also for herpes rhinitis.

Ethnobotanical investigation conducted showed that the five species of *Senna* (*Senna podocarpa*), *Senna tora*, *Senna occidentalis*, *S. alata*) have been actively involved in local medicine (Ogunkunle, 2006). Silver *et al*., 1997 equally observed that extracts from *S. podocarpa* inhibited growth of herpes simplex virus and African swine fever virus and their infections. This finding established the fact that the plant is virucidal.

2.2.2 PHARMACOLOGY OF *SENNA SIAMEA*

As *S. siamea* is a mixture of various groups of chemicals, it is of no surprise that it exhibits different modes of actions. Its major actions include, anti-malarial, anti-diabetic, anti-tumoral or anti-cancer, hypotensive, diuretic, antioxidant, laxative, anti-inflammatory, analgesic, antipyretic, anxiolytic, antidepressant, sedative, and antimicrobial activities. *Senna* is a potent laxative. Its cathartic effects can be obtained from a tea prepared from 1 or 2 teaspoons of dried leaves or standardized commercial dosage forms. *Senna's* use in treating constipation is well documented. It is one of the most popular laxatives, especially in elderly patients (Heaton and Cripps, 1993)

Approximately 90% of *sennosides* are excreted in the feces as polymers. Only 3% to 6% of the metabolites of *sennosides* are excreted in urine (Vanderperren *et al*., 2005)

An in-vitro study using Caco-2 monolayers as a model of the human intestinal mucosal barrier suggests that sennosides are transported in a concentration-dependent manner. Transport was higher in the secretory direction compared with the absorptive direction, suggesting the involvement of efflux pumps in the intestine (Waltenberger *et al*., 2008)

2.2.2.1 ANTI-MALARIAL EFFECTS

Various extracts of leaves, stem bark, and flowers of *S. siamea* were screened for its anti-malarial activity (Jun D *et al*., 2012).Most of the activities described were determined in vitro on Plasmodium falciparum strains. Specified and bio-guided fractionation was also based on this antimalarial test. Activities were assessed on different strains, among which are chloroquine sensitive (3D7), chloroquine resistant (W2, FcM29-Cameroon) and multidrug resistant (K1) in order to find effective compounds against resistant malaria. In all studies, alkaloids fraction of the leaves exhibited better anti-plasmodial activity than other extracts (Hussian, 1991).

In addition, the effectiveness of *C. siamea* leaves' aqueous extract on mosquitoes larva was investigated against *Aedes aegypti* by determining the median lethal concentration (LC50) within 24, 48, 72, and 96 hours. The results indicated that this extract exhibited 50 % inhibition of mosquito larvas at 419.65 mg/mL for 24 hours and at 218.43 mg/mL for 96 hours, respectively (Pavananunda *et al*., 2013). Also, in chronic administration within 21 days, chloroform extract of the stem bark including coumarin and betulinic exhibit 100 % and 90% of mortality on *Aedes aegypti* (Nsonde-Ntandoua *et al*., 2010). So, *C. siamea* could be used effectively as indigenous mosquito control agents alternatively to conventional mosquito chemicals.

2.2.2.2 ANTI-DIABETIC AND ANTI-LIPEMIC EFFECTS

The potential effects of *S. siamea* (leaves, roots) on endocrinological system were evaluated by several methods. Ethanolic, ethyl acetate and hexane extracts of *S. siamea*'s leaves at doses 150 and 300 mg/kg were tested for antidiabetic activity in alloxan induced diabetes model and the plasma blood glucose levels were estimated by GOD-POD method at 0, 2, 4, 6, 8 and 12hr. So, ethyl acetate extract of *S. siamea*'s leaves at both different doses produced significant reduction when compared to ethanol and hexane extracts ($P<0.001$) (Luangpirom and Saenbuaphan, 2006).

Ethanolic extract of leaves of *S. siamea* exhibits a hypoglycemic and antihyperglycemic effect in non-diabetic rats after induction of hyperglycemia with 2 g/kg/bw of glucose feeding within 1-5 hours. Indeed, this extract administered orally at the doses of 500 and 750 mg/kg/bw significantly decreased blood glucose by 50.32 and 47.29 % per hour with glibenclamide (10 mg/kg/bw) as positive control ($P< 0.05$). The aqueous extract of *S. siamea*'s root (1000 - 3000 mg/kg, orally) caused improvement in blood glucose level and body weights within 24 hours in alloxan-induced hyperglyceamic rats, significantly ($P< 0.05$). It was reported that sun-dried and freshly uprooted root have the same anti-diabetic potential (Odason, and Kolawole, 2007). In addition, administrations of leaves' methanolic extract (250, 500 mg/kg, orally) within three week induced a significant decrease in streptozotocin (STZ) diabetic rats with high blood glucose levels. It also reduced their serum cholesterol and triglycerides and improved their HDL-cholesterol level ($P<0.01$) (Kumar *et al*., 2010) and (Patel *et al*., 2012).

2.2.2.3 OTHER USES

The *Senna* constituents, aloe-emodin and beta-sitosterol possess inhibitory activity against cancer cells in mice (Robbers and Tyler, 1999, Ralevic *et al*., 1990). *Senna* did not have antidiabetic activity when tested in diabetic mice (Swanston-Flatt *et al*., 1989). *Senna* extract was not found to be cytotoxic or mutagenic against strains of Escherichia coli. However, *Senna* was able to induce single and double-strand breaks in plasmid DNA suggesting *Senna* may only be toxic to DNA in cell-free systems (Silva *et al*., 2008).

2.2.3 LAXATIVE STUDIES ON *SENNA SIAMEA*

2.2.3.1 ANIMAL DATA

Senna has also been studied for long-term laxative treatment in rats (Ralevic *et al*., 1990). Perivascular nerve stimulation caused a vasoconstrictive effect on the mesenteric vascular bed. However, it is thought that myenteric neurons in the rat colon are not destroyed by sennosides, as had been previously suggested (Kiernan and Heinicke, 1989). Anthraquinone purgatives in excess were said to have caused degeneration of neurons.

Sennosides exert their cathartic effect through alterations in colonic motility, which occurs indirectly by damage to the epithelial cells. They also change colonic absorption and secretion to cause fluid accumulation. Enhanced permeability is the result of disruption of the tight junctions between the colonic epithelial cells (Soyuncu *et al*., 2008).

Metabolism of anthranoid laxatives (Dewitte and Lemli, 1990) and sennosides have been reported (Lemli, 1988).

2.2.3.2 CLINICAL DATA

Many reports are available discussing *senna's* role in constipation (Marlett *et al*., 1987, Godding, 1988) ; its use in elderly patients (Maddi, 1979, Passmore *et al*., 1993) psychiatric patients (Georgia, 1983) and spinal cord injury patients (Cornell *et al*.,1973) ; and in pregnancy, in which it is the stimulant laxative of choice (Gattuso and Kamm, 1994). In cancer treatment protocols, *senna* has also been noted to reverse the constipating effects of narcotics, and may prevent constipation if given with the narcotic (Cameron, 1992). However, it may cause more adverse effects than other laxatives, primarily abdominal pain (Sykes, 1996). In terminally ill patients with cancer, it has also demonstrated efficacy in preventing constipation, which may not only be

attributable to opioids but potentially due to administration of tricyclic antidepressants or phenothiazines, physical inactivity, deficient nutrition, and/or inadequate fluid intake (Agra *et al*., 1998). *Senna* has also been studied in long-term constipation (Mishalany, 1989). Castor oil was superior to *Senna* for long-term constipation sufferers in another report (Pawlik *et al*., 1994). In a study of children younger than 15 years of age with constipation, the efficacy and adverse effects of *Senna* were compared with lactulose. Lactulose use resulted in patients passing more normal stools than on the corresponding day of the *Senna* week. Additionally, *Senna* was associated with more adverse effects with greater frequency compared with lactulose (Perkin, 1977).

Patients who underwent reconstructive pelvic surgery were randomized to receive *Senna* 8.6 mg/docusate 50 mg or placebo following the surgery. There was a significant difference in time to first bowel movement (3 ± 1.5 vs 4.05 ± 1.5 days; $P < 0.002$) for patients receiving *Senna* and docusate compared with placebo. Additionally, significantly more patients receiving placebo required magnesium citrate ($P< 0.001$) (Patel, 2010). *Senna* may influence intestinal transit time (Rogers *et al*., 1978, Sogni *et al*., 1992, Ewe *et al*., 1993). Its effectiveness as part of a cleansing regimen to evacuate the bowels in preparation for colonoscopies or barium enemas is documented (Staumont *et al*., 1988, Han, 1989, Hangartner *et al*., 1989). Results from these studies include reduced ingestion of commercial golytely solution and simethicone when given with *Senna* (Wildgrube and Lauer, 1991) and more effective colon cleansing with *Senna* in combination with polyethylene glycol electrolyte lavage solution compared with the solution alone (Ziegenhagen *et al*., 1991). However, a study comparing the efficacy of PEG 2 L and *Senna* syrup 120 mg with PEG 4 L found that the combination therapy was not as effective as the larger quantity of PEG, though it was better tolerated (i.e., 38% could not finish the PEG 4 L compared with 6% receiving combination therapy) (Hookey *et al*., 2006).

A study of 345 patients undergoing colonoscopies assessed the efficacy of magnesium citrate combined with *Senna* versus magnesium citrate alone. In patients receiving magnesium citrate only, 6.9% of the patients had to reschedule the colonoscopy due to inadequate bowel evacuation. In those patients receiving combination therapy, 4.4% required rescheduling (P = 0.44). Adequate visualization during the colonoscopy occurred in 81.3% of patients receiving combination therapy compared with 67.5% receiving magnesium citrate alone (P = 0.004) (Vradelis *et al*., 2009). When *Senna* 180 mg was compared with 95 ml of sodium phosphate solution for bowel evacuation prior to colonoscopy, *Senna* was not equivalent to sodium phosphate. However, *Senna* was better accepted compared with sodium phosphate based on taste, and was associated with less nausea and vomiting (Kositchaiwat *et al*., 2006).

Capsule endoscopies are useful for assessing obscure GI bleeding, suspected small-bowel Crohn disease, celiac disease, polyposis syndromes, and small-bowel tumor detection. Compared with standard preparation (i.e. restriction to clear fluids, fasting, simethicone),the addition of purgatives, such as magnesium citrate and *Senna*, did not improve completion rates or view quality and were associated with less patient acceptance (Postgate *et al*., 2009).

Because sennosides are water-soluble polar molecules with a high molecular weight, they are not re-absorbed in the small intestine. Specifically, the beta-glycosidic bond provides protection against acid digestion and to alpha-glucosidase activity. Instead, they act as pro-drugs as they pass through the small intestine. They are later converted in the large intestine by gut bacteria to the active metabolite, rheinanthrone, which increases colonic motility and fluid secretion (Chevallier 1996, Vanderperren *et al*., 2005).

Prostaglandins may also be involved in the laxative actions (Newall *et al*., 1996). The kinetics of *Senna* constituents rhein and aloe-emodin have been investigated in humans (Krumbiegel and Schulz, 1993)

Dulcolax (bisacodyl or sodium picosulphate) offers one such highly effective and safe solution. It is a widely available contact laxative licensed for the treatment of constipation and is commonly used by millions of people worldwide. The active ingredients of dulcolax act only where they are needed, in the colon, and stimulate the natural movements of the bowels to alleviate the symptoms of constipation. Dulcolax is clinically proven to be a safe and effective treatment for constipation even over a long-term (Wald *et al*., 2006).

2.3 EPIDEMIOLOGY OF CONSTIPATION

Bowel habits and perception of constipation vary widely among populations. The prevalence of constipation in the UK define by Rome11 criteria, has been estimated at 8.2%.However, in another UK survey, 39% of men and 52% of women reported straining at stool on more than one in four occasion. The prevalence of chronic constipation in the US varies from 2-3%.In other parts of the world (Italy, France, Germany, Brazil, and South Korea) the overall prevalence of constipation is estimated at 12.3%.Constipation is at least twice as common in women as in men, with an estimated ratio of 2:2:1.It occurrence increases with advancing age, particularly after age 65.Its prevalence is 2- fold higher in black patients (in those of lower socio-economic status), and in nursing home residents (Fork *et al.,* 1982). Pregnancy is also associated with higher prevalence of constipation, estimated in one systematic review to be between 11% and 35%. Chronic constipation is frequently associated with other functional gastrointestinal motility disorders including chest pain, gastro-oesophageal reflux disease (GORD), irritable bowel syndrome, and functional dyspepsia.

Chronic constipation imposes a substantial burden to health-care resources. In the US, $821 million was spent on laxatives in 2002.Constipation was a reason for seeking care in an estimated 5.7million ambulatory physician visits per year. Another survey in the US reveals that on average,40% of sufferers attempt to treat their constipation by changing their nutrition despite extensive research showing that diet and lifestyle are not necessarily to blame for the occurrence of constipation and increasing fluid and fibre intake will not definitely provide effective relief from the condition.(New omnibus data-article, 2006).The new evidence from the survey has revealed that there is still a considerable unmet need in the treatment of constipation. It is our responsibility to make people aware of it, and to offer the best solutions for constipation, by publicizing the facts and correcting these misunderstanding.

Constipation is a highly prevalent functional gastrointestinal disorder affecting 3-15% of the general population (Jones and Lydeard, 1992). In South Africa, 29% of the population, consisting of both black and white suffer from constipation especially in the elderly (Meiring and Joubert, 1985). The menace has a substantial impact on morbidity and quality of life (Drossman *et al.,* 1993), which may be characterized by unexplained abdominal pain, discomfort and bloating in association with altered bowel habits (Thompson *et al*., 1999).

The use of chemical drugs such as *Senna*, correctol, exlax, senokot and gaviscon is very common as a means of treating constipation. Statistics have shown that 43% of whites and 76.6% of blacks in South Africa indulge in the use of laxatives, out of which 14.3% and 21.5% respectively use more than one laxative at a time for the treatment of constipation (Meiring and Joubert, 1985). The use of these orthodox drugs is however, limited due to their high cost and undesirable side-effects (Erasto *et al*., 2005). Consequently, majority of the affected persons in South Africa rely on herbal preparations for the treatment of the menace. For instance, some plant extracts are

known to exhibit antispasmodic effects by stimulating water absorption in the intestine (Palombo, 2006). Apart from being fast acting, cheap and readily available, the users of medicinal plants for the treatment of constipation also believe that they have some control in their choice of medication (Joshl and Kaul, 2001).

2.4 ANATOMY OF COLON AND EFFECTS OF OPIATES THAT RESULT IN CONSTIPATION

The cause of constipation in opiate users is multi-factorial (De Schepper *et al*., 2004).Opioids interfere with normal gastrointestinal motility by delaying transit, stimulating non-propulsive motility, segmentation and tone, and stimulation of sphincters such as the pylorus and ileocecal sphincter through their effects on enteric neurons (Wood and Galligan, 2004). Opioids stimulate the absorption of fluids, mainly by delayed transit increasing contact time for absorption, and by stimulating mucosal sensory receptors that activate a reflex arc that facilitates further fluid absorption (Kurz and Sessler, 2003). These multiple effects lead to opioid-induced constipation.

2.4.1 THE ANATOMY OF THE LARGE AND SMALL INTESTINE

The digestive system processes the food you eat. Food travels via the esophagus into the stomach and then into the small and large intestines. The small intestine starts at the pylorus of the stomach and ends at the cecum of the large intestine. The main function of the small intestine is continued digestion and absorption of nutrients.

2.4.1.1 THE JEJUNUM AND THE ILEUM

The jejunum is the middle portion of the small intestine. It starts at the duodenojejunal junction and changes into the ileum, which is the third portion. The jejunum takes up about two-fifths of

the length of the small intestine, but no clear line demarcates where it turns into the ileum. The ileum ends at the ileocecal junction. The ileum and jejunum are attached to the posterior abdominal wall by the mesentery.

Sympathetic and parasympathetic nerves are brought by the superior mesenteric plexus. Blood is brought to the jejunum and ileum by branches from the superior mesenteric artery. Blood is drained by the superior mesenteric vein. Lymph nodes that drain this area include the juxtaintestinal lymph nodes, mesenteric lymph nodes, and central nodes. Lacteals are specialized lymphatic vessels found in the small intestine that absorb fat from the foods you eat.

2.4.1.2 THE LARGE INTESTINE

Most of the large intestine is located in the abdomen; the sigmoid colon and rectum are in the pelvic cavity. The abdominal portion of the large intestine includes the cecum and the ascending, transverse, and descending colon. The main function of the large intestine is to absorb water from fecal material before it's eliminated from the body. The colon is also home to friendly bacteria that synthesize vitamin K and keep bad microbes in check. The large intestine is much larger in diameter than the small intestine and has omental appendages attached to it.

2.4.1.2.1 THE CECUM

The cecum is a pouch of intestine that hangs below the ileocecal junction in the right lower quadrant of the abdomen. Folds of mucosal tissue form the ileocecal valve that covers the ileal orifice. The appendix extends from the posteromedial part of the cecum.

Sympathetic and parasympathetic nerves come from the superior mesenteric plexus. Blood supply to the cecum comes via the ileocolic artery, a branch of the superior mesenteric artery. The

appendicular artery branches from the ileocolic artery. Lymphatic vessels pass to the ileocolic lymph nodes and the superior mesenteric lymph nodes.

2.4.1.2.2 THE ASCENDING COLON

The ascending colon travels from the cecum upward on the right side of the abdominal cavity to the right colic flexure near the right side of the liver. This part of the colon is retroperitoneal. Nervous supply is brought to the ascending colon by the superior mesenteric plexus. The ileocolic and right colic arteries supply blood. Blood is drained away by the ileocolic and right colic veins. Lymph is drained by the epicolic and paracolic lymph nodes, and then it travels to the ileocolic and right colic lymph nodes.

2.4.1.2.3 THE TRANSVERSE COLON

The transverse colon crosses from the right side of the abdomen to the left, ending at the left colic flexure. The sympathetic nerves that serve the transverse colon come from the superior and inferior mesenteric plexuses; the parasympathetic nerves arise from the vagus nerves and the pelvic splanchnic nerves.

Blood is brought to the transverse colon primarily by the middle colic artery. The distal portion of the transverse colon is served by the left colic artery, a branch of the inferior mesenteric artery. Venous blood is removed by the superior mesenteric and inferior mesenteric veins. Lymph is drained into the colic lymph nodes and into the colic nodes.

2.4.1.2.4 THE DESCENDING COLON

The descending colon travels behind the peritoneum and downward from the left colic flexure to the left iliac fossa where it continues as the sigmoid colon. Sympathetic nerve supply comes from

the lumbar splanchnic nerves, the inferior mesenteric plexus, and the periarterial plexuses that surround the inferior mesenteric artery. Parasympathetic nerve supply comes from the pelvic splanchnic nerve.

Blood is brought to the descending colon by the left colic and sigmoid arteries, branches of the inferior mesenteric artery. Blood is drained away by the inferior mesenteric vein. Lymph is drained into the epicolic and paracolic lymph nodes, which drain into the intermediate colic lymph nodes. From here the lymph drains into the inferior mesenteric lymph nodes.

