EFFECTS OF ORAL AND SUBCUTANEOUS ADMINISTRATION OF ACALYPHA WILKESIANA MACAFEEANA LEAF EXTRACT ON HISTOPATHOLOGICAL PATTERN OF SELECTED ORGANS OF MALE ALBINO RATS

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FIELD OF STUDY: HISTOPATHOLOGY

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF MEDICIAL LABORATORY SCIENCE. IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (MSC) HONOURS DEGREE IN MEDICAL LABORATORY SCIENCE

SUPERVISOR: DR. P.U.O. ACHUKWU

JULY, 2013.

DEDICATION

The research work is dedicated first to God almighty. To Cheche and Sasafi; and to my darling pretty Osarogie Joyce.

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Above all, I thank the almighty God, who made me all my way comfortable and do everything to me previously through and in the future throughout my life. To God alone be the glory and Jesus must be honoured in my life on daily basis.

ODIGIE, B. E.

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ABSTRACT

Acalypha wilkesiana belongs to the family of Euphobiaceae with common names as copperleaf and fire dragon. The juice of the leaf can be robbed on fungal skin infections and oral consumption has been reported to be antimycotic and antibacterial in action. Therefore, the general objective for this study was to investigate the histological effects of oral and subcutaneous administration of *Acalypha wilkesiana* leaf extract on organs of white rats. The specific objectives were to: (i) investigate possible histo-morphological alteration of selected organs of white rats exposed to *Acalypha wilkesiana* leaf extract, (ii) examine the integumentary system for possible deleterious effects and (iii) critically evaluate the histological indices of the

Liver and Kidney of the test animals. Sixty (60) albino male rats, with average mean weight of 199.34g were randomly selected into ten (10) groups of 6 rats per cage and was labeled as cages A_1, B_1, C_1, D_1 E_1 and A_2, B_2, C_2, D_2, E_2 respectively. They were housed in wire gauze cages with saw dust as beddings to acclimatize under standard condition of temperature (25 ± 5^{oC}) and a light/dark periodicity of 12:12hrs. Commercially purchased drinking water, standard Top feed(R) was given *ad libitum*. The plant was authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Edo State. Preparation and extraction of plant extract as well as the phytochemical analysis of the leaf was done in line with conventional protocols. Cages A_1, B_1, C_1 and D_1 were treated orally, cages A_2, B_2, C_2, and D_2 subcutaneously both in order of 200, 400, 600 and 800mg/kg body weight with regards to the $L.D_{50}$ Ó6, 344mg/kg (oral) and Ó5, 766mg/kg (subcutaneous). In addition, cages E_1 and E_2 serves as the untreated control group without extract administration. Empirical and physical measurements were carried out before and after commencement of extract administration. At the end of the experiment, all animals were sacrificed by cervical dislocation. Grossing was done, while cut tissues at 3-5mm were processed in an automatic tissue processor. Sections were obtained at 3-5microns using the hertz digital rotary microtome (Hestion ERM 4000 Germany). Furthermore, staining of the sections was according to haematoxylin and eosin (H&E) method and the Periodic Acid Schiff (PAS) for specific organs. Sections were examined using Swift(R) Binocular microscope with an in built lighting system. Data was presented in Means ± SD and analyzed using one way ANOVA and Duncan post hoc test. Animal acclimatization, test administration, grossing, tissue processing, microtomy, staining and microscopic examination of histology sections lasted for 6 months. The results showed that all treated animals showed varying degree of weight loss which was marked in the animals treated subcutaneously (C_2 and D_2 respectively). Difference in weight of the test animals were statistically significant ($p < 0.05$). Phytochemical analysis revealed presence of alkaloids, terpenes, tannins, saponins, flavonoids, anthraquinones and cardiac glycosides. Grossly, all

organs both test and control showed no variation in colour, size and consistency. Grossing revealed a stable architecture while histological findings were normal. There was no evidence of necrosis and no histo-morphological changing pattern. Therefore, this study showed that oral and subcutaneous administration of *Acalypha wilkesiana* boiled decoction in rats may have effect on body weight without deleterious / cellular degeneration on human organs in a similar circumstances.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Medicinal Plants

Acalypha wilkesiana belongs to the family of *Euphobiaceae,* with common names as copperleaf, Josephôs coat, fire dragon, and match-me-if-you-can (Oladunmoye, 2006). This *Species* is named after Admiral Charles Wilkes who explored the South Pacific in the 1800s. In St. Louis, It is typically best grown as annual bedding plants or in containers which can be overwintered indoors, where it grows in a single growing season (Stephen *et al.,* 2009). In the ground, it grows in moist. It is average to moderately fertile, well-drained soils in full sun to part shade with best foliage colour in full sun (Iniaghe *et al.,* 2009). Soils must be kept consistently moist. If soils dry out, rapid leaf drop usually occurs. Stems may be pinched to control size and shape and to promote bushiness. Take tip cuttings in late summer to overwinter. Container plants may also be overwintered as house plants as long as they receive intense bright light and soils are kept uniformly moist. As the common name suggests, this shrub is grown for its attractive foliage with shinning features. Its major problems are White fly, mealy bugs, spider mites and various caterpillars are occasional visitors. The use of the plant, plant extract or plant-derived chemicals to treat tropical, subcutaneous and systemic diseases, has stood the test of time (Oladunmoye, 2006).

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported to be safe and without adverse side effect especially when compared with the synthetic drugs use (Iniaghe *et al.,* 2009). In Africa, particularly in the west African sub-region, new drugs are often beyond the reach of the vast majority that are poor such that up to 85% of the population now turn to medicinal plants as remedy against diseases including infections (Hostettmann and Maston, 2002).

Traditional believes have likely played some roles in that, the poor who are unable to afford the high cost of synthetic drugs also turn to their villages to consult with their aged parents who have some knowledge about roots and herbs as alternative measures for the treatment of some ailment, believing that the use of roots and herbs has being dated back to their fore fathers in the time past and it doesnôt by any means fail them (Madziga *et al.,* 2010). Hence, when people from these remote communities get an infectious disease, they are usually treated by traditional healers because of their expertise in such procedures as making traditional / native way of diagnoses, treating wounds, setting bones and making herbal medicines (Madziga *et al.,* 2010). Traditional healers claim that their medicine is cheaper and more effective than modern medicine. Patients of these communities have a reduced risk to get infectious diseases from resistant pathogens than people from urban areas treated with traditional antibiotics (Madziga *et al.,* 2010).

However, such medicinal plants (such as Acalypha wilkesiana) are found all-over the world especially in the tropics of Africa, America and Asia. The weeds are wild and can be found growing everywhere, while the ornamental species must have been introduced into West Africa from other parts of the world and are cultivated as foliage plants in gardens and greenhouses (Madziga *et al.,* 2010). Some of the species are well known in traditional medicine and a few have actually appeared in the homeopathic pharmacopoeia of United States (1941) and India (1971). *Acalypha wilkesiana* was reported to be used in the treatment of hypertension, especially in Managing the abnormal sodium and potassium metabolism that accompany hypertension (Ikewuchi *et al.,* 2009).

Medicinal plants have enjoyed a great popularity in the treatment of various diseases for many centuries. They are plants which are used for therapeutic purposes. The discovery of these useful plants was as a result of manôs inquisitive and inventive nature as well as necessity to feed

(Sofowora, 1992). They include all higher plants that have been alleged to have medicinal properties, that is, effects that relates to health or which have been proven to be useful as drugs by western standards or which contain constituents that are used as drugs (Akinniyi *et al.,* 1996). The active principles of medicinal plants are able to alleviate illnesses (Akinniyi *et al.,* 1998). Based on their physiological and pharmacological actions and uses, medicinal plants are classified as; CNS active plants, anti-inflammatory agents, anti-allergic plants, anti-diabetic plants, cyto protective plants, antioxidants as well as antibiotics. A medicinal plant may have multidimensional effects falling into more than one of the classes mentioned above (Dahanukar, 2000).

During the last two decades, there has been a considerable increase in the study and use of medicinal plants all over the world especially in advanced countries. There was also increase in the international commerce and commercial exploitation of herbal medicines through over the counter labeled products. In some countries, herbal medicines are still a central part of the medical system e.g. China (Liu, 1987), Ethiopia (Desta, 1993), Argentina (Anesini and Perez, 1993) and Papua New Guinea (Nick *et al*., 1995). The renewed interest in the use of medicinal plants may be attributed to cheapness, availability, and accessibility by the local populace, high incidence of side effects of synthetic medicines and environmental friendliness of plant extracts. Medicinal plants also serve as the starting point for the discovery of semi synthetic chemical compounds. The chemical structures derived from plant substances can also be used as models for new synthetic compounds (Sofowora, 1989; Akerele, 1992). Medicinal plants have been used in Africa before the introduction of antibiotics and other modern drugs (Kabir *et al*., 2005). About 80% of the populations in developing countries still use traditional medicine for their healthcare. Modern pharmacopoeias contain at least 25% of drugs derived from plants and many others which are synthetic analogues are built on prototype compounds isolated from plants (De Silva, 2005). Nature has been a source of medicinal agents for thousands of years and an

impressive number of modern drugs have been isolated from natural sources. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Bobbarala *et al.*, 2009). Plants are the sources of natural pesticides that make excellent leads for new pesticide development (Bobbarala *et al.*, 2009). The leaves of *Acalypha wilkesiana* are popularly used in the north eastern Africa in the treatment of skin infections.

The juice of the leaf can also be robbed on fungal skin infections and oral consumption of boiled decoction has also been reported to be antimycotic and antibacterial in action (Oliver-Bever, 1986). Leaf poultice is used for headache, swelling, cold and wound dressing (Iwu, 1993). Chopped pieces of the dried stem and root were steeped in alcohol and used for stomach ache and as worm expellant in man in the Delta region of Nigeria (Iwu, 1993; Burkill, 1985). In traditional medicine, the leaves of this plant have diuretic effects and are eaten as vegetables in the management of hypertensions. The aqueous extract is also used in the management of fever in infants as well as abnormal sodium and potassium metabolism that accompanies hypertensions (Oladunmoye *et al.,* 2006). This plant has been reported to specifically have anti parasitic properties as the expressed juice or boiled decoction is locally used within Nigeria and some other parts of West Africa for the treatment of malaria parasite infection, dermatological and gastrointestinal infections (Akinde and Odeyemi, 1987). The reputed efficacies of these plants have been experienced and passed on from one generation to the other (Madziga *et al.,* 2010).