2.4.2 ENTERO-ENDOCRINE CELLS AND GOBLET CELLS: It is part of the enteric endocrine system that monitors the luminal environment and secrets hormones such as cholecystokinin and gastrin into the blood. Secrets a lubricating mucus into the intestinal lumen

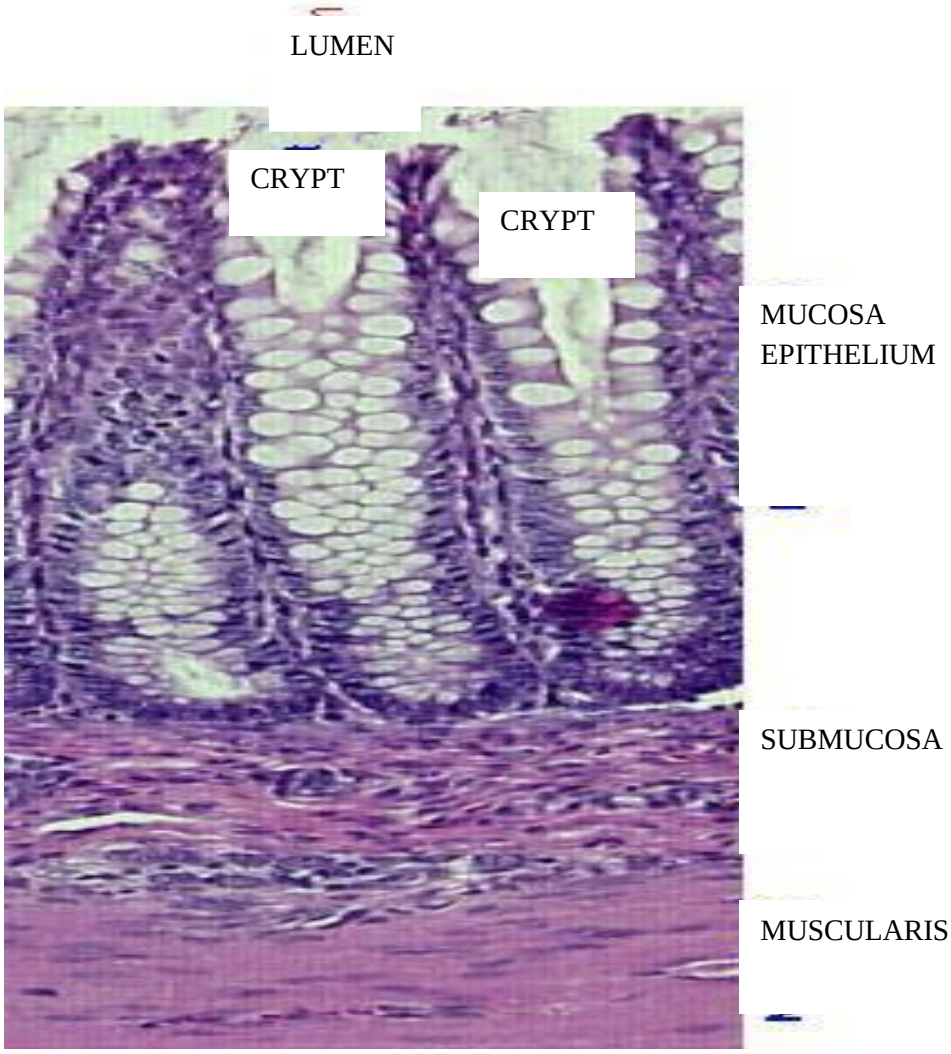


Figure 2: Section of colon from a dog. Crypts extending from the ileum, and the numerous, foamy goblet cells that populate the epithelium of the crypts (Richard Bowen, 2014)

2.5 DRUG EFFECTS ON COLON

The small and large intestine are one of the most common sites for the adverse action of drugs, accounting for 20-40% of all drug side effects. The most important factor in the diagnosis of drug-induced intestinal side effects is awareness. The mechanisms of damage are invariably complex, but may be due to topical effects, a known pharmacologic action of the drug on motility

(for instance cholinergic/anti-cholinergic effect) and/or secretion, immune suppression and in the case of cytotoxic drug treatment, a combination of many actions (Zeino Z. *et al*., 2010).

2.5.1 *SENNA SIAMEA* AND ADVERSE REACTIONS

Senna may cause loss of fluids, hypokalemia, diarrhea, and abdominal pain and cramping (Soyuncu *et al*., 2008)

Prolonged use may alter electrolytes and thereby increase the risk for cardiac complications. Patients with intestinal obstruction should avoid *Senna* (Newall *et al*., 1996)

Long-term use of any laxative in particular irritant laxatives such as *Senna*, often results in laxative dependency syndrome, characterized by poor gastric motility in the absence of repeated laxative administration. Other reports of laxative abuse include laxative-induced diarrhea (Cummings *et al*., 1974, Morris and Turnberg, 1979) and osteomalacia and arthropathy associated with prolonged use of the product (Frier and Scott, 1977)

The long-term use of anthraquinone glycosides has been associated with pigmentation of the colon (melanosis coli). Several cases of reversible finger clubbing (enlargement of the ends of the fingers and toes) have been reported following long-term abuse of *Senna*-containing laxatives (Prior and White 1978). One report described a woman who developed finger clubbing following ingestion of 4 to 40 senokot tablets per day for approximately 15 years (Fitz Gerald and Redmond 1983). Clubbing reversed after the laxative was discontinued. The mechanism has been postulated to be related to either increased vascularity of the nail beds or a systemic metabolic abnormality secondary to long-term laxative ingestion. A case report describes a patient with anorexia nervosa using 50 to 100 tablets of *Senna* daily for weight loss. She developed nephrocalcinosis, finger clubbing, and hypertrophic osteoarthropathy. Nephrocalcinosis was likely due to long-term

ingestion of calcium (each *Senna* tablet contain calcium 12.5 mg) in the presence of dehydration, resulting in low calcium excretion. This, in addition to a low body mass index, contributes to calcium phosphate retention (Lim *et al*., 2008).

Senna abuse has been associated with the development of cachexia and reduced serum globulin levels after long-term ingestion (Levine *et al*., 1981)

Case reports include occupational asthma and rhino-conjunctivitis from a factory worker exposed to *Senna*-containing hair dyes (Helin and Mäkinen-Kiljunen, 1996) and asthma and allergy symptoms from workers in a bulk laxative manufacturing facility (Marks *et al*., 1991).Another report describes urticaria, rhinoconjunctivitis, and wheezing occurring within 2 hours of an occupational exposure to airborne *Senna* despite wearing a protective suit and respirator (Wong *et al.*, 2009).

Senna may cause hepatotoxicity. This may be attributed to the exposure of the liver to high amounts of toxic metabolites of anthraquinone glycosides (Vanderperren *et al*., 2005)

In a case report, a woman 42 years of age who boiled dried *Senna* leaves and consumed 200 ml of the product each day for 2 years presented with a 5-day history of epigastric pain, vomiting, anorexia, fever, mildly elevated liver function tests, and iron deficiency anemia. She was diagnosed with portal vein thrombosis based on doppler findings. Additionally, fluid loss and dehydration associated with long-term use of *Senna* may have exerted negative effects on coagulation (Soyuncu *et al*., 2008)

Another case report describes the development of subacute cholestatic hepatitis in a man 77 years of age who used *Senna* 15 to 30 mg/day for 3 months. Dis-continuation of the product resulted in a progressive decline in liver enzymes and bilirubin levels (Sonmez *et al*., 2005).Children,

particularly those wearing diapers, may experience severe diaper rash, blister formation, and skin sloughing. In a study of 88 exposures to *Senna,* 33% displayed severe diaper rash, which was significantly worse for those wearing diapers ($P < 0.05$). The presence of blisters and skin sloughing was also worse in children wearing diapers ($P < 0.05$). Diarrhea occurred 5 to 6 hours after ingestion of *Senna*, with skin lesion appearing 14 to 15 hours following ingestion. Thus, the dermatologic manifestations could be attributed to prolonged skin contact with stool or *Senna* being present in later stools, causing an irritant effect on the skin (Spiller *et al*., 2003).

Patients who are homozygous for the CYP2D6∗4 variant, and thus poor metabolizers for phase 1 hepatic detoxification reactions, may be at risk of hepatitis. The CYP2D6∗4 variant is common in approximately 10% of white people (Seybold *et al*., 2004).

2.5.2 OPIOIDS

The constipating effect of opioids is through their action on mu opioid receptors in the submucosal plexus of the gastrointestinal tract(De Luca and Coupart, 1996).This decreases gastrointestinal motility by decreasing propulsive peristalsis (at the same time increasing circular contractions), decreases secretions (pancreatic and biliary), and increases intestinal fluid absorption(De Luca and Coupart, 1996). There is also a central descending opioid-mediated effect so that even spinally administered opioids cause decreased gastric emptying and prolonged oral–caecal transit time. The opioid-induced increase in circular muscle contractions causes colicky pain. There is good evidence from RCTs (Clark *et al*., 2004) (Ahmedzai and Brooks, 1997) and animal studies(Meert, and Vermeirsch, 2005) that, compared with water-soluble opioids such as morphine and oxycodone, the more lipid-soluble opioids such as fentanyl and buprenorphine are less likely to cause constipation while maintaining the same degree of analgesic effect. This is probably caused by their much reduced time in the systemic circulation. Other risk factors for

constipation and bowel dysfunction in people taking opioids for advanced cancer include hypercalcaemia, reduced mobility, reduced fluid and food intake, dehydration, anal fissures, and mechanical obstruction. Lack of privacy for defecation may also play a part for people in hospital. Drugs that can cause or exacerbate constipation include anticholinergics. In the treatment of cancer, thalidomide, vinca alkaloids, and $5HT_3$ antagonists can all cause constipation. Additionally there is an increased risk of constipation in people with autonomic neuropathy caused by diabetes mellitus, for example, and in people with neuromuscular problems such as spinal cord compression (Tamayo, and Diaz-Zuluaga, 2004)

2.5.3 OPIOID INDUCED CONSTIPATION

Constipation is the most common adverse effect occurring with chronic opioid use. Prophylactic treatments are essential to minimize this complication. Opioids have various effects on the gastrointestinal tract, including decreases in motility, secretions, and blood flow, which lead to hard, dry feces (De Luca and Coupar, 1996). The constipating effects of opioids are considered to be dose-related, and tolerance to this symptom rarely develops. A common goal of therapy is for patients to have one bowel movement every one to two days (Pappagallo, 2001)

Nondrug treatments, such as increasing fluid and dietary fiber intake, increasing physical activity, and establishing a toileting routine, should be implemented to minimize the risk of constipation (Canty, 1994). Monotherapy with stool softeners is considered ineffective, and use of a scheduled stimulant laxative often is required (American Pain Society, 2003). There are no studies showing superiority of one laxative over another. However, one common approach is the scheduled use of *senna* with or without a stool softener (Cherny *et al*., 2001). If patients do not have an adequate response, a trial of an osmotic agent (e.g., sorbitol) may be used. Bulk-forming laxatives also are an option, although these agents require adequate fluid intake that may not be appropriate in all

patient populations (Pappagallo, 2001). Periodic use of saline laxatives or administration of suppositories or enemas may be needed.

Transdermal fentanyl (Duragesic) is considered an option for patients who have difficulty with the constipating effects of oral opioids. Although not free of constipating adverse effects, transdermal fentanyl has been shown to have fewer such effects compared with various oral opioids (Canty, 1994, Staats *et al*. 2004). A retrospective cohort study found a significantly higher risk of developing constipation with oral oxycodone (Roxicodone) compared with transdermal fentanyl (Staats, 2004). A randomized crossover trial found a significant reduction in constipation in the transdermal fentanyl group compared with sustained-release oral morphine (29 and 48 percent, respectively) (Allan *et al*., 2001)

2.5.4 SELECTED MEDICATIONS FOR TREATING OPIOID-INDUCED CONSTIPATION

One concept to reduce the adverse effects of opioids is the use of very small doses of opioid antagonists (Gan *et al*., 1997,Cepeda *et al*., 2004, and Hirayama *et al*., 2001). The rationale is that agents such as naloxone (Narcan) have a biphasic effect whereby very low doses reduce the incidence of opioid adverse effects and may augment the analgesic effect (Gan *et al*.,1997 and Hirayama *et al*. 2001). Much of the data are limited to the inpatient setting with intravenous administration of the opioid antagonist (Gan *et al*., 1997). Concomitant administration of intravenous naloxone with morphine infusions has been studied, but the results have been mixed (Gan *et al*., 1997). More research is needed before this treatment is implemented as part of routine practice.

2.5.5 TOXICOLOGICAL STUDIES ON *SENNA SIAMEA*

Concerns regarding the carcinogenicity of anthranoid laxatives have been raised. In a 2-year study, rats receiving *Senna* dosages of 25, 100, and 300 mg/kg/day did not show any changes in several assessments, including hematology measures, tissue histology, and mortality ratio, when compared with the control rats. High doses of *Senna* were associated with increases in water consumption, electrolyte changes, and increases in tubular basophilia and tubular pigment deposits in the kidneys. In fact, other animal data suggest that *Senna* may have anticancer action (Mitchell *et al*., 2006, Borrelli *et al*., 2006).

An analysis of the literature from 2009 suggests: (1) *Senna* is not associated with structural and/or functional changes in the enteric nerves, (2) long-term administration of *Senna* is not associated with GI tumors or any other type in rats, (3) when dosed up to 300 mg/kg in rats for 2 years, *Senna* was not carcinogenic, and (4) evidence does not show an increased risk of genotoxicity in patients treated with *Senna* (Morales *et al*., 2009).

Risk assessment for *Senna's* use during pregnancy has been addressed (Dobb and Edis, 1984). One review suggests *Senna* to be the stimulant laxative of choice during pregnancy and lactation (Gattuso and Kamm, 1994). Uterine motility was not stimulated by *sennosides* in one report in pregnant ewes (Garcia-Villar, 1988). None of the breast-fed infants experienced abnormal stool consistency from their mothers' ingestion of *Senna* laxatives. The constituent rhein, taken from milk samples, varied in concentration from 0 to 27 mg/ml, with between 89% and 94% of values no more than 10 mg/mL (Faber and Strenge-hesse, 1988). Non-standardized laxatives are not recommended during pregnancy (Newall *et al*., 1996).

Toxicity studies separating toxic components of *Senna's* anthraquinone derivatives have been performed (Dobb and Edis, 1984).

Various case reports of *Senna* toxicity are available and include coma and neuropathy after ingestion of a *Senna*-combination laxative (Dobb and Edis, 1984) and hepatitis after long-term use of the plant (Beuers *et al*., 1991).

CHAPTER THREE

MATERIALS AND METHODS

3.0 MATERIALS

3.1 EXPERIMENTAL ANIMALS

Thirty female Wistar rats were obtained from the animal house of a local dealer at Sabon-gari, Zaria, Kaduna. The animals were acclamatized for two weeks and were fed with vital feed and clean water ad libitum. The behavioural state of animals, were adequately monitored to ensure they are in good health condition.

3.1.1 PLANT

Senna siamea leaves were obtained from locally grown shrubs on the main campus of Ahmadu Bello University in the month of July year 2013. The plant was authenticated by Usman Gallah in Biological Sciences Herbarium, Ahmadu Bello University, Zaria with voucher number 90017.

3.1.2 REAGENTS

Haematoxylin and Eosin stain (H and E), Acid Schiff (PAS), Normal saline, Loperamide Capsule (2 mg) (Pramo Life Science Pharmaceuticals, Mumbai, India), Bisacodyl Tablets (5 mg) (Medrel Pharmaceuticals, India, PVT.LTD.), Chloroform, Distilled-water, Ethanol, Xylene.

3.1.3 INSTRUMENTS

Glassjar, Glass slides, Cover slips, Beakers (10 mls), Measuring cylinder (250 mls). Thirty plastic containers, Plastic test-tubes (Afro-Asia Automobile and Plastics Limited), Scalp Vein set (Anhui Kangda Medical Products CO.,LTD. China), Water bottles, and Orogastric tubes, Recording Microdynamometer 7050(Ugo Basile Biological Research Apparatus, Milan- Italy), Pyrex (containing tyrode solution),1 Litre, France, Spiral Condenser, Thermo-circulator (Churchill

Instrument CO. LTD., Walmgate road, Perivale, MIDDX, England- Volts 220/210/50, Watts-1000), Microscope, Digital thermostatic water bath, Evaporating dish, Insulin syringes, Micro-weighing scale, Dissecting set, Standard whatman blotting-papers (150 mm in diameter), Medlax Glooves

3.2 METHODOLOGY

3.2.1 PLANT EXTRACTION

Fresh leaves of *S. siamea* were harvested from locally grown shrubs on the main campus of Ahmadu Bello University in the month of July year 2013 and dried under the shade to preserve its phytochemical elements. The dried leaves weigh 300g after which they were ground into powder. The leaves were macerated by soaking in distilled water for 24 hours and then filtered through a filter paper using a funnel, and allowed to settle. The aqueous extract was transferred into an evaporating dish and placed inside the water bath, in order to evaporate it to dryness. The extract weighs 36.66g and the yield was estimated to be 12.2%.

3.2.2. IN VITRO EXPERIMENT

The effect of aqueous extract of *S. siamea* on the ileum was investigated in order to measure changes in spontaneous ileal contraction or relaxation generated by the extract on smooth muscle contractility in an organ bath. An experiment that was conducted in the Pharmacology laboratory of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria in the month of August, year 2015.

3.2.2.1. PREPARATION OF ISOLATED ILEUM

Two healthy wistar rats were obtained from the animal house of the Department of Human Anatomy. They were kept in the animal house of the Department of Human Human Anatomy,

Ahmadu Bello University, Zaria. They were fed with vital feeds and water ad libitum. The animal was starved for 24 hours before the experiment to allow for rapid contraction of the ileum.

At the first phase of the experiment, one of the rats was sacrificed by a blow to the head and dissected to remove a segment of the ileum. After the ileum strips had been carefully freed from the fat and connective tissue, the strips were mounted to an organ baths filled with tyrode solution (15mls) at room temperature (37 °C).

3.2.2.2. GENERAL PROCEDURES

A digital thermostatic water-bath was connected to a power source, the pyrex (1 litre) attached to the set-up was filled with tyrode solution of up to 1 litre volume and this was also connected to a spiral condenser that links to the tissue bath (containing 15mls of tyrode solution). Another inlet to the tissue bath supplies the tissue with oxygen through a thermocirculator. At about 20-30 minutes of constant voltage supply, the whole set-up was connected to a recording microdynamometer 7050. A myograph recording sheet was attached to the microdynamometer to record the tonic contraction and relaxation of the ileum. The speed and time of the set-up was regulated at 0.025cm/sec and 5sec respectively. A piece of the harvested segment of the ileum was carefully suspended inside the tyrode solution and as soon as the resting tension had stabilized, a solution of acetylcholine (0.1, 0.2, 0.4, and 0.8mls) prepared in distilled water at a concentration of 10ug/ml was added to the bathing buffer and a rapid increase in ileum tone followed by stable constriction (tonic contraction) was induced. The tonic contraction induced by the introduction of acetylcholine was recorded on the myograph sheet. The tissue (ileum strip) was then washed three times using tyrode solution, and as soon as this was done, a stabilized resting tension was restored. Atropin solution (0.1mls) at a concentration of 20ug/ml was then added to the bathing buffer and a rapid decrease in ileum tone followed by tonic relaxation was

observed. The tissue was again washed with tyrode solution and allowed to maintain a stabilized resting tension. The ileum strip was then exposed to *Senna siamea* at different concentrations of 100mg/ml, 250mg/ml and 500mg/ml. Solution of *Senna siamea* prepared at these concentrations were introduced into the tyrode solution at a volume of 0.2mls, 0.4mls, and 0.8mls. The tissue was washed three times at every stage to avoid or remove the effect of previously administered drug or extract. As soon as this was done, the ileum strip was further exposed to loperamide (1mg/ml concentration) at 0.1 and 0.2mls respectively and a decrease in tonic contraction was observed.