However, there is paucity of information on the effect of oral consumption and subcutaneous administration of *Acalypha wilkesiana* leaf extract using boiled decoction from this cultivar, hence the need to investigate the possible histo-morphological alterations of selected organs of white rats under the use of *Acalypha Wilkesiana* leaves obtained from Benin City, Edo State,

Nigeria. Apparently, lack of scientific proof of efficacies claimed by traditional herbal practitioners in Nigeria called for this study.

1.1 Aim and objectives

The general objective was to investigate the histological effects of oral and subcutaneous administration of *Acalypha wilkesiana* leaf extract on organs of albino rats. The specific objectives were as follows: (i) to investigate the possible histo-morphological alteration of selected organs of white rats including the gastrointestinal tract exposed to *Acalypha wilkesiana* leaf extract, (ii) examine the integumentary system for possible deleterious effects and (iii) critically examine the histological indices of the liver and kidney of the test animals.

CHAPTER TWO

2.0 LITERATURE REVIEW

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Doughari *et al.,* 2008). The plant *Acalypha wilkesiana* is widely used in various classical and herbal Formulations worldwide. Apart from its diverse uses, it has been reported to be antimycotic and antibacterial in action (Oliver-Bever, 1986). *Acalypha wilkesiana* has been shown to have anti-bacterial and anti-fungal properties. The pressed juice or boiled decoction is used for the treatment of gastrointestinal disorders and fungal skin infections. In traditional medicine, the leaves of this plant are eaten as vegetables in the management of hypertensions (Ogundaini, 2006; Oladunmoye, 2006; Adesina *et al.,* 2000). The aqueous extract is also used in the management of fever in infants and abnormal sodium and potassium metabolism that accompanies hypertensions (Oladunmoye, 2006). The juice of the leaf is smeared on fungal skin infections and decoction of leaves when drunk is reported to be antimycotic and antibacterial in action (Oliver-Bever, 1986). Leaf poultice is used for headache, swelling, cold and wound dressing. Chopped pieces of the dried stem and root were steeped in alcohol and used for stomach ache and as worm expellant in man in the Delta region of Nigeria (Iwu, 1993; Burkill, 1985).

The effect of *Acalypha wilkesiana* leaves on the blood pressure and aortic contractile and relaxation responses of salt-loaded rats was also investigated (Ikewuchi *et al.,* 2009). The pressure necessary to enable the blood to circulate is provided by the pumping action of the heart [cardiac output (CO)] and the tone of the arteries [peripheral resistance, (PR)]. The contraction of the heart propels blood through the arterial tree. However, it is the dynamic regulation of arterial diameter, especially in the smaller branches of the network, which controls

blood pressure (BP) and flow in the periphery (Huang *et al*., 2005; Zhang *et al*., 2005; Blaustein *et al.*, 2006). Blood flow in small arteries is governed by Poiseuille's law (Berne and Levy 2001; Blaustein *et al*., 2006) which states that the resistance to flow (R) is inversely proportional to the fourth power of the internal radius, (i.e., R Ŭ1/r4). Small increases in myogenic tone (decreases in r) should, therefore, have a profound effect on R (or total peripheral R). There is a direct relationship between myogenic tone in isolated arteries and BP in intact animals (Blaustein *et al*., 2006). The maximal contractile response of the aorta from the treated animals was significantly ($p<0.05$) higher than both the test-control and control, while their maximal relaxation response was significantly ($p<0.05$) lower. The result suggests that the mechanism of action of the leaves may be alteration of the contractile responses of the aortas; and also supports the use of the leaves in the management of hypertension (Ikewuchi *et al.,* 2009).

Owoyele *et al.,* (2011) carried out a study to determine whether the ethanol extract of *Acalypha wilkesiana* has analgesic, anti-inflammatory and antipyretic as well as anti-ulcer effects. The hot plate latency assay and formalin- induced paw licking models were used to evaluate analgesic effects. Animals were divided into groups comprising of five rats each. There were control (administered saline) and reference (administered indomethacin) groups. There were three extract groups administered 25, 50 or 100 mg/Kg body weight of extracts. Ulcer was induced using absolute ethanol followed by pylorus ligation in all animals; inflammation was induced using carrageenan while pyrexia was induced by injecting brewerôs yeast intramuscularly into the dorsal part of the abdominal cavities of the rats. Different sets of rats were used for the anti-ulcer, anti-inflammatory and antipyretic studies although animal grouping for extract administration were as in analgesic studies. The results showed that the extract produced dose-dependent and significant ($p<0.05$) analgesic and anti-inflammatory activities. The extract also significantly protected against ethanol induced ulcer. Likewise, the extract significantly ($p<0.05$) reduced the pyretic states of the animals. Hence, the study further provides evidences that may

support the ethnomedicinal uses of the ethanolic extracts of *Acalypha wilkesiana* leaves. The study fully established the analgesic, anti-inflammatory, antipyretic and anti-ulcer effect of *A. wilkesiana* in laboratory animal and thus justifies the local uses of the plant for the treatment of these conditions in humans. The antipyretic study showed that the extract of *A. wilkesiana* produced significant inhibition of brewerôs yeast induced pyrexia. Brewerôs yeast is commonly used to induce pyrexia because the yeast serves as a foreign organism within the biological system of the animals and this has pyrogenic effect which ultimately increases the temperature of the body (Adesokan, *et al*, 2008).

Nonetheless, the results of the study indicate that the extract has the potential to inhibit the elevated body temperature induced by pyrogens within biological system. Results obtained from the anti-ulcer study showed that the extract of *A. wilkesiana* has the ability to inhibit ulcers induced by oral administration of ethanol. Similarly, in 2011; the effect of aqueous extract of the leaf of *Acalypha wilkesiana* on the hematology, plasma biochemistry and ocular indices of oxidative stress was investigated in alloxan induced diabetic rats. Compared to test control, the treatment lowered (significantly, $p < 0.05$) plasma glucose, triglyceride, conjugated bilirubin levels, atherogenic index of plasma, plasma alanine transaminase activity, total protein and total bilirubin, aspartate transaminase activity and unconjugated bilirubin, plasma urea, blood urea nitrogen and ocular malondialdehyde contents, lymphocyte and monocyte counts, and not significantly, plasma very low density lipoprotein cholesterol, but increased (significantly, $p < 0.05$) plasma calcium contents, total white cell and platelet counts, mean cell volume and ocular ascorbic acid content, and (though not significant) plasma high density lipoprotein cholesterol level, red cell and neutrophil counts. The hypoglycemic nature positively affected the hemopoietic system and integrity and function (dose dependent) of the liver and kidney of the diabetic rats; but improved the lipid profile and had no deleterious effect on red cell morphology and also protected against oxidative stress in ocular tissues. The study also revealed the presence

of pharmacologically active compounds in the leaf extract. All of these highlighted the cardioprotective potential of the leaf of *Acalypha wilkesiana* and supported its use in traditional health practices for the management of diabetes mellitus (Ikewuchi *et al.,* 2011).

Alli *et al.,* (2011) studied the effect of aqueous extract (10%w/v) of the leaf of *Acalypha wilkesiana* in the liver and kidney of albino rats showing the activities of Alkaline Phosphatase (ALP), Alanine Transaminase (ALT) and Aspartate Transaminase (AST). The effect of the extract on glucose and protein concentrations was also studied. The administration of the aqueous extract resulted in a significant reduction in the enzymes activities ($p<0.05$) in the liver and kidney which was complimented by an increased activity of these enzymes in the serum. However, the result obtained showed that prolonged usage or overdose of the aqueous extract of *Acalypha wilkesiana* could exhibit a dose dependent toxicity. Interestingly, the aqueous extract of copper leaf causes reduction of blood glucose level in normoglycemic rats suggesting the possibility of its usage in managing diabetes or hyperglycemia. It was also discovered that the aqueous extract exhibit transient hypoglycemic effect. Hence, repeated administration of the aqueous extract of copper leaf or its prolonged usage can lead to the damage of the two vital organs, which is the liver and kidney. But there were paucity of information on the possible histo-morphological alteration and or deleterious effect on the vital organs.

In Nigeria, *Acalypha Wilkesiana* plant has been documented as a large, fast growing and ever-green shrubs which provides a continuous splash of colours in the landscape with the bronze red to muted red. The plant is about 4 to 8 inch long, heart-shaped leaves, available in varying mottled combinations of green, purple, yellow, orange, pink, or white; depending on the cultivar (Gilman, 1999). Although, there are quite a reasonable number of cultivars worldwide; the macrophylla, hoffamanu, godseffiana, Macafeeana, hispida, marginata and racemosa are peculiar cultivars within Nigeria (Oladunmoye, 2006; Yushaôu *et al*. 2008; Iniaghe *et al*. 2009).

Plate 1: *Acalypha wilkesiana* leaf investigated X5 Optic-shot (Source: UBTH horticultural garden)

CHAPTER THREE

3.0 MATERIALS AND METHODS

Standard histological methods and materials were used with improved modifications in histochemical techniques (Avwioro, 2010; Bancroft and Marilyn, 2002; Baker *et al.,* 2001).

3.1 Animal care ethics.

The animal study was carried out in compliance with policies outlined in the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). The rats were obtained and housed in wire gauze cages with saw dust as beddings to acclimatize, in the animal house of the Department of Medical Biochemistry, University of Nigeria, Enugu Campus for 2 weeks, under standard condition of temperature (25 ± 5ºC) and a light/dark periodicity of 12:12 hrs. Enough food (Standard Top Feed® and commercially purchased UNIBEN table water with NAFDAC Reg. no 01-4597 was provided and given ad libitum.

3.2 Experimental animals.

For this study, sixty (60) in-bred male wistar rats (*Rattus norvergicus*) of about 2-3months old, weighing between 180 - 200g body weight were randomly selected into ten (10) groups of 6 rats per cage and was labeled as cages A_1, B_1, C_1, D_1 E_1 and A_2, B_2, C_2, D_2, E_2.

Plate 2: Animal grouping with the required dose regimen, Optic-shot X5 (Source: UNTH Animal House)

3.2.1 Empirical measurement.

The method described by (Ajiboso *et al*., 2007) was used to determine body weight of experimental rats. Each of the rats was monitored for daily gain in body weight using digital electronic balance (Gilbertini, Italy). Gain in weight was calculated from the relationship given below: Daily gain in weight= Final day Weight ï Initial day Weight, while the average mean weight of 199.34g was noted.