3.2.3 – IN VIVO EXPERIMENT

3.2.3.1 EXPERIMENTAL DESIGN

Thirty experimental animals (wistar rats) were divided into five groups of six animals per group. Group 1 were treated with normal saline throughout the duration of the experiment. Group 2 were administered loperamide (opiate) orally for 6 days only until constipation was induced and group 3 were administered *Senna siamea*(orally) for 7 days before giving them opiate for 6 days, this was done to check for the protective effect of *Senna siamea* against opioid induced constipation in the colon. Group 4 received opiate for 6 days and extract for 7 days while group 5 were administered bisacodyl for 7 days after the animals in the group received opiate (loperamide) for 6 days. Extract and drugs were orally administered to all experimental animals at different doses and concentrations using orogastric tubes.

TABLE 3: EXPERIMENTAL DESIGN

GROUPS	TREATMENT(ORAL ADMINISTRATION)	DURATION
GROUP1	Normal saline (1ml/kg bw)	All through the experiment
GROUP2	Opioid (loperamide)-3mg/kg (Wintola,2010)	6days induction
GROUP3	*Senna siamea*-300mg/kg(Zhong Xi Yi Jie Za Zhi 1986)+ Opioid-3mg/kg	7days treatment+6days induction
GROUP4	Opioid-3mg/kg+ *Senna siamea*-300mg/kg(Zhong Xi Yi Jie Za Zhi 1986)	6days induction+ 7days treatment
GROUP5	Opioid + Standard laxative(bisacodyl) 5mg/kg	6days induction + 7days treatment

3.3 MORPHOLOGICAL STUDY

Body weights before and after administration were measured to observe changes in body weight. Organ/body weight ratio was determined. The stool consistency was also observed using stool texture (stool dryness, wetness, softness or hardness) and stool weight.

3.4 TISSUE PROCESSING

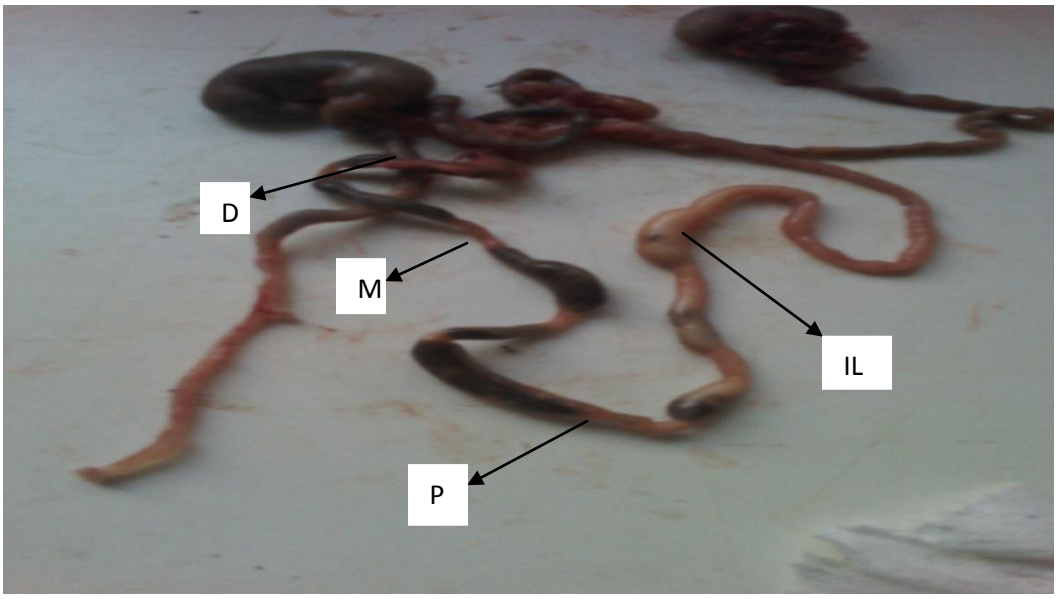


Figure 3: Excised portion of colon of wistar rats. Note: IL-ileocecal junction, P-proximal part, M-middle part, D-distal part.

The distal portion of the colon was excised and fixed in 10% formolsaline immediately to avoid post-mortem changes, after which the tissue was processed by passing it through ascending grades of alcohol for dehydration; 90%, 95%, 100%, and chloroform in two changes for 2 hours each. The tissue was then allowed to pass through 2 changes of molten paraffin wax to enhance infiltration. The tissue was then embedded in paraffin wax to help solidify the tissue. The tissue was mounted on a wooden block and trimmed. Sections were made at a thickness of 5 microns using a rotary microtone. These sections were floated in warm water bathe from which suitable sections were selected and mounted on slides. These sections were stained and left to dry for 3 hours. The tissues were cleansed in two changes of xylene for 3 minutes each after which they were passed through descending grades of alcohol (100%, 95%, and 75%) for 3 minutes each. This was done to hydrate the tissue.

3.5 HISTOLOGY AND HISTOCHEMISTRY

Haematoxylin and Eosin stain was used to histologically access the microscopic architecture and structure of the large intestine (colon). Here, the arrangement of goblet cells around the crypt, aggregation of lymphoid cells within the intestinal lumen and the muscularis mucosa are well-expanciated as observed in the photo-micrograph of table of results below. PAS was used to detect the presence or absence of neutral mucin in goblet cells of the intestine. This is an indication that reveals the rate at which mucoid secreting cells respond to stimulatory activities within the intestinal lumen.

CHAPTER FOUR

RESULTS

4.0 IN VITRO STUDIES

Results showed that acetylcholine (0.1, 0.2, 0.4, 0.8mls) which was the standard drug used contracted the ileum of the rabbit while atropine (0.1mls) produced a relaxation effect (Figure4&5). On a similar note, the extract (*Senna siamea*) at a concentration of 100mg/ml, 250mg/ml and 500mg/ml relaxed the contraction of the ileum in a dose dependent manner. This is shown in figure 6, 7 and 8. Introduction of loperamide (1mg/ml) into the organ-bath solution resulted in relaxation of the ileum by reducing the amplitude of contraction by almost 50%. Responses elicited by the extract and loperamide were summarized in Figure 8-11.

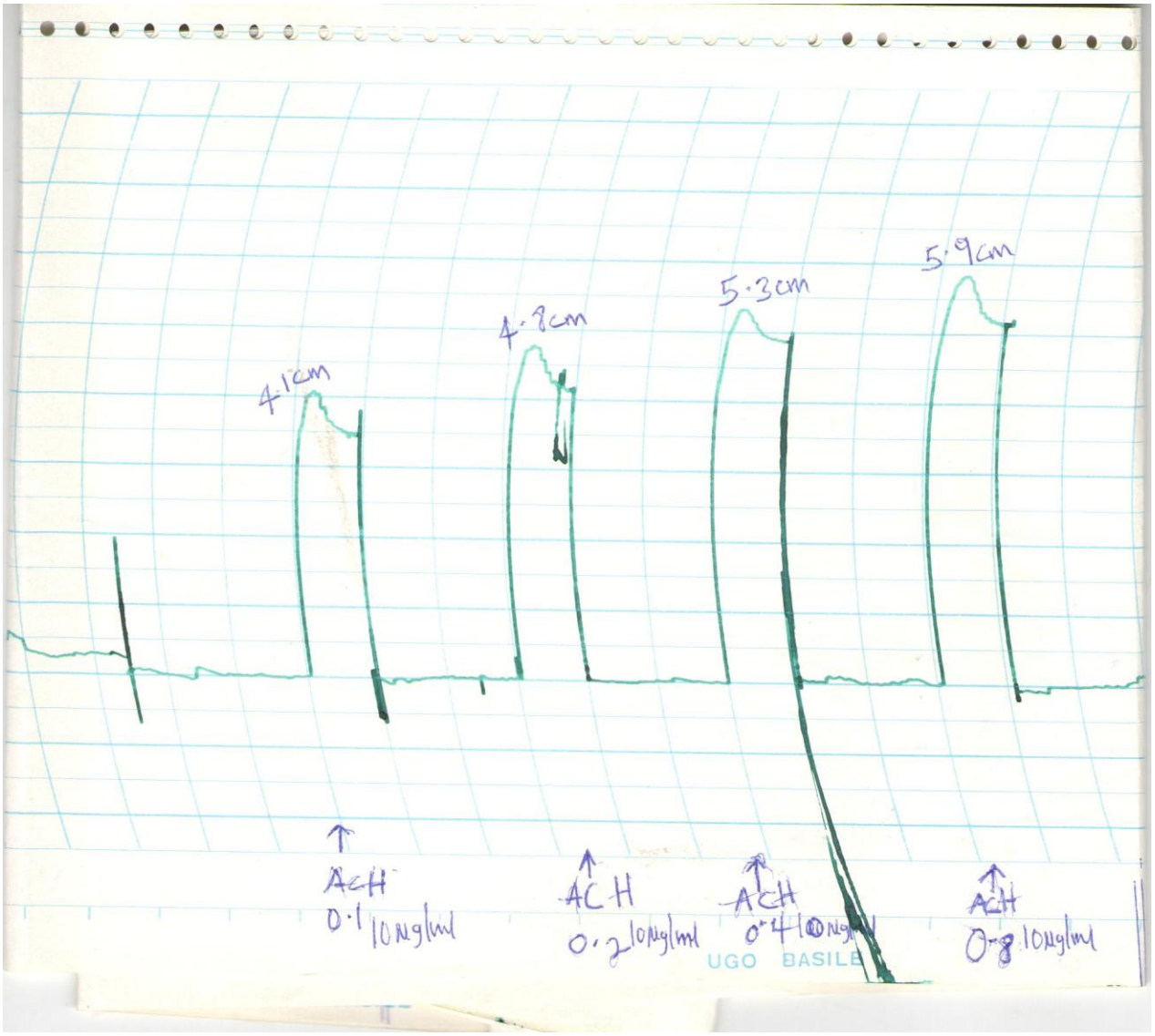


Figure 4: Standard solution of ACH at 10µg/ml concentration of 0.1ml, 0.2ml, 0.4ml and 0.8ml potentiated the contraction of the ileum of an experimental rabbit in an organ-bath, and a potentiation height of 4.1, 4.8, 5.3 and 5.9cm were recorded. Note: ACH-acetylcholine.

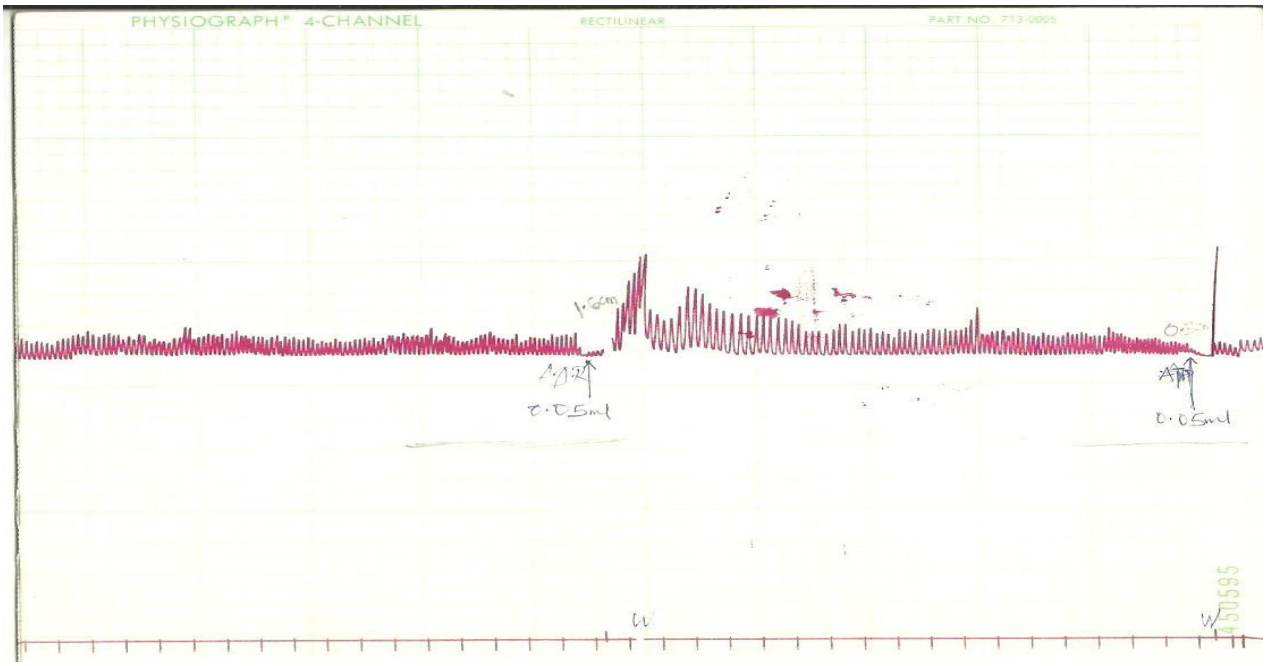


Figure 5: Standard solution of ADR and ATP at a concentration of 100µg/ml and 20µg/ml respectively of the same volume (0.05ml). ADR potentiated the contraction of ileum,recording a potentiation height of 1.6cm while ATP relaxed the contraction,recording a height 0.3cm. ADR-adrenaline, ATP-atropin

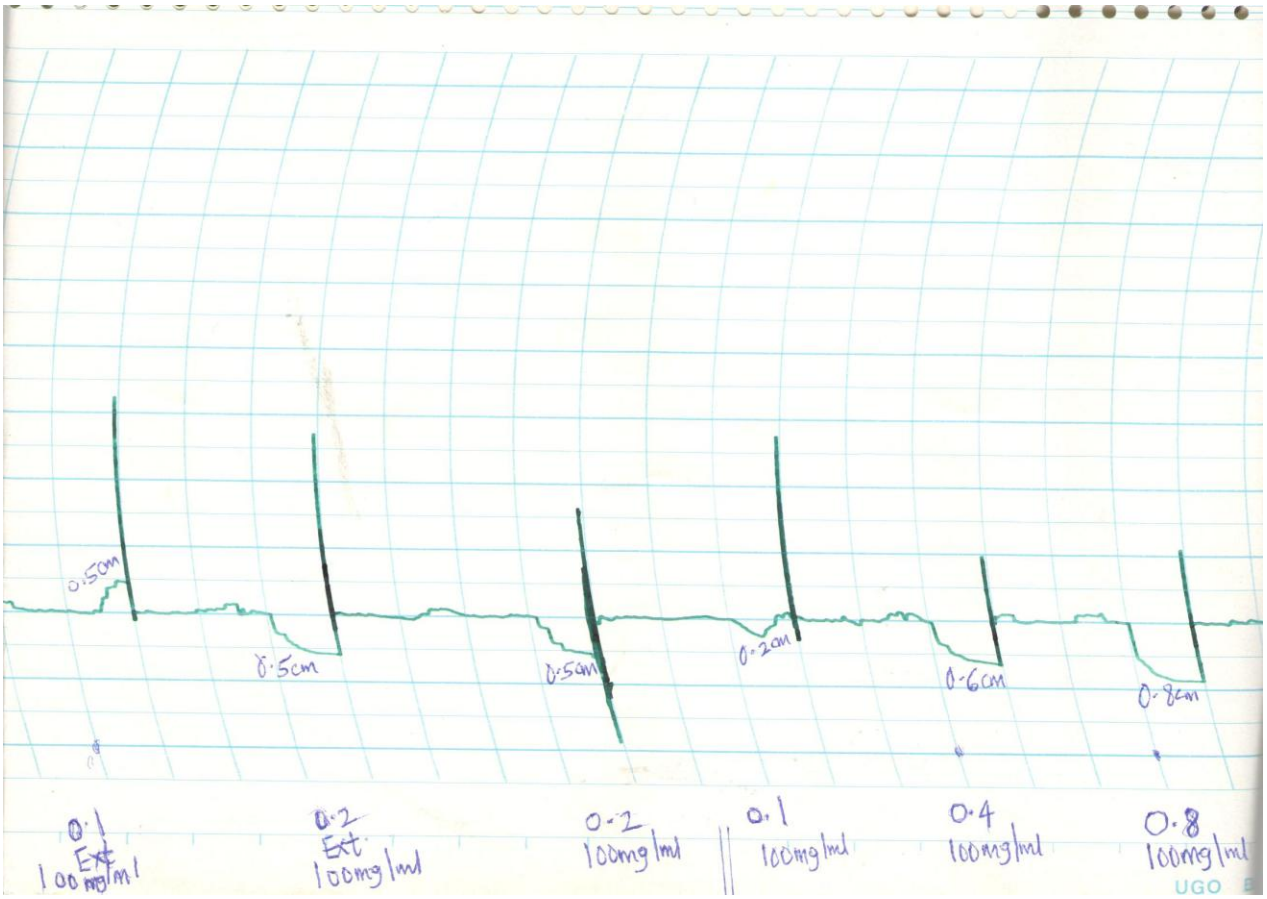


Figure 6: Smooth muscle relaxation of ileum at extract concentration of 100mg/ml (0.1,0.2,0.4, and 0.8mls)

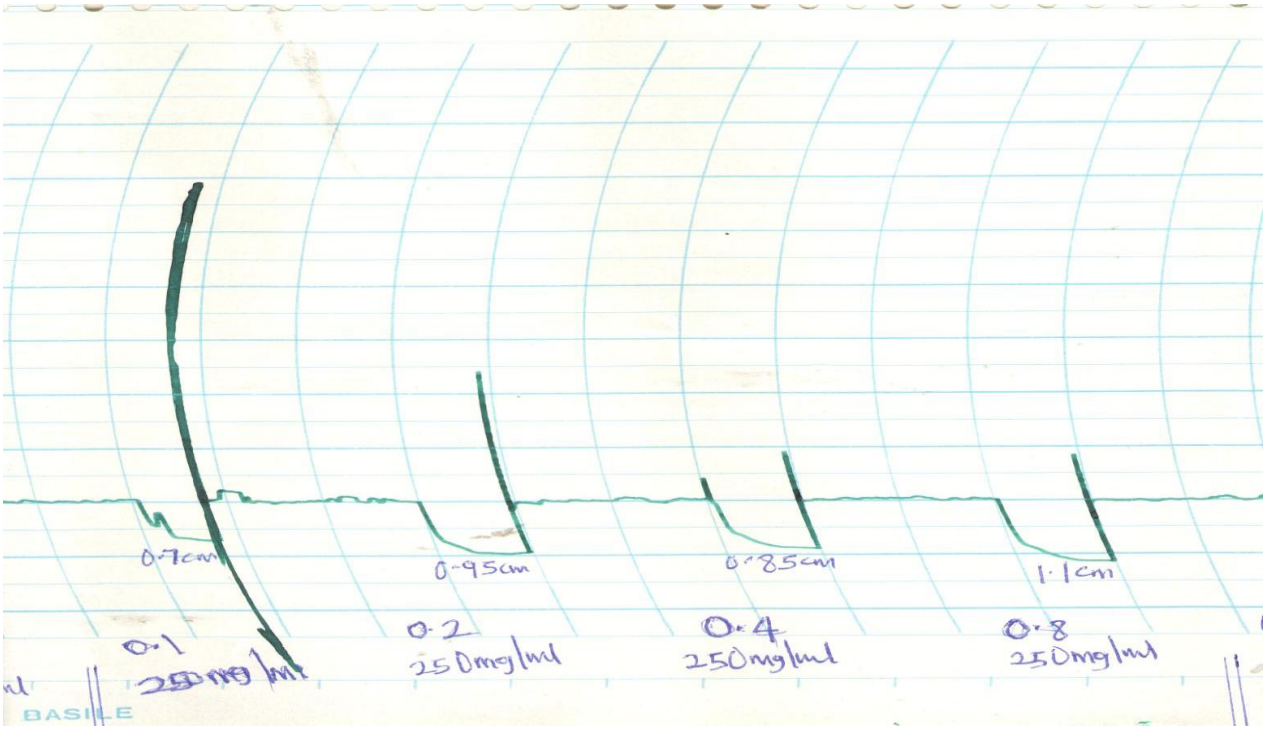


Figure 7: Smooth muscle relaxation of ileum at extract concentration of 250mg/ml (0.1, 0.2, 0.4, and