3.2.2 Physical measurement.

Behavioral signs of acute toxicity were observed in experimental rats and were noted; such as: dullness and reduced behavioural activities in the first few hours of the dose administered orally.

Plate 3: Showed dullness and reduced activity of high dose treated animals (600 and 800mg/kg b.w.) especially in subcutaneous administration (C_2 and D_2) Optic-shot X5 (Source: UNTH Animal house).

3.3 Acute toxicity test ($L.D_{50}$)

Acute toxicity of *Acalypha wilkesiana* crude leaf extract was obtained using modified Lorkeôs method (1983) presented in (Madara *et al.,* 2010) and was further modified in the present study. The study was carried out in two phases.

Phase 1: Sixteen (16) rats were randomly selected into four groups (K, L, M and N) of 4 rats per cage and were administered 500, 1500, 2500 and 3500mg/kg body weight of the decoction orally. The rats were observed for signs of toxicity which include but were not limited to paw licking, salivation, stretching, weakness, reduced activities, dullness, sleep, respiratory distress, coma and death in the first four (4hr) and subsequently daily for 7days.

Phase II: Fresh set of Sixteen (16) rats were again randomly selected into four groups (O, P, Q and R) of 4 rats each per cage and were administered 5500, 7500, 9500 and 11500mg/kg body weight of the extract orally, based on the result from the first phase. They were observed for signs of toxicity and mortality for the first critical four (4hr) and thereafter daily for 7 days. The oral median lethal dose was calculated using the formula: $L.D_{50}$ = ã $\frac{\text{Minimum toxic dose}}{\text{Maximum tolerated dose}}$ (Madara *et al.,* 2010). Fresh batch of animals were acquired and the procedure was repeated using subcutaneous route of administration.

Table 3.1: Determination of dose-mortality relationship of *Acalypha wilkesiana* leaf extract in male wistar rats (Oral administration).

GROUP	NO. OF RATS	DOSE (mg/kg)	RESULTS	NO DIED/ NO USED	% MORTALITY
Phase I					
K	4	500	-	0/4	0
L	4	1,500	-	0/4	0
M	4	2,500	±	0/4	0
N	4	3,500 Minimum	+	0/4	0
Phase II					
O	4	5,500	++	0/4	0
P	4	7,500	+++	0/4	0
Q	4	9,000	++++	0/4	0
R	4	11,500 Maximum	+++++	0/4	0

Note: L.D_{50} was calculated based on ã Minimum toxic dose x Maximum tolerated dose

Where 3, 500 mg = Minimum toxic dose

11, 500mg = Maximum tolerated dose

ã3500 x 11500 = 6,344

Therefore, L.D_{50} = Ó6,344mg/kg b.w. (oral)

Table 3.2: Determination of dose-mortality relationship of *Acalypha wilkesiana* leaf extract in male wistar rats (Subcutaneous administration).

GROUPS	NO. OF RATS	DOSE (mg/kg)	RESULTS	NO DIED/ NO USED	% MORTALITY
K	4	500	-	0/4	0
L	4	1,500	±	0/4	0
M	4	2,500	±	0/4	0
N	4	3,500 $_{Minimum}$	+	0/4	0
Phase II					
O	4	5,500	++	0/4	0
P	4	7,500	++++	0/4	0
Q	4	(9,500) $_{Maximum}$	+++++	0/4	0
R	4	11,500	+++++	0/4	0

L.D_{50} = ã3500 x 9500 = 5,766mg/kg b.w. (subcutaneous).

Acute gross behavioral signs of toxicity for evaluation in this study include the following: paw licking, salivation, stretching, weakness, reduced activities, dullness, prolong sleep, respiratory distress, coma and death.

Key: Mode of Scoring

±	Ÿ	One of the sign of acute behavioral toxicity
+	Ÿ	Two of the signs of acute behavioral toxicity
++	Ÿ	Three of the signs of acute behavioral toxicity
+++	Ÿ	Four of the signs of acute behavioral toxicity
++++	Ÿ	Five of the signs of acute behavioral toxicity
+++++	Ÿ	More than six of the signs of acute behavioral toxicity
~~Ź~~	Ÿ	All signs of acute behavioral toxicity listed above
-	Ÿ	Absence / none of the signs of acute behavioral toxicity
b.w.	Ÿ	body weight

3.4 Collection, Identification and Authentication of plant

Sample of the fresh leaf of *A. wilkesiana* (Plate 1) was collected from University of Benin Teaching Hospital (UBTH), horticultural garden, Benin City, Nigeria. It was identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. Thereafter, a sample plant was deposited at the Department of Plant Biology and Biotechnology; University of Benin (Herbarium), Benin City, Nigeria and a voucher number was assigned.

3.5 Preparation and Extraction of Plant Decoction.

The leaves of *Acalypha wilkesiana* was collected and thoroughly washed with tap water and allowed to dry under normal atmospheric condition for a period of one week. The dried leaves were pulverized into uniform powder using a house hold blender and were sieved to obtain a pure fine powdered particle (Plate 4). Two hundred grams of the pulverized sieved, fine powdered particle was measured using a weighing balance. One litre (1L) of commercially purchased distilled de-ionized water was measured and added with a measuring cylinder. Heat was applied for 10minutes at 95^0C in a hot air oven. The heated, boiled mixture was transferred to the GFL shaker (No 3017 MBH, Germany) while the mixture was mechanically agitated for 8hrs. It was left on the bench for 12hrs before filtration under suction pressure with a Whatmanôs filter paper. Thereafter, filtrate was concentrated under reduced pressure in a rotary evaporator (Buchi, Switzerland) and was lyophilized using (Christ Alpha 1-2 LD, Germany) and stored at 4°C. The process was repeated to obtain a total extract yield of about 21.2g. For experimental use, 2g was dissolved in 20mls of commercially purchased distilled and deionized water to give a concentration of 100mg/ml. To ensure that the active principles are preserved, fresh preparation were made from the refrigerated stock every-other day.

Plate 4: Freshly pulverized, sieved; fine powdered form of *Acalypha wilkesiana* particles

Optic-shot-X5

3.6 Design and Conduct of experiment.

Cages A_1, B_1, C_1 and D_1 were treated with *Acalypha wilkesiana* boiled decoction orally and cages A_2, B_2, C_2, and D_2 were treated subcutaneously, both in order of 200, 400, 600 and 800mg/kg body weight respectively with regards to the $L.D_{50}$ Ó6, 344mg/kg bw (oral) and Ó5, 766mg/kg b.w. (subcutaneous) of the decoction while Cages E_1 and E_2 served as the control groups without experimental dose. $L.D_{50}$ was calculated based on ã $\overline{\text{Minimum toxic dose x Maximum tolerated dose}}$ i.e. ã$\overline{3500 \text{ x } 11500}$ = 6,344mg/kg body weight (Oral) and ã$\overline{3500 \text{ x } 9500}$ = 5,766mg/kg body weight (subcutaneous). Note; the animals were weighed before and after administering the decoction orally for group caged A_1, B_1, C_1, D_1 and subcutaneously for A_2, B_2, C_2, D_2, while the average mean weight of 199.34g was noted. The decoction was administered using both routes of administration as specified above for 31days at the intervals of 2days. On day 32, all animals were sacrificed by cervical dislocation; organs of interest were excised, studied and preserved in 10% neutral buffered formalin for tissue processing.

3.7 Administration of extract

Two hundred (200) grams of the extract obtained from copper leaf was dissolved in 1000ml of distilled-deionized water and was administered orally to the rats at a dose of 200, 400, 600 and 800mg/kg body weight in rats. Rats in cage A_1 were given 1ml (200mg/kg) orally while rats in cage A_2 received the same dose subcutaneously. Rats in Cage B_1 received 2ml (400mg/kg) orally while B_2 received the same dose subcutaneously, Rats in cage C_1 received 3ml (600mg/kg) orally and rats in cage C_2 were administered the same dose subcutaneously. D_1 and D_2 received 4ml (800mg/kg body weight) respectively using oral for D_1 and subcutaneous route for D_2. Mathematically: Two hundred (200) g of the extract from *Acalypha wilkesiana* powered leaf is contained in 1000ml of distilled-deionized water used. Therefore, 1ml of the decoction contains 200mg/1000ml and so on from the first principle.

3.8 Phytochemical screening.

The powdered leaf of *Acalypha wilkesiana* plant was examined for the presence of the following phytochemicals: alkaloids, tannins, saponin, steroid, terpenes, flavonoids, phlobatannin, anthraquinones and cardiac glycosides using conventional protocols as established (Borokini and Omotayo, 2012; Anokwuru *et al.,* 2011 and Owoyele *et. al.,* 2011).

3.8.1 Alkaloids

0.5 g of extract was stirred with 3 mL of 1% aqueous hydrochloric acid on a steam bath and filtered; 1 mL of the filtrate was treated with few drops of Picric acid solution. Colour changes / precipitate were taken as preliminary evidence to draw inference (Owoyele *et. al.,* 2011).

3.8.2 Saponin

Using a sterile test tube, 30ml of tap water was added to 1 g of the powdered leaf sample. The mixture was vigorously shaken and heated concurrently. The heated mixture was observed for the formation of froth / foams which was taken as preliminary evidence to draw inference (Owoyele *et. al.,* 2011).

3.8.3 Phlobatannin

0.2 g of the powdered leaf sample was dissolved in 10 ml of distilled water and filtered. The filterate was boiled with 2% HCl solution. The sample was observed for the formation and colour of precipitate to draw inference (Owoyele *et. al.,* 2011).

3.8.4 Tannins

0.5 g of the powdered leaf sample was dissolved in 5 ml of distilled water, followed by gentle boiling and was allowed to cool to a considerable temperature. 1 ml of this solution was

dispensed into a sterile test tube while 3 drops of ferric chloride solution was added immediately. The colour of the sample was observed to draw inference (Anokwuru *et al.,* 2011).

3.8.5 Terpenes/ terpenoids

The Salkowski test was used to check out for terpenes by making use of 5 ml of the powdered leaf sample, which was added to 2 ml of chloroform and was mixed evenly, followed by the addition of 3 ml concentrated sulphuric acid (H_2SO_4), which was carefully added to form a layer. Colour changes were observed to draw inference (Anokwuru *et al.,* 2011).