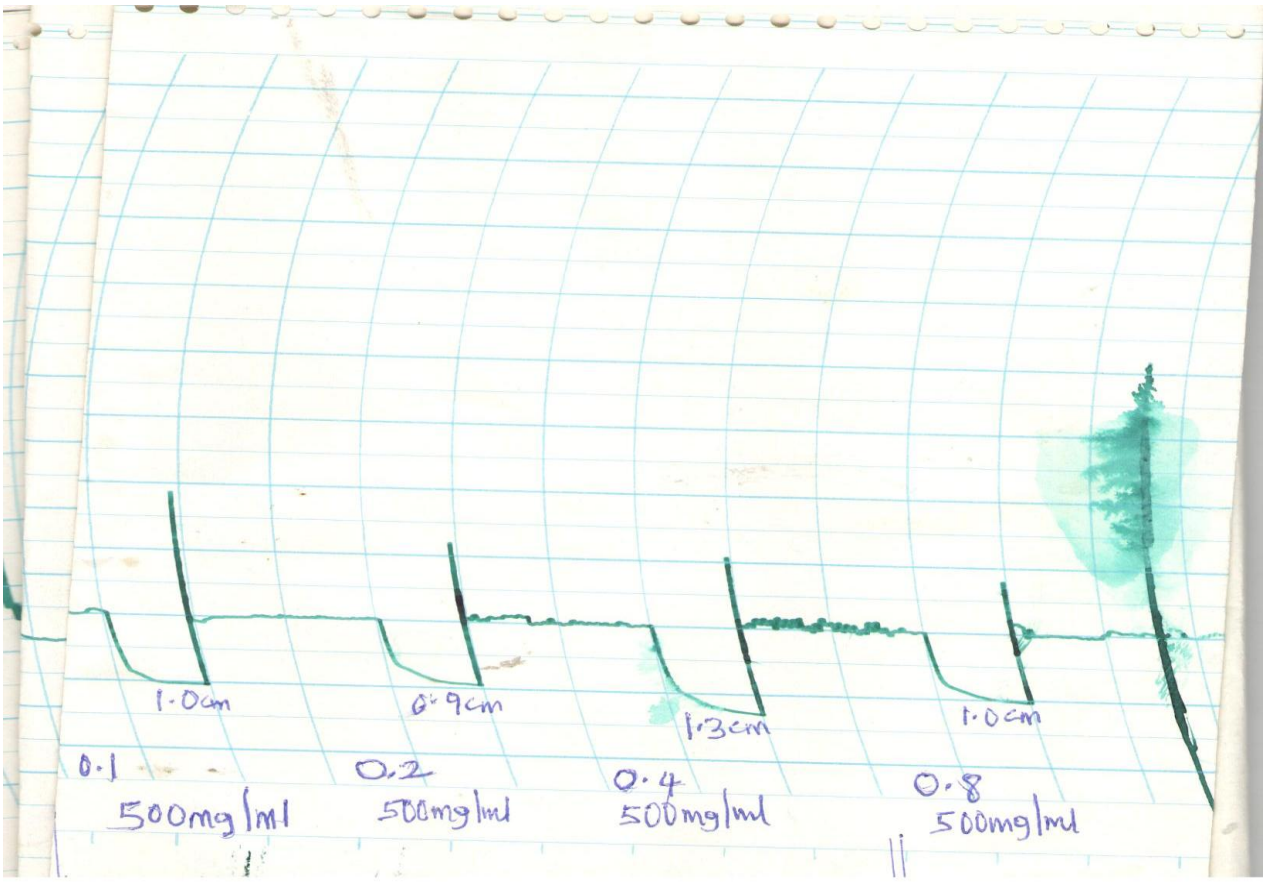


Figure 8: Smooth muscle relaxation of ileum at extract concentration of 500mg/ml (0.1, 0.2, 0.4, and 0.8mls)

4.0.1 Drug Interaction (extract + loperamide)

Introduction of extract at concentration of 250mg/ml (0.2mls) and loperamide hydrochloride at 1mg/ml (0.1mls) initiated amplitude height of 0.5cm below the base-line as seen in Figure 9. Results here showed that loperamide did not produce any antagonistic effect on the extract at this particular dose. Extract (250mg/ml) at 0.2mls and loperamide (1mg/ml) at 0.2mls produced amplitude height of 0.65cm below the contraction base-line. Result obtained from interaction between extract (250mg/ml) at 0.8mls and loperamide at 0.1mls showed a reduced relaxation

effect of the extract at a recorded amplitude height of 0.5cm below the contraction base-line (figure 10).

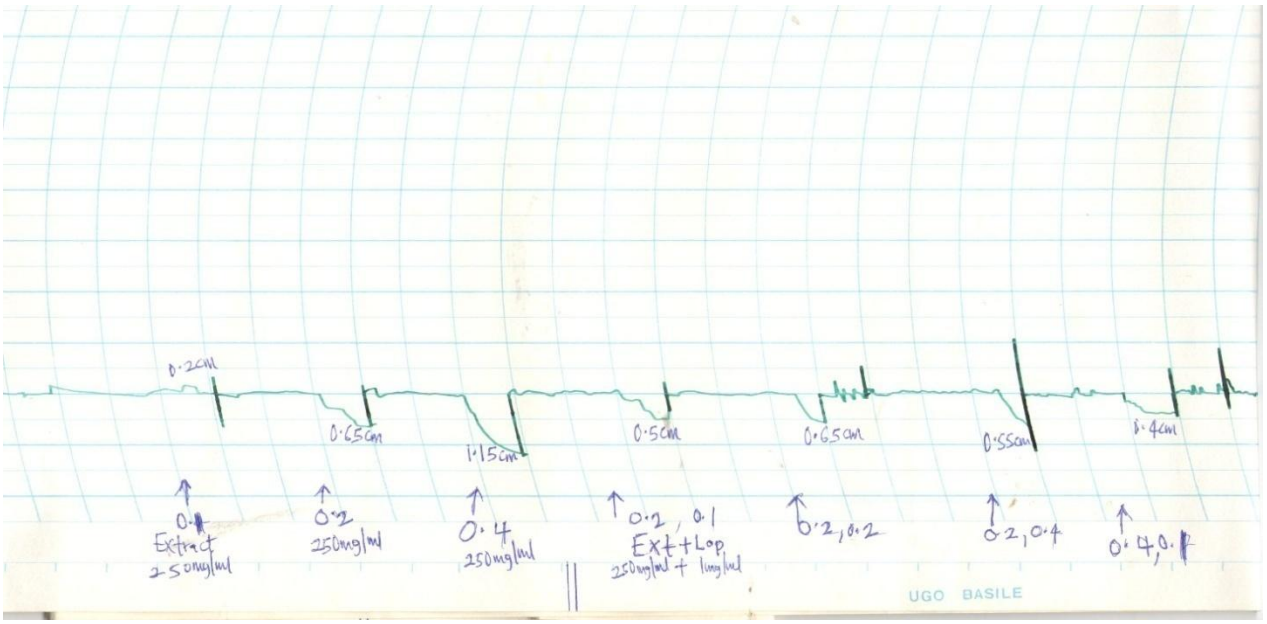


Figure 9: Action of extract(250mg/ml) and loperamide(1mg/ml) on the ileum at 0.2, 0.1mls. Action of extract(250mg/ml) and loperamide(1mg/ml) on the ileum at 0.2, 0.2mls. Action of extract(250mg/ml) and loperamide(1mg/ml) on the ileum at 0.2, 0.4mls.Note:W-washed tissue with distilled water. Ext.-extract, Lop.-loperamide hydrochloride.

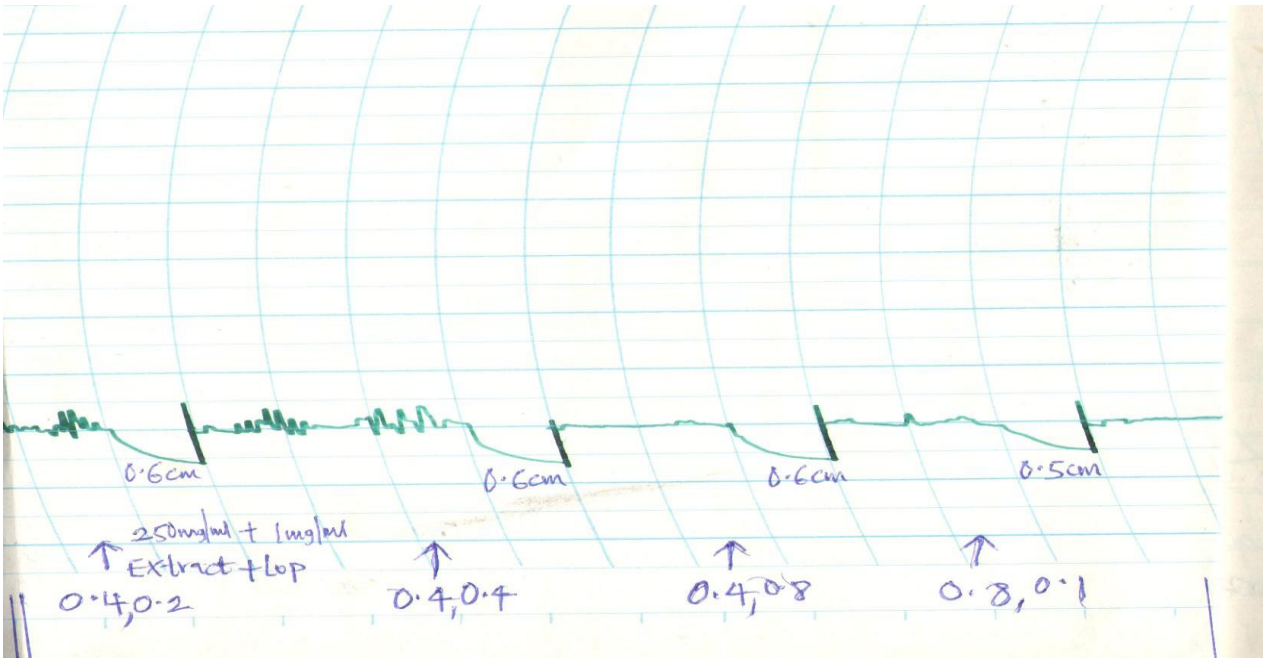


Figure 10: Drug(Loperamide) interaction with extract(*S.siamea* 250mg/ml) on the ileum of experimental rat in an organ-bath. Here, the extract was introduced into the organ-bath before loperamide.

4.0.2 Drug interaction (loperamide+ extract)

Introduction of Loperamide(1mg/ml) at 0.4mls and *Senna siamea* (250mg/ml) at 0.2mls produced an amplitude height of 0.5cm(figure 11a). A similar result was obtained when loperamide(1mg/ml) at 0.4mls and *Senna siamea*(250mg/ml) at 0.2mls produced an amplitude of 0.5cm as seen in figure 11b.

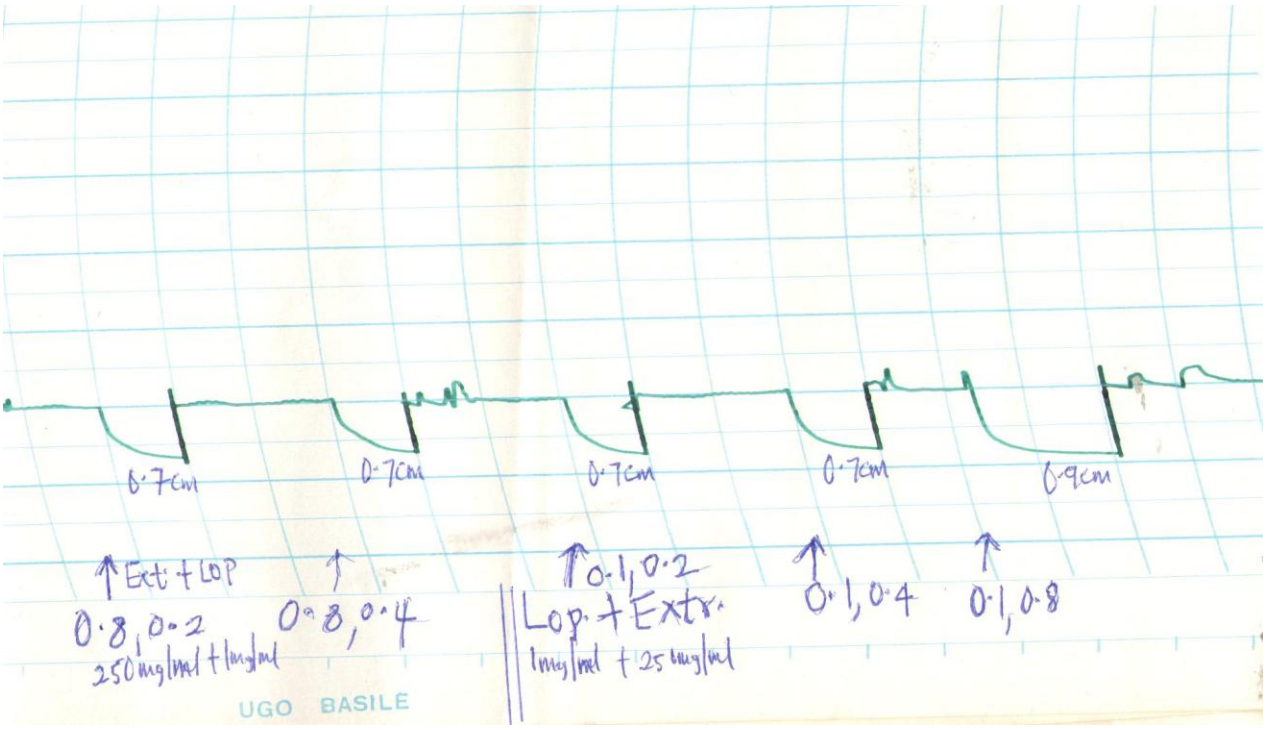


Figure 11a: Drug(loperamide hydrochloride) interaction with extract(*S.siamea*) on the ileum at 0.1, 0.2mls, and 0.1, 0.4mls. Here, loperamide was introduced into the organ-bath before the extract. Lop.-loperamide hydrochloride, Ext.-extract, W-washed tissue with distilled water.

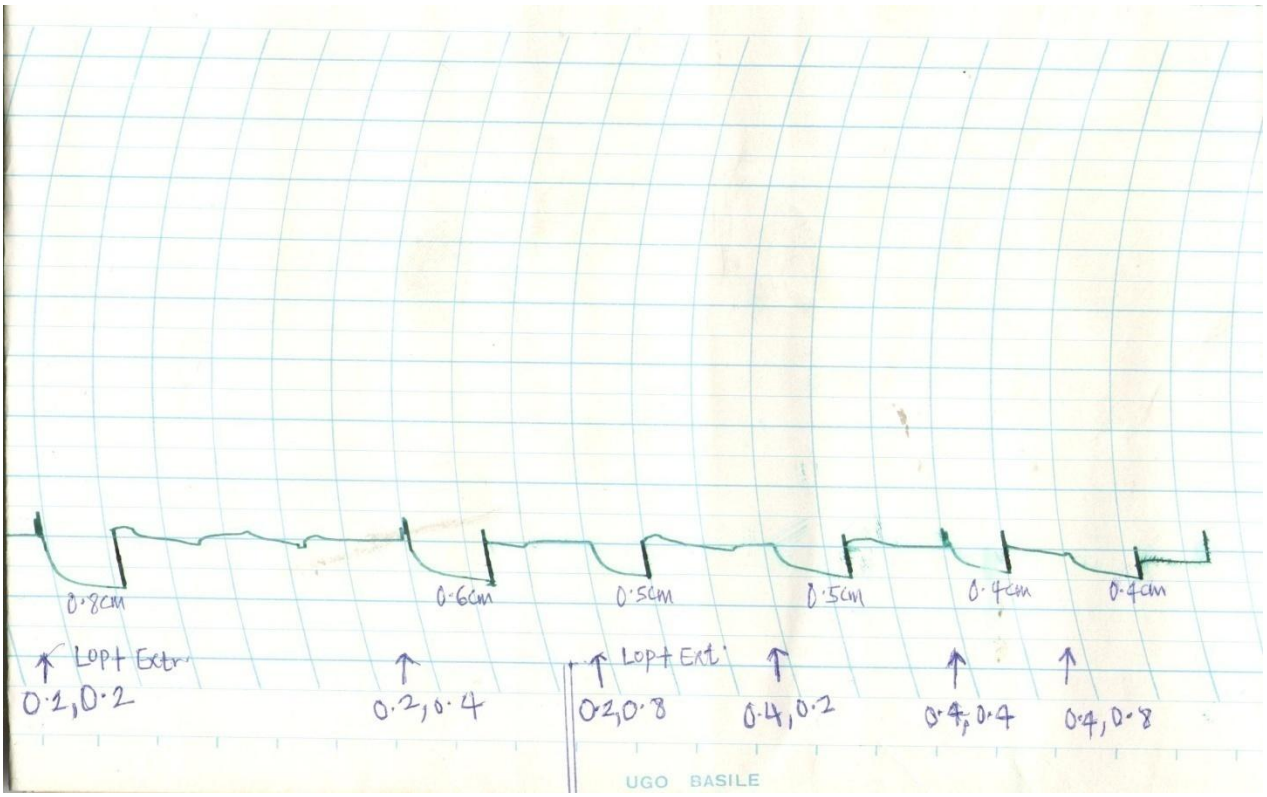


Figure 11b: Drug(loperamide hydrochloride) interaction with extract(*S.siamea*) on the ileum at 0.2, 0.8mls, and 0.4, 0.2mls. Here, loperamide was introduced into the organ-bath before the extract. Lop.-loperamide hydrochloride, Ext.-extract, W-washed tissue with distilled water.

4.1 IN VIVO STUDIES

4.1.1 MORPHOLOGY

There was no significant change in body weight across experimental groups as shown in table 1. In addition to this, the organ-body weight ratio across experimental groups also showed little or no significant difference as recorded in table 1.

TABLE 4: Mean values for Stool weight, Intestine-Weight Ratio and Change in body weight across experimental groups.