3.8.6 Steroids

2 ml of acetic anhydride was added to 0.5 g powdered leaf of each plant sample, followed by 2 ml of sulphuric acid. Colour changes were observed to draw inference (Borokini and Omotayo 2012).

3.8.7 Cardiac glycosides

5 ml of the powdered leaf sample of the plant studied was treated with 2 ml of glacial acetic acid, containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. Colour changes were observed to draw inference (Keller-Killani test) (Anokwuru *et al.,* 2011).

3.8.8 Flavonoids

5 ml of diluted ammonia solution was added to a portion of the aqueous filtrate of the plant extract, followed by addition of concentrated sulphuric acid. Colour changes were observed to draw inference (Borokini and Omotayo 2012).

3.8.9 Anthraquinones

5 mL of chloroform was added to 0.5 g of extract in a dry sterile test tube and was shaken for 5 minutes. It was filtered and the filtrate shaken with equal volume of 100% ammonia solution. Pink to violet colouration in the ammoniacal layer (lower layer) was observed and was used to draw inference being indicative of the presence of free anthraquinones (Anokwuru *et al.,* 2011).

The phytochemicals present in the samples from the leaves of *Acalypha wilkesiana* were juxtaposed with their ethno-medicinal significance and from these; several suggestions were deduced on the secondary metabolites responsible for the pharmacological actions of the plant (Borokini and Omotayo 2012).

Table 3.3: Qualitative phytochemical analysis of *Acalypha wilkesiana* leaf extract

S/N	Secondary Metabolites	Positive Indicators	Present / Absent
01	Alkaloids	Orange precipitate	Present
02	Tannins	Greenish-black colouration	Present
03	Saponins	Presence of froths/foams	Present
04	Phlobatannins	Red precipitates	Absent
05	Terpenes	Reddish-brown coloration of the - interface	Present
06	Flavonoids	Yellow precipitate	Present
07	Cardiac glycosides	Brown interface, violet ring below and greenish ring at lowest part.	Present
08	Steroids	Blue colouration	Absent
09	Anthraquinones	Pink, violet or red coloration at the lower layer (ammoniacal layer)	Present

3.9 Methodology

Table 3.4: Oral and subcutaneous administration of *Acalypha wilkesiana*_crude leave extract (boiled decoction) into 60 albino Wistar rats.

Cages	Number of rats	Average Weight	Dosage mg/kg Body Weight	Route of Administration	No of Days
A_1	6	196.40	200	Oral	31 Days
B_1	6	198.88	400	Oral	31 Days
C_1	6	200.58	600	Oral	31Days
D_1	6	201.60	800	Oral	31 Days
E_1	6	198.48	-	Oral	31 Days
A_2	6	198.26	200	Subcutaneous	31 Days
B_2	6	198.80	400	Subcutaneous	31 Days
C_2	6	199.65	600	Subcutaneous	31 Days
D_2	6	200.28	800	Subcutaneous	31 Days
E_2	6	200.44	-	Subcutaneous	31 Days

Average weight of rats =199.34g

Each of the animals in cages A_1 to D_1 and A_2 to D_2 were picked one at a time with a hand towel and appropriate volumes of the decoction in ml was administered using a sterile 5ml syringe through oral and subcutaneous route of administration.

3.9.1 Processing of histology samples.

For each of the organs studied, the tissues were fixed for 24 hours with 10% neutral buffered formalin and were cut during grossing at 3-5mm. Thereafter, the tissues were processed in an automatic tissue processor for dehydration, clearing, and impregnation using molten paraffin wax, while embedding was done with the aid of the embedding machine. Sections of the tissues were obtained at 3-5microns using the digital (hertz) rotary microtome (German mode) to produce serial ribbons. Staining of the sections was according to Haematoxylin and Eosin (H&E) staining method and the Periodic Acid Schiff (PAS) as described (Avwioro, 2010; Bancroft and Marilyn, 2002; Baker *et al.,* 2001).

3.9.2 Protocol for Cole's and Mayer's Haematoxylin and Eosin (H&E) method

- Dewax and hydrate section in water
- Stain section in Coleôs Hematoxylin (10mins) or Mayerôs Haematoxylin (5mins).
- Wash section thoroughly in running tap water.
- Differentiate section in 1% acid alcohol briefly
- Blue in Scotôs water for 5minutes
- Counter stain section in eosin for 3minutes
- Wash section in running tap water until excess eosin has been removed.
- Dehydrate section in ascending grades of alcohol (70%, 90% and absolute)
- Clear section in xylene, mount in DPX. (Avwioro, 2002; Baker *et al.,* 2001)

3.9.3 Protocol (SOP) for Periodic Acid Schiff (PAS)

- Dewax and hydrate section in water
- Oxidize section in 1% periodic acid for 5 minutes
- Wash section thoroughly in running tap water.
- Apply Schiff reagent for 10 minutes
- Wash in tap water
- Counter stain nuclei with Mayerôs haematoxylin for 5minutes
- Rinse section in water
- Differentiate in 1% acid-alcohol for 5 seconds
- Blue in Scott water for 5 minutes or running tap water for 10 minutes
- Dehydrate section in ascending grades of alcohol (70%, 90% and absolute)
- Clear section in xylene, mount in DPX. (Avwioro, 2010)

3.10 Reagents and chemicals

Scottôs water, distilled water, haematoxylin dye, eosin dye, 1% acid-alcohol, 1% Periodic acid, Schiff reagent, xylene, ethanol, distrene plasticizer, 10% neutral buffered formalin and normal saline. (All reagents were distilled prior to use).