GROUPS	STOOL WEIGHT (g)	INTESTINE-WEIGHT RATIO	CHANGE IN BODY WEIGHT (g)
	Mean± SEM	Mean± SEM	Mean± SEM
1	0.307 ± 0.180	10.717±1.67	14.333± 9.244
2	0.333±0.233	9.233±0.89	17.166± 9.250
3	0.267±0.211	9.333±1.43	11.016± 3.982
4	0.299±0.253	9.833±1.00	12.200± 5.043
5	0.260±0.239	8.340±1.15	3.720± 3.307

4.1.2 STOOL ANALYSIS

Loperamide significantly increased the weight of the fecal pellets. The opiate-induced stool was smaller and dried pellets when compared to stool obtained from the control group as shown in plate 2. It was also observed that some of the constipated animals did not pass stool at all. There

was an improvement in the stool pattern of groups treated with *S.siamea*. Stool from the group appeared normally-shaped and softened as compared to stool collected in the control group. Stool collected from bisacodyl treated group appeared lumped and slightly reduced in size. The texture and general morphology of stool sample obtained from experimental groups is summarized in Plate 1-5.



Plate 1: Picture of stool sample collected from animal treated with normal saline (control). From this it can be observed that the stool appeared normal in size and texture when compared to the stool sample obtained in plate 2.



Plate 2: Picture of stool sample collected from animal treated with loperamide(group 2). Stool sample appeared dried and reduced after 6 days of loperamide administration, an indication that constipation was induced.



Plate 3: Picture of stool sample collected from animal treated with extract and then loperamide(group 3). Stool sample appeared moisty and large indicating that loperamide had little or no effect on the laxative effect of *Senna siamea*.



Plate 4: Picture of stool sample collected from animal treated with loperamide and then extract(group 4). Stool sample appeared normal and of similar size when compared to the stool sample in Plate 1(control group).

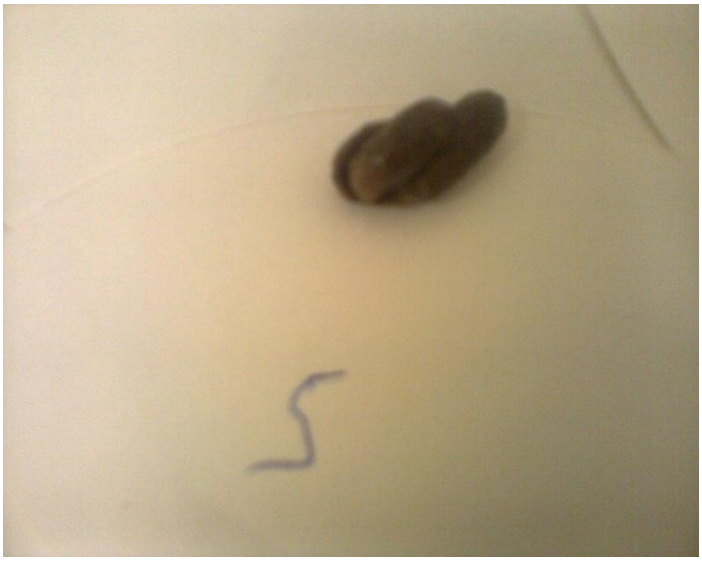


Plate 5: Picture of stool sample collected from animal treated with loperamide and then standard laxative (bisacodyl) group 5. Stool sample appeared lumped and slightly reduced in size when compared to sample obtained in Plate 1(control group)

4.2 HISTOLOGY

Histological assessment of colon in control group showed that the glands (crypts) are closely packed and well arranged in a particular fashion within the mucosa as shown in plate 6. The crypts are numerous and the mucosa folds appeared straight. A view of the colon at X250 magnification revealed a more defined cell boundary around the crypt as seen in plate 7 with prominent but few goblet cells. Opiate (loperamide) treated colon have larger but fewer crypts that are not closely arranged in the mucosa when compared to what was seen in the control group (plate 10). The mucosa folds of colon in this group appeared oval and bent as compared to what was obtained in the control group (having a straight and oval-shaped mucosa folds). The goblet cells of opiate treated group also appeared prominent, numerous and more visible at X250 magnification as seen on plate 11. At the same magnification, cells linning the mucosa appeared more basal as compared to what was observed in control group, enterocytes also appeared to have lost their cellular integrity, cells lining the crypt appeared to have slurred off, making the glands to gradually loss its definite shape.

Results obtained from animals treated with extract at first and then opiate (loperamide) showed that the colon had a similar cellular cyto-architecture with the constipated group (groups treated with the opiate only) as seen in plate 14. In this group, the glands (crypts) appeared larger, not closely arranged with fewer goblet cells as compared to what was observed in constipated colon (having numerous goblet cells). A detailed presentation of these cells could be seen on plate 15 at X250 magnification using H/E stain. In the extract treated group, the glands (crypts) are smaller, numerous and closely packed in the mucosa as shown in plate 18. The cellular cytoarchitecture appeared similar to what was obtained in the control group. On the contrary,colon of animals

treated with standard drug (bisacodyl) have larger and numerous crypts with enlarged goblet cells(plate 22). The mucosa fold is less, giving the cells a well-defined boundry.

4.3 HISTOCHEMISTRY

Mucin content in goblet cells of constipated group stained red with PAS (PAS positive) as shown in plate 13. The goblet cells are large and sparsely distributed within the crypt. Mucin content in goblet cells of extract treated group is reduced and PAS positive with few stained cells (plate 21). This is in sharp contrast as compared to the result obtained from bisacodyl treated colon. The mucin content is higher as the goblet cells are prominently stained with PAS (plate 25).

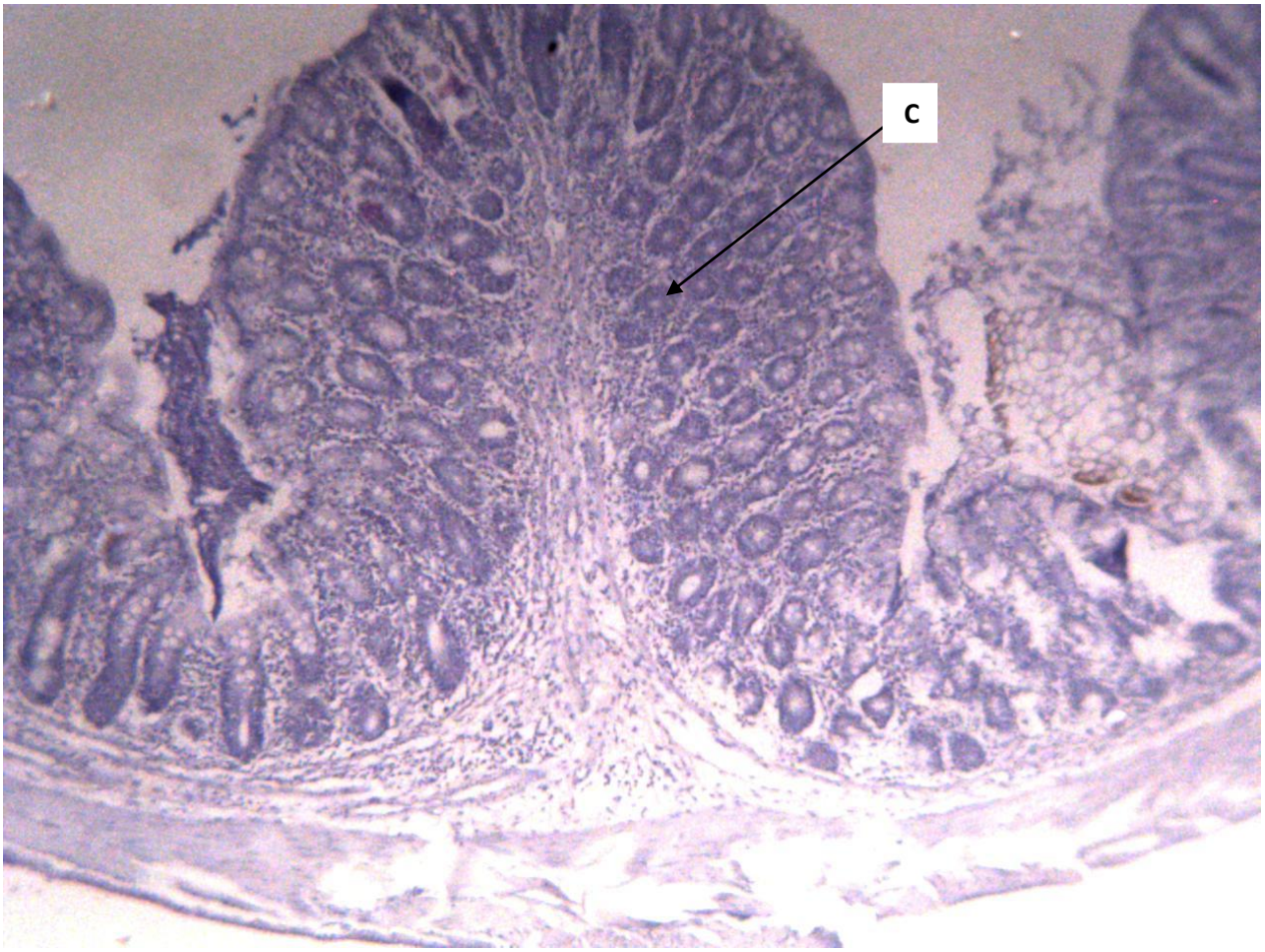


Plate 6: Photo-Micrograph of the transverse section of colon in group that were administered normal saline only (control group), showing a normal histoarchitecture of the colon Note: C-crypt. H/E X40

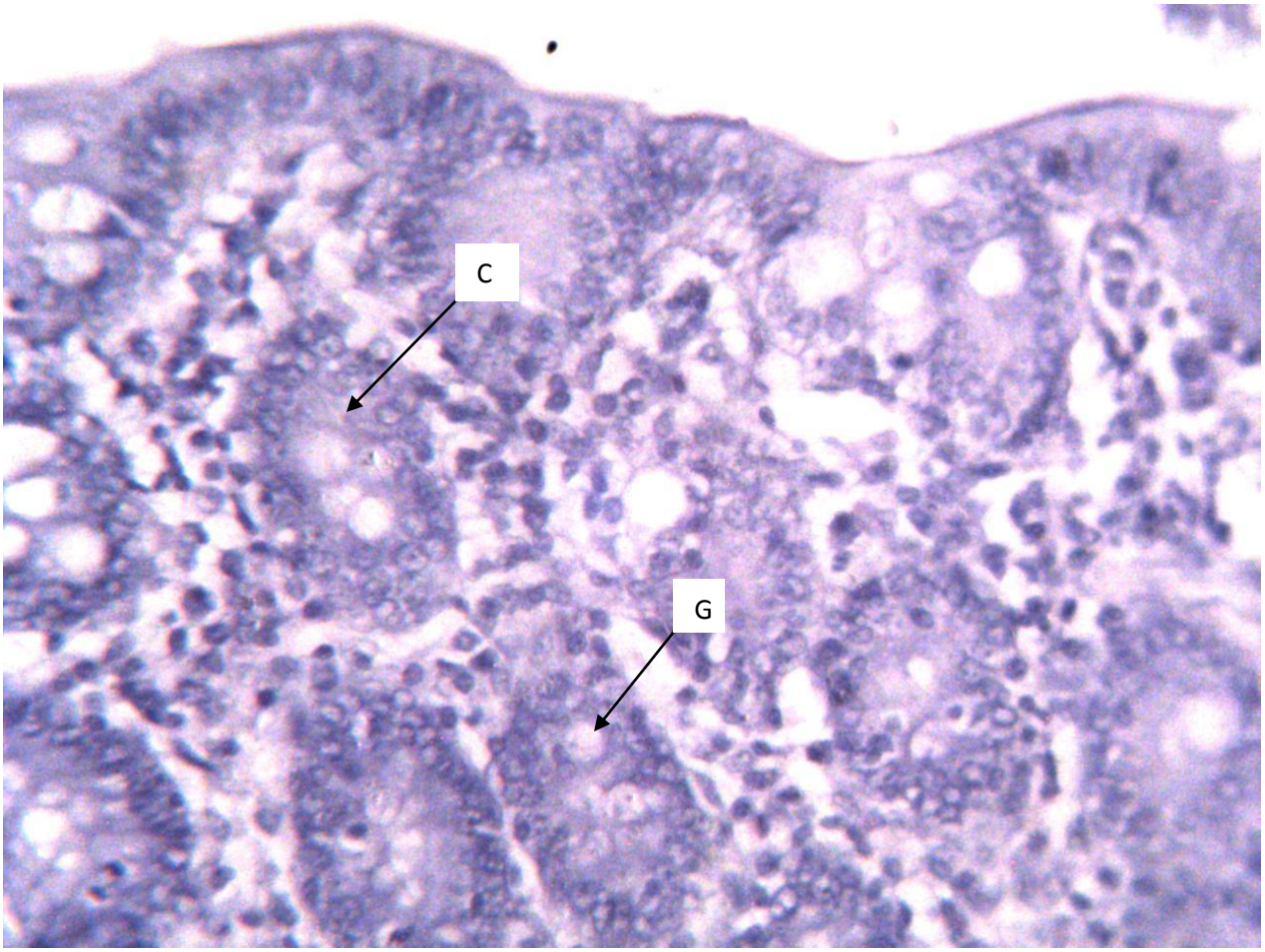


Plate 7: Photo-Micrograph of the transverse section of colon in control group Note: C-crypt, G-goblet cells. H/E X250

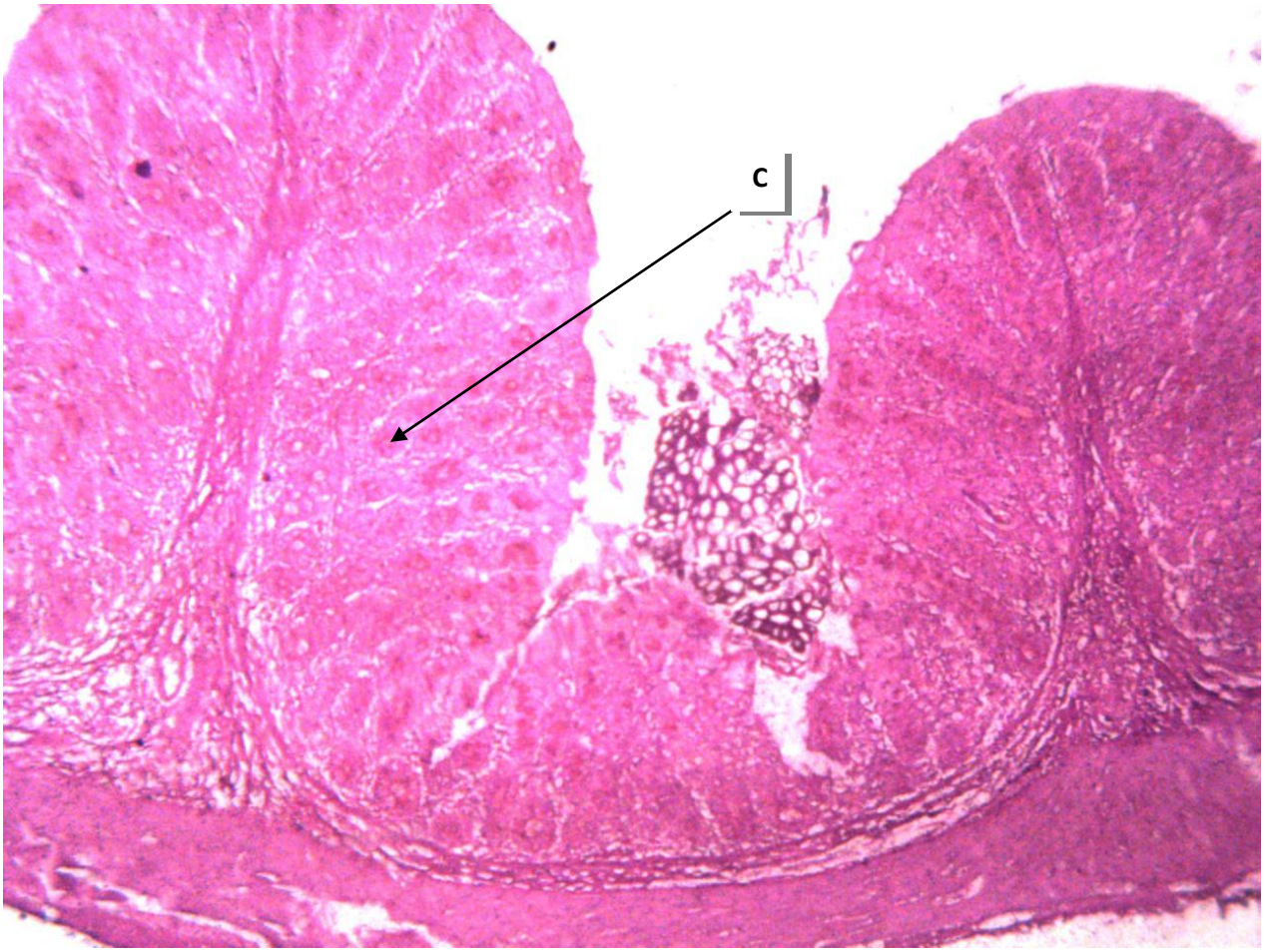


Plate 8: Photo-Micrograph of the transverse section of colon in control group Note: C-crypt. PAS X40

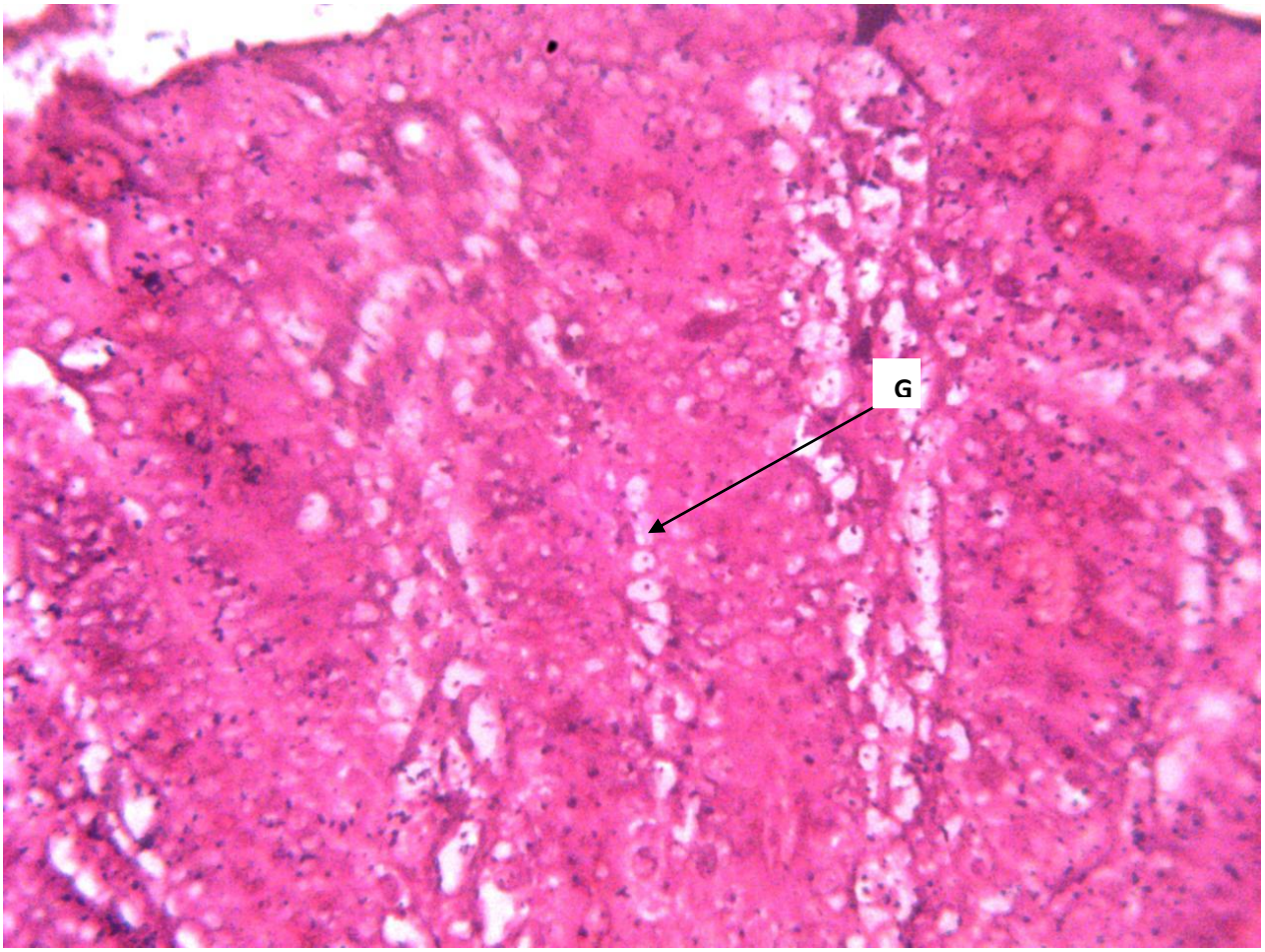


Plate 9: Photo-Micrograph of the transverse section of colon in control group Note: G-goblet cells. PAS X250

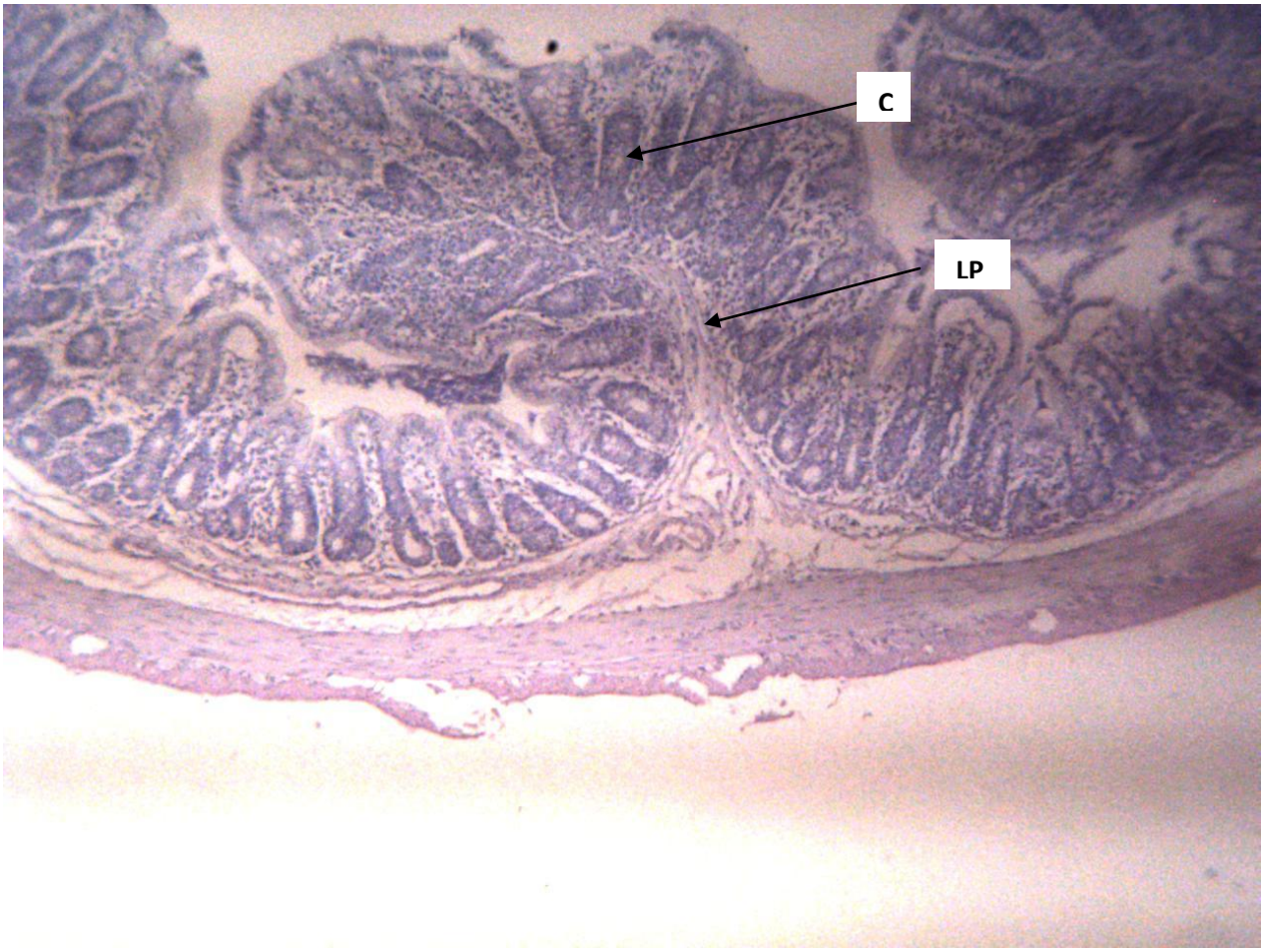


Plate 10: Photo-micrograph of the transverse section of the colon in constipated group (group 2)
Note: C-crypt, LP-lamina propria. H/E.X40

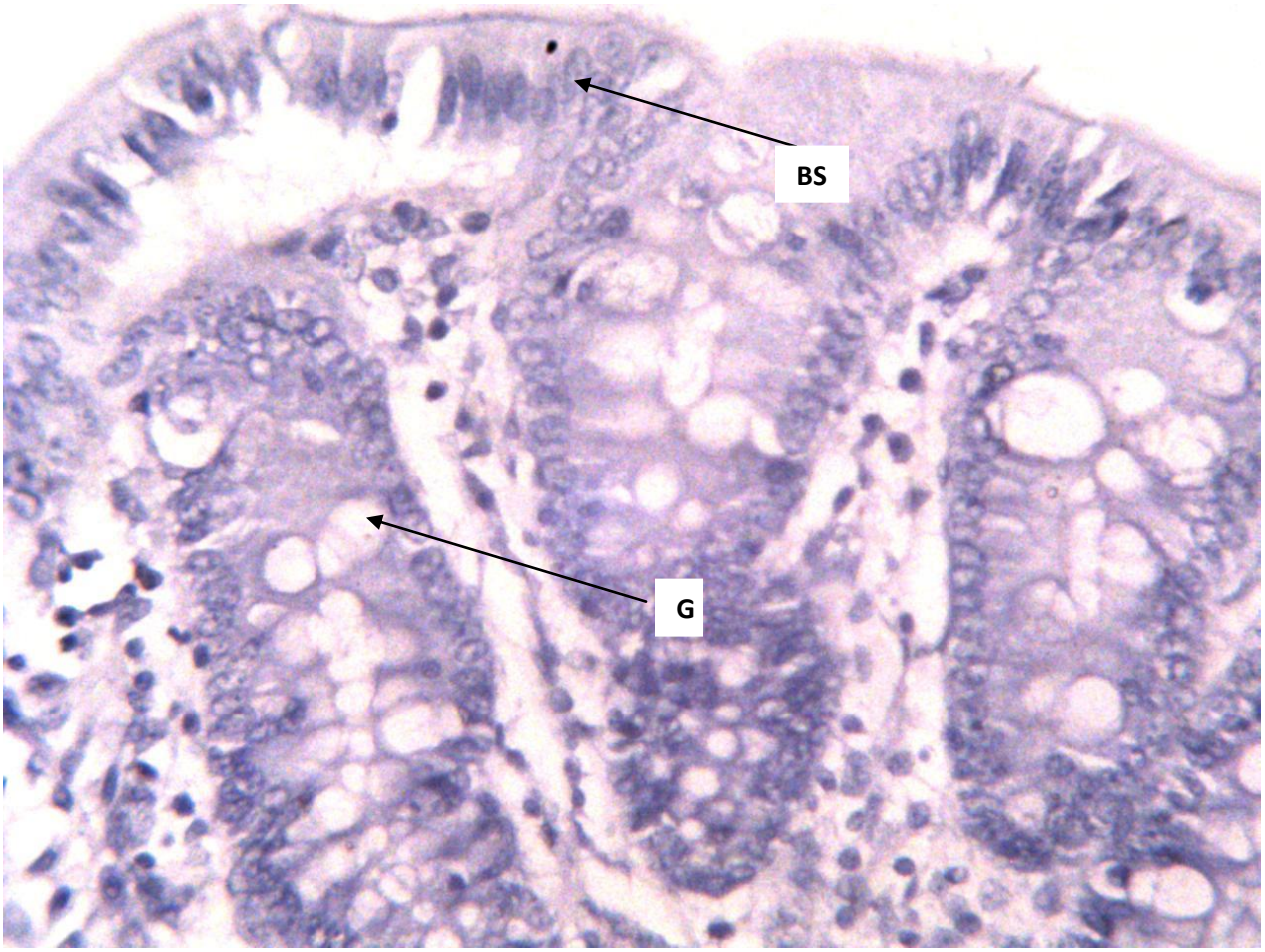


Plate 11: Photo-micrograph of the transverse section of colon in constipated group (group 2) Note: BS-basal cells G-goblet cells (enlarged and numerous). H/E.X250



Plate 12: Photo-micrograph of the transverse section of the colon in constipated group (group 2)
Note: C-crypt. PAS.X40

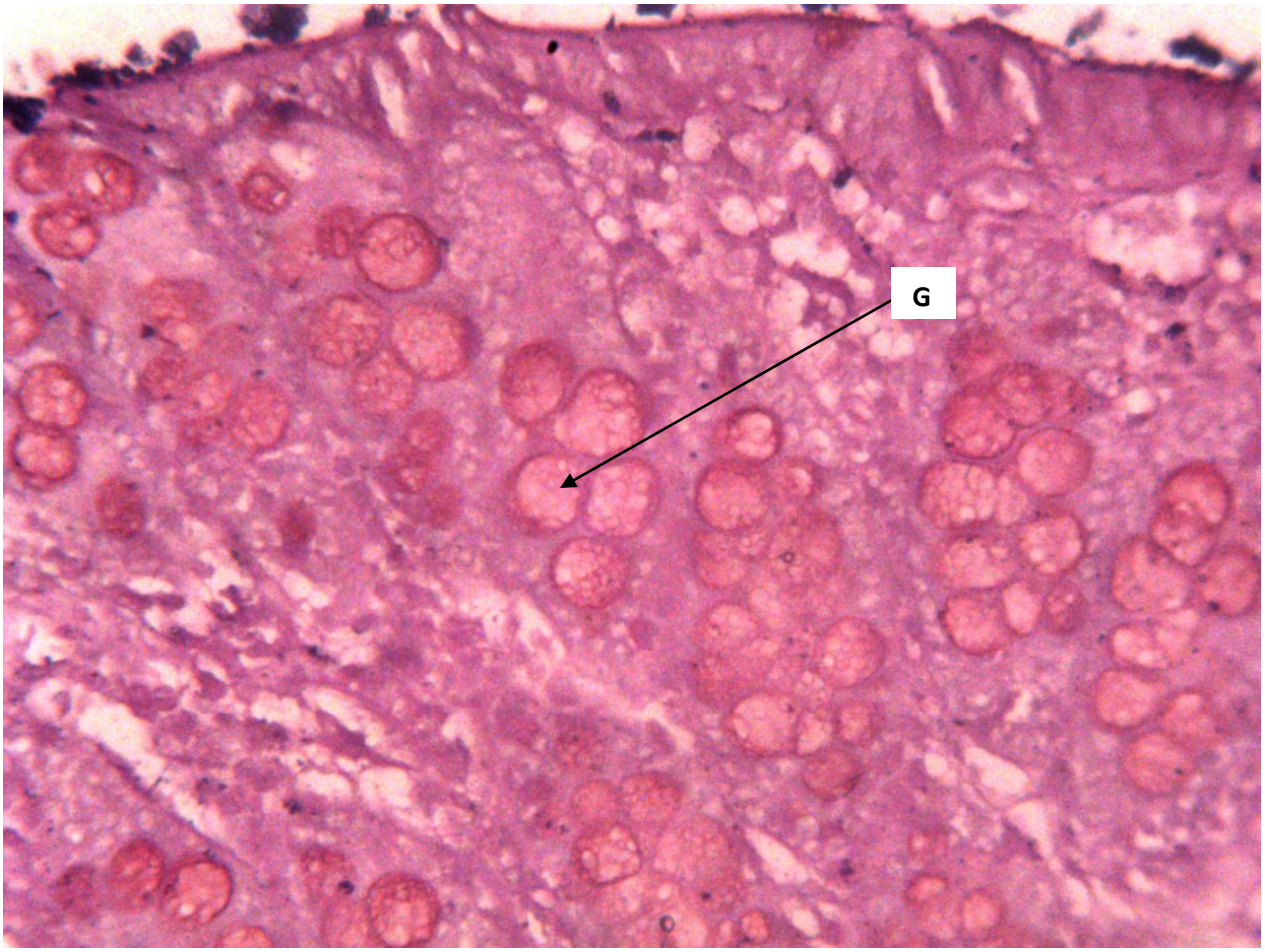


Plate 13: Photo-micrograph of the transverse section of colon in constipated group (group 2) Note: G-goblet cells (numerous and enlarged). PAS.X250



Plate 14: Photo-micrograph of the transverse section of colon in opiate treated group after the administration of extract (group 3) Note: LP-lamina propria C-crypt. H/E.X40

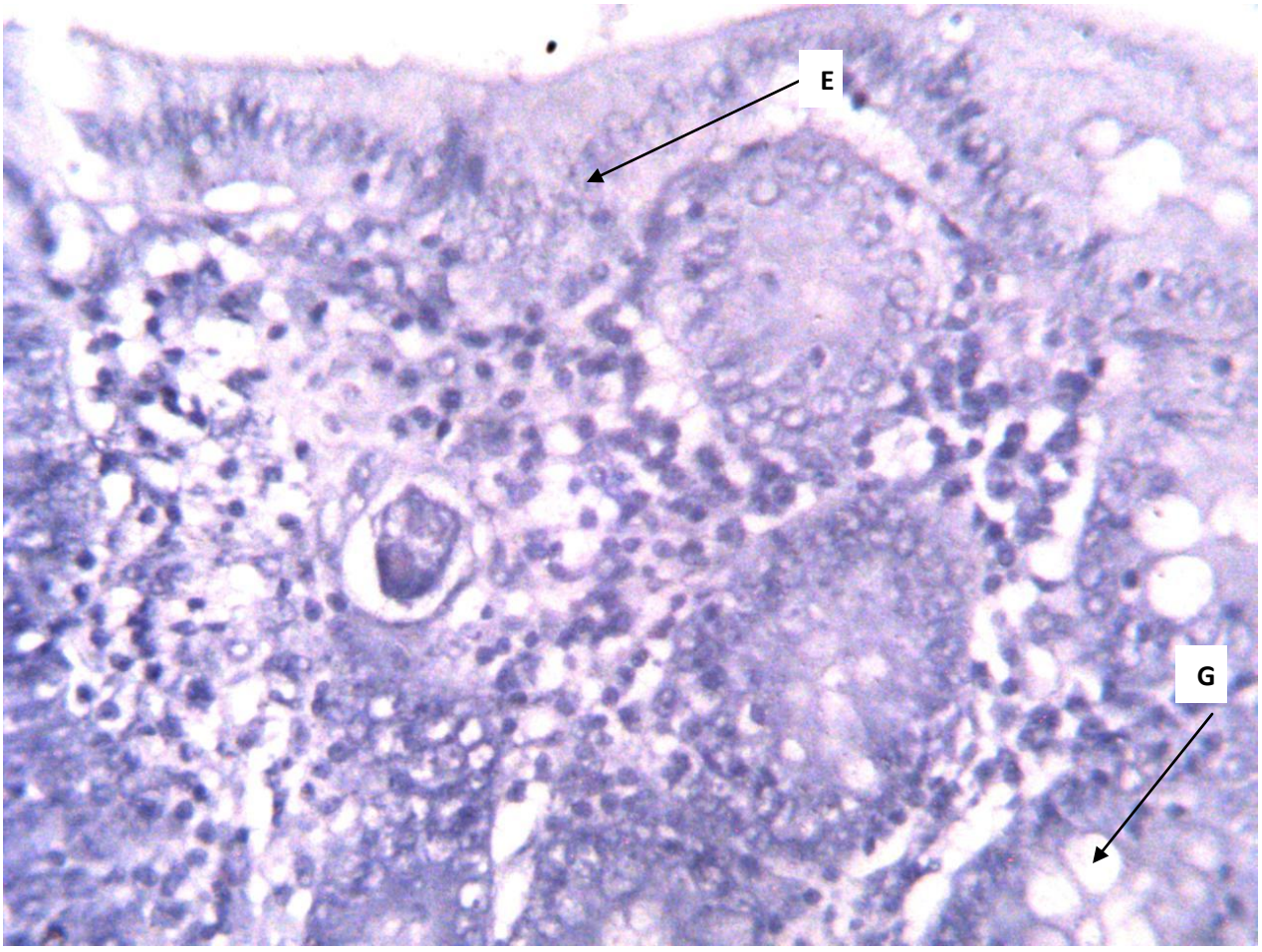


Plate 15: Photo-micrograph of the transverse section of colon in opiate treated group after the administration of extract (group 3) Note: E-enterocytes (slurring off), G- goblet cells (large but few) H/E X250

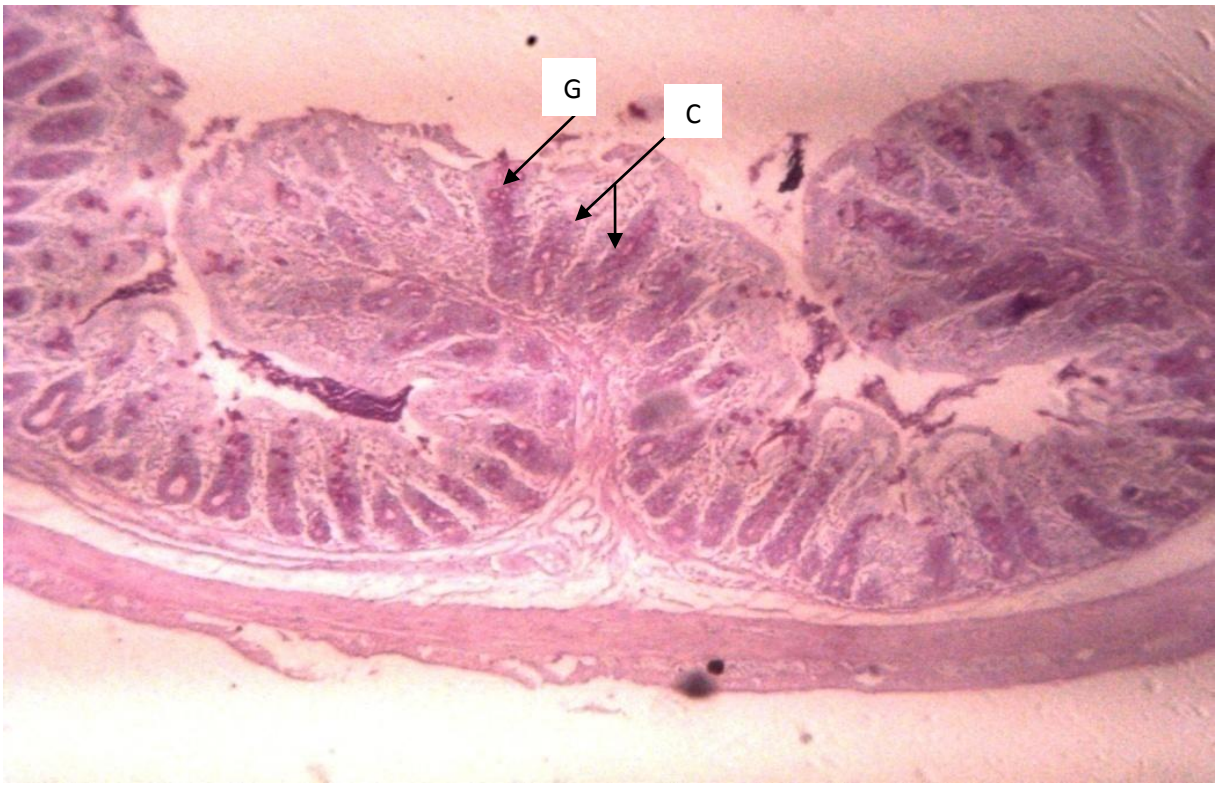


Plate 16: Photo-micrograph of the transverse section of colon in opiate treated group after the administration of extract (group 3) Note: G-goblet cells enlarged, C-crypt. PAS X40

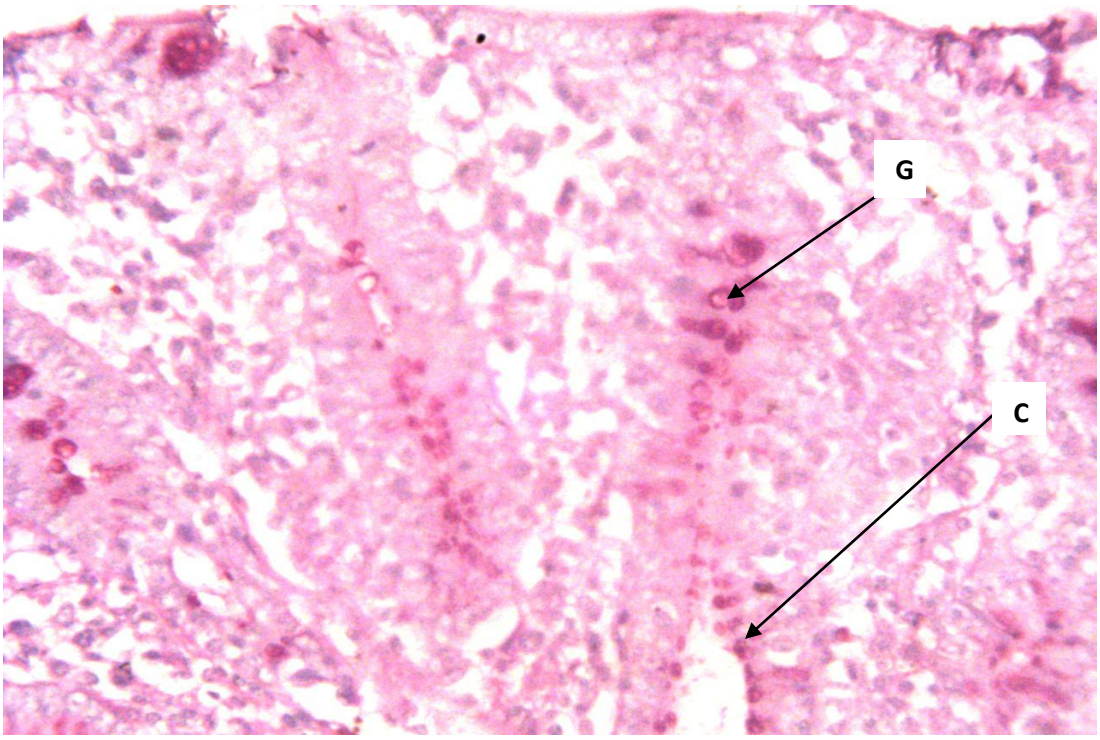


Plate 17: Photo-micrograph of the transverse section of colon in opiate treated group after the administration of extract (group 3) Note: G-goblet cells enlarged, C-crypt. PAS X250

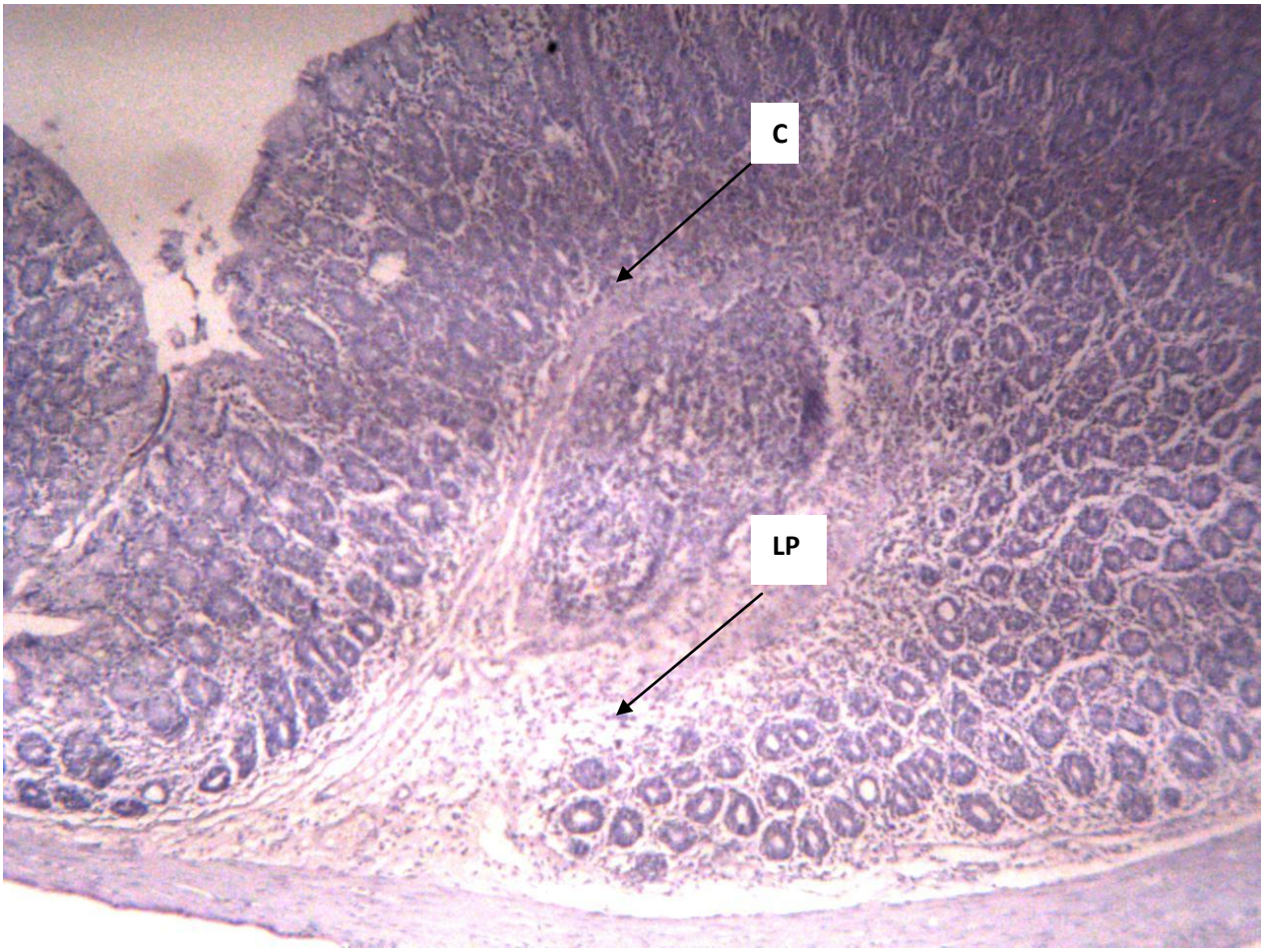


Plate 18: Photo-micrograph of the transverse section of colon in extract treated group after the administration of opiate (group 4) Note: C-crypt LP-lamina propria. H/E X40

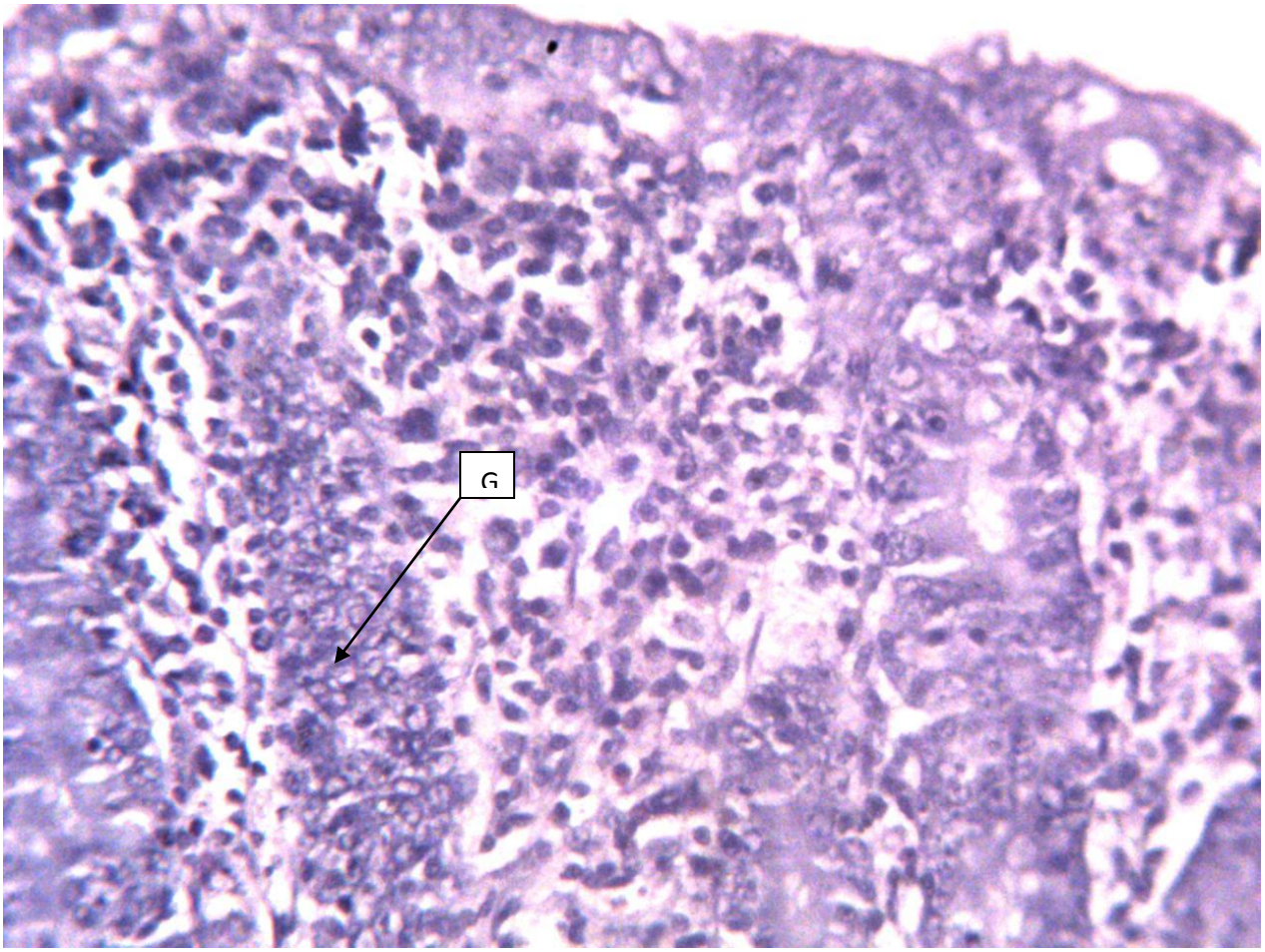


Plate 19: Photo-micrograph of the transverse section of colon in extract treated group after the administration of opiate (group 4) Note: G- Goblet cells (scanty). H/E X250



Plate 20: Photo-micrograph of the transverse section of colon in extract treated group after the administration of opiate (group 4) Note: C-crypt. PAS X40

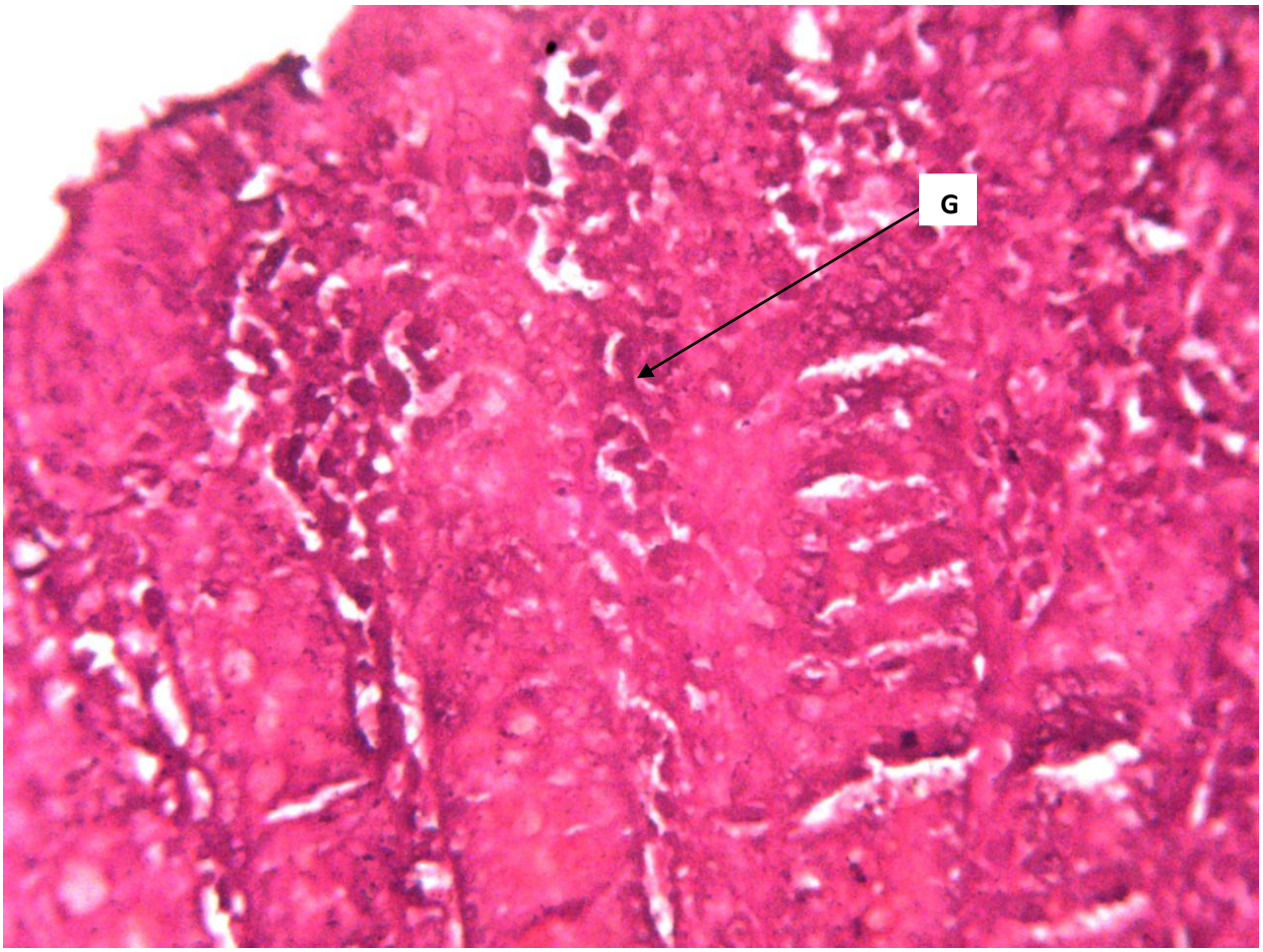


Plate 21: Photo-micrograph of the transverse section of colon in extract treated group after the administration of opiate (group 4) Note: G-goblet cells. PAS X250

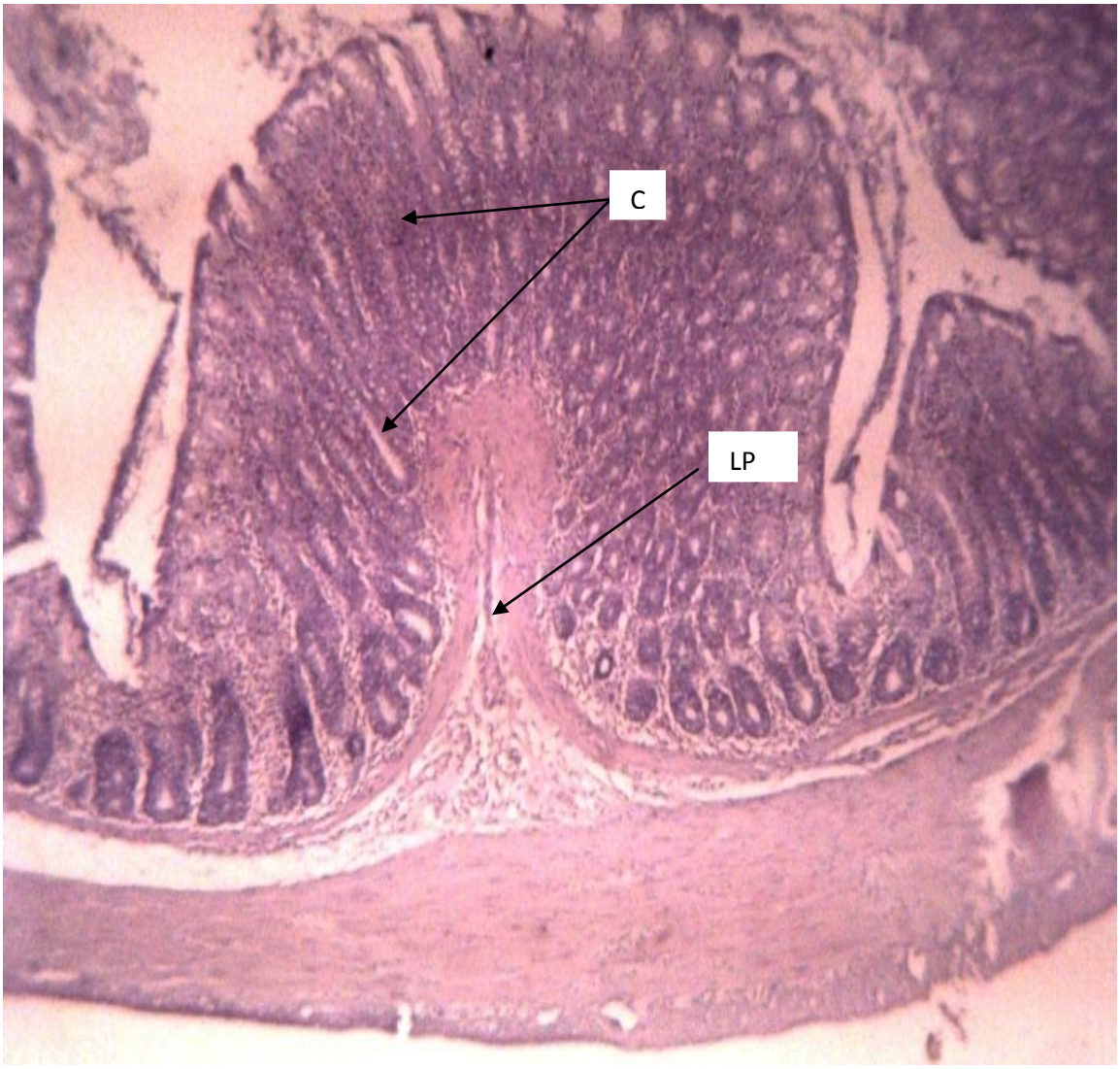


Plate 22: Photo-micrograph of the transverse section of colon in bisacodyl treated group (group 5)
Note: LP-lamina propria C-crypts. H/E X40

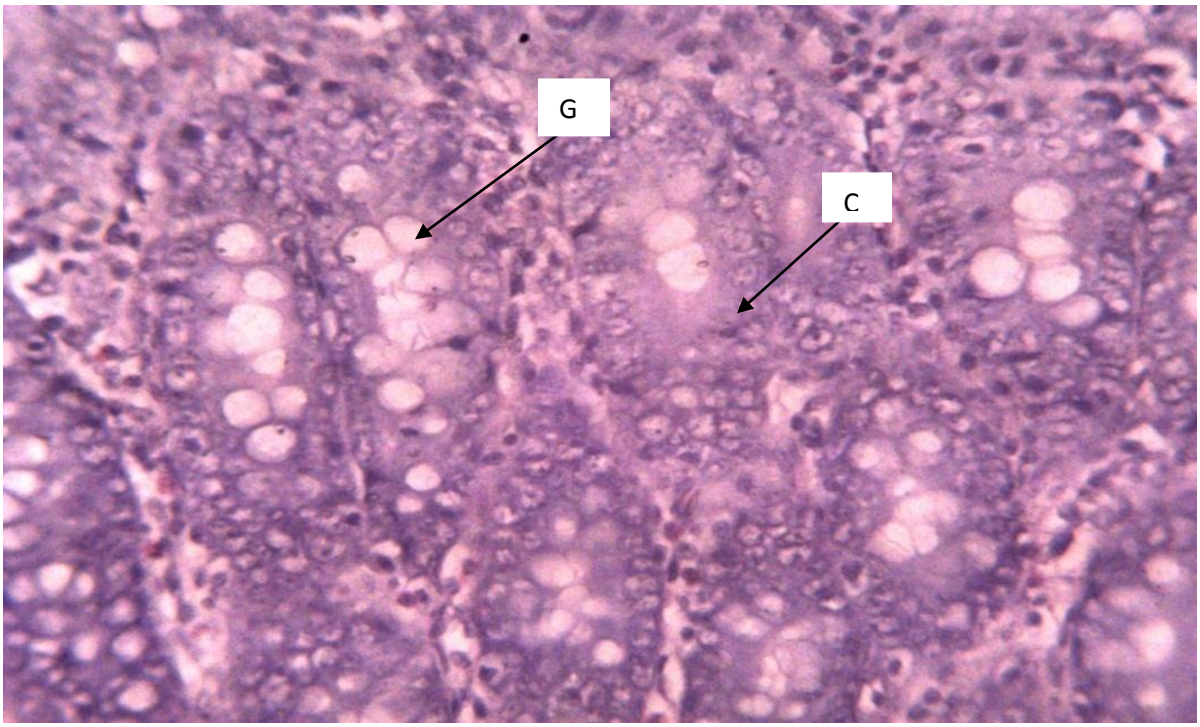


Plate 23: Photo-micrograph of the transverse section of colon in bisacodyl treated group (group 5)
Note: G-goblet cells C-crypts. H/E X250

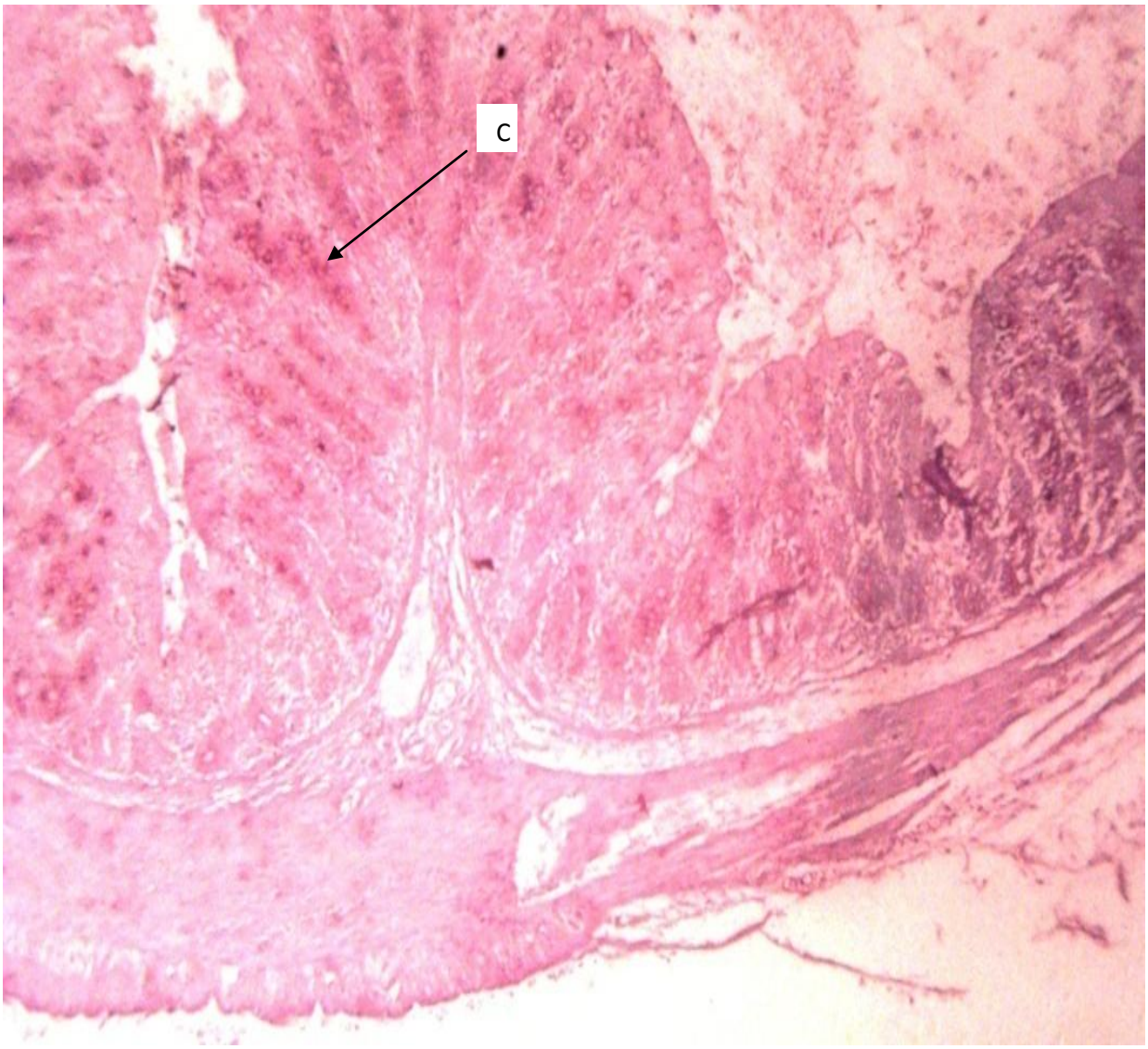


Plate 24: Photo-micrograph of the transverse section of colon in bisacodyl treated group (group 5)
Note: C-crypts. PAS X40

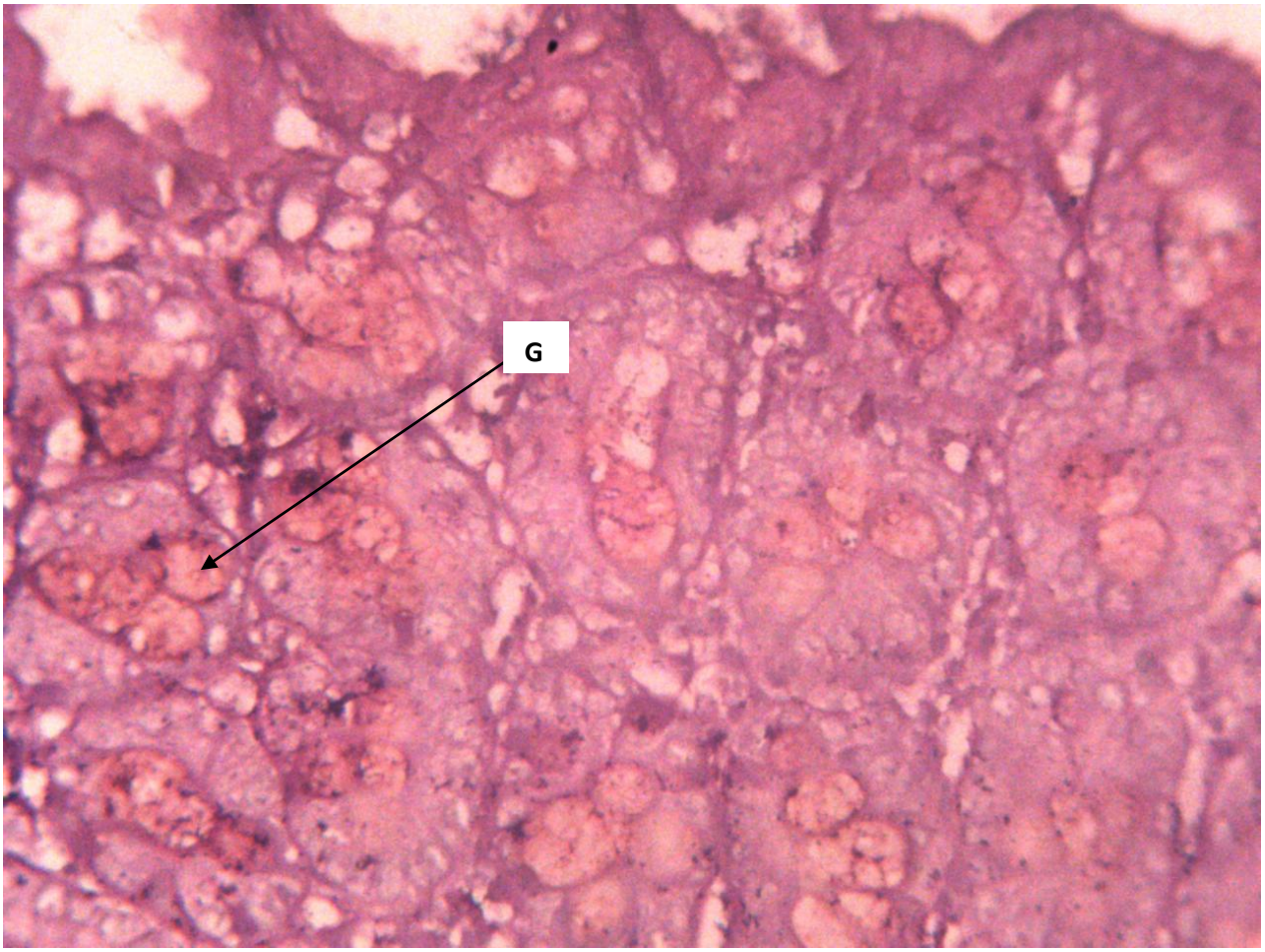


Plate 25: Photo-micrograph of the transverse section of colon in bisacodyl treated group (group 5)
Note: G-goblet cells (enlarged) PAS X250

CHAPTER FIVE

DISCUSSION

Senna is one of the well-known anthranoid-containing herbal drugs used as laxatives. Although the stimulant laxatives including *senna* have been recommended for short-term treatment of acute constipation, many people use them for long periods without medical supervision. The concerns about possible health problems resulting from chronic abuse of anthranoid laxatives prompted a number of studies aimed at investigating their laxative, genotoxic and potential carcinogenic effects. Some studies indicated that anthranoids could be carcinogenic in rodents. Although epidemiologic studies on cancer risk assessment in humans for exposure to anthranoids failed to confirm the carcinogenicity of these drugs so far, they are still considered as potential carcinogens based on the results of *in vitro* toxicity and *in vivo* animal tests (Al-Dakan *et al*., 1995). Results obtained from this study showed that *senna siamea* exhibit laxative property as demonstrated in the histological and histochemical assessment of opioid-induced constipation in the colon. This could be attributed to the presence of anthraquinone glycosides (especially sennosides A and B), saponins, glycosides, alkaloids like barakol, and tannins (Wiam *et al*., 2005). This is also in line with the study conducted by Stoll *et al*., 1950 which states that the laxative quality of *senna* is due to the presence of sennosides A and B in its leaves and pods, which were isolated in pure form. Chaichantipyuth, 1979; and Gritsanapan, 1983 also reported that the laxative effect comes from anthraquinone glycosides while the somnolent effect comes from barakol, which is a major chemical constituent of *S. siamea*. On a similar note, Ewe 1980, Wanitschke 1980 and Van Gorkom *et al*., 1999 reported that anthraquinones stimulate chloride secretion and inhibit sodium absorption, resulting in an accumulation of fluid and subsequent increased colonic motility. They further stated that the increased chloride secretion by anthranoid laxatives is due to disruption of epithelial tight junctions leading to increased permeability of the epithelium.

The *senna* sennosides have been widely used but the information relating to their mode of action remains scant. In the last decades, various possible mode of action of sennosides as laxative has been

explained including (a) stimulation of colon nerve plexuses thereby leading to defecation (Dobbs *et al*., 1975); (b) sennosides and their metabolites acting directly on large intestine motility (Garcia *et al*., 1980; Leng, 1986a); (c) changes in the colon motility and colonic fluid absorption (Leng, 1986b , 1989); and finally (d) involvement of prostaglandin E2 in secretagogue action of the sennosides in small intestine has been suggested (Nijs *et al*., 1991).

S. siamea relaxed the contractility of ileum in water bath (tyrode solution) while loperamide further reduced the amplitude of contractions induced by *Senna siamea* and vice versa. This demonstrates that the extract is an opiate (loperamide) inhibitor or opioid blocker and causes decreased contractions. It is likely that the extract exerts its action by specific interference at the receptor level and may not be due to the direct action on ileal smooth muscles.

Results obtained at the in vivo phase of this study demonstrated that oral administration of *Senna siamea* has lesser or no significant effect on the stool and body weight of experimental animals. However, *Senna siamea* exerted its laxative effect of the colon as observed in the nature and texture of animal stool. It was observed that the stool size of *Senna* treated animals appeared normal as compared to stool samples collected from the control. The stool was well-formed and softened which is attributable to the high moisture content and crude fibre contained in the plant as documented by David 2002 and Alli Smith 2009. Studies conducted by Rogers *et al*.,1978, Sogni *et al*., 1992 and Ewe *et al*., 1993 also suggested that *Senna* may influence intestinal transit time. This is to further buttress the fact that *Senna siamea* eased the evacuation of stool in opioid-induced constipation as seen in this study. However, animals treated with loperamide at 5mg/kg orally, craved for more water and their stool was observed to be very dried and reduced (an evidence that the animals were constipated) while some animals in the same group did not pass stool at all. The effect of bisacodyl (a stimulant laxative) on the colon was observed to be similar

to the effect exerted by *S.siamea* on the colon both morphologically and histologically (though *Senna siamea* proved to be a better stimulant as demonstrated in this study). This is due to their peristaltic inducing potential (their potential to induce peristalsis within the colon).

Histological analysis using H and E stain reveals many enlarged goblet cells around the crypt of Lieuberkuen in the constipated group compared to that seen in the control group. This is an indication that constipation was induced, and when also compared to treated groups (Group IV and V) it was evident that mucous secretion from goblet cells was reduced. Massive mucous secretion appeared in the histology of constipated group to protect the walls of the intestine from injury or pain when passing constipated stool.

Epithelial cells around the crypts are well-defined in control group, giving the gland a definite oval-shape. This is not so as seen in the constipated group, but a restored architecture was observed in the treated groups (extract treated and bisacodyl treated groups) and there was no evidence of toxicity in the tissue histology. On a similar note, a 2-year study by Mitchell *et al*., 2006 and Borrelli *et al*., 2006 showed that rats receiving *Senna* dosages of 25, 100, and 300 mg/kg/day did not show any changes in several assessments, including hematology measures, tissue histology, and mortality ratio, when compared with the control rats.

PAS was used to specifically stain neutral mucin. Tissues of animals treated with loperamide are PAS positive with massive mucoid secretions within the crypts. This is in sharp contrast to animals treated with extract and bisacodyl. The reason for this is that the extract was able to block the opiate-induced constipation by antagonising the effect of loperamide at the receptor level thereby easing or relieving the evacuation of stool. This in turn reduces the level of mucoid secretion since the stool being passed is soft and easy to evacuate

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

SUMMARY

- The laxative property of *S. siamea*, as evaluated in this study was well observed and appreciated both at the in vitro and in vivo phase. At the in vitro phase, the interaction between *S. siamea* and loperamide at different doses and concentration revealed a detailed picture of how *S. siamea* decreased the amplitude of contraction of the smooth muscle of the ileum and how loperamide further relaxed the smooth muscle of the ileum. On the other way round, the introduction of loperamide into the tyrode solution before *senna* extract described an interaction whereby the loperamide could not block the effect elicited by *S. siamea*, and this further buttress the fact that drug interaction between loperamide and the extract has no significant effect on the laxative property of *S.siamea*.
- Deductions can however be made that *S.siamea* did not act on a similar receptor with acetylcholine to elicit the relaxation of the smooth muscle of the ileum while loperamide acted on a similar receptor as atropine to elicit the relaxation observed in the study.
- At the in vivo phase, the morphometric study of the stool nature showed how loperamide at 3mg/kg constipated the experimental animals in group 2 after 6 days of administration and how *S.siamea* at 300mg/kg relieved constipation by loosening the stool after 7 days of administration. It improved evacuation of stool by reducing muscle tone and stool appeared smooth and well-formed
- Statistical analysis of the stool weight, body weight and organ-body weight ratio showed no significant difference between experimental groups. A standard curve was plotted

showing response (height of potentiation and relaxation of smooth muscle of the ileum) and the logarithm of organ-bath concentration.

6.1 CONCLUSION

This study demonstrated that *Senna siamea* is effective in preventing and treating opiate-induced constipation through suppressing goblet cell production of mucous and improvement in faecal evacuation. It also blocked the effect of loperamide on the isolated rabbit ileum.

6.2 RECOMMENDATION

Further studies should be conducted on the ligand interaction and the molecular pathway of *Senna siamea* interaction with loperamide within the colon

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