3.11 Equipment and Apparatus

Analytical weighing balance, stainless steel cages, dissecting materials: Dissecting Board, Dissecting Set, Cotton Wool. Glassware (Pyrex): Measuring Cylinder, Conical Flask, Cover slip, Slides, Universal Containers, 5ml syringes. Tissue Processing Materials: Automatic Tissue Processor (Hestion -ATP7000 tissue processor-Germany), Embedding Machine (Hesition- E500 Germany), Leuckhart molds, Digital rotary microtome (Hestion ERM 4000 Germany), Water

Bath (Gallenkamp), Hot plate, Staining rack, Forceps and Swift(R) Binocular Microscope (Olympus England). GFL shaker (No 3017 MBH, Germany)

3.12 Location and Duration of Study.

This study was conducted in the Department of Medical Laboratory Science, Histopathology Sub-Departmental Laboratory, Faculty of Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus. Collection and extraction of plant material, animal acclimatization, determination of $L.D_{50}$, test administration, animal sacrifice, gross examination, fixation, processing, microtomy, staining, microscopic examination of histological sections and photomicrography lasted for 6 months (October 2012 to April 2013).

3.13 Microscopy and Photomicrography

The sections were examined using Swift(R) Binocular Microscope with an in built lighting system and white films with an Olympus photomicroscope® (Opticshot- 2; Nikon, Tokyo, Japan) at x 400magnification.

3.14 Statistical Analysis.

Data were analyzed using one way analysis of variance and Duncan post hoc test. Values were presented in means and standard error of the mean. Probability values less than 0.05 was considered statistically significant.by the use of Statistical package for social sciences (SPSS) version 16.0 (Inc Chicago, Illinois, USA).

CHAPTER FOUR.

4.0. RESULTS.

Physically, all high dose treated animals (C_1, D_1 and C_2, D_2) representing 600 and 800mg/kg of rat both oral and subcutaneous administration showed signs of dullness and reduced activities especially animals from the subcutaneous route of administration (Plate 3). Phytochemical screening of the leaf of A*calypha wilkesiana* was discovered to contain important secondary metabolites; such as alkaloids, terpenes, tannins, saponins, flavonoids, anthraquinones and cardiac glycosides. Meanwhile, steroids and Phlobatannins were not present (Table 3.3). These secondary metabolites exhibit various biological activities such as anti-oxidative, analgesic, antipyretic, anti-microbial, anthelmintic, antimycotic, antibacterial actions, antimalarial actions, anti-inflammatory and diuretic activities which have been associated with their intrinsic reducing capability as pro-oxidants. The presence of these secondary metabolites especially alkaloids, tannins and flavonoids justifies the use of *Acalypha wilkesiana* in ethno medicine. Empirical measurement showed that low dose (200 and 400mg/kg) treated rats both (oral and subcutaneous) had slight weight loss as compared to the severe weight loss observed in the high dose (600 and 800mg/kg) treated rats (Table 4.1).

Grossly, all organs both test and control showed no variation in colour, size and consistency. The mean weight of the right kidney in this study was 1.1g, while the left kidney was 0.95g. The mean length, width and thickness of the right kidney were 1.24cm, 0.86 and 0.82 cm respectively while those of the left were 1.21 cm, 0.84 and 0.80 cm. When compared with the control the mean weight of the right kidneys was 1.2, the left 0.95g and the mean length of right kidney of the rat was 1.25 cm and the left was 1.22 cm (Oral administration). For the subcutaneous route of administration, the mean weight of the right kidney was 1.0gm, the left one was 0.92 gm. The mean length, width and thickness of the right kidney were 1.22cm, 0.82

and 0.78 cm respectively while those of the left were 1.21 cm, 0.82 and 0.81 cm. When compared with the control the mean weight of the right kidneys was 1.2, the left 0.93 g and the mean length of right kidney of the rat was 1.15 cm and the left was 1.22 cm (Subcutaneous administration).

Furthermore, the mean weight of the liver of rats on oral administration of *Acalypha wilkesiana* decoction (Groups A_1, B_1, C_1 and D_1) was 0.44g when compared to the control (group E_1) which was 0.46g and the mean weight of the liver of rats on subcutaneous administration (Groups A_2, B_2, C_2 and D_2) was 0.47g when compared to the control (group E_2) which was 0.48g. The cut surface of the liver was smooth and shiny (Control group E_1 and E_2) while the colour was reddish brown. For the treated groups (A_1, B_1, C_1 D_1 and A_2, B_2, C_2 D_2) the cut surface was also smooth like the control groups but glistening. The histo-morphological patterns of all the organs examined were consistent without change in pattern and or variation in the morphology of the treated animals when compared to the control (Plate 5-18). Histopathology findings were in keeping with normal histology of organs examined (Plate 21-81) and there was no evidence of necrosis or deleterious effect seen at the cellular level.

Table 4.1: Toxicity analysis of Albino rats treated with *Acalypha wilkesiana* leaf extract for 31 days (oral and subcutaneous administration).

Cages	Dose in mg/kg	Mean Average weight b4 Administration of decoction	Mean Average weight after Administration of decoction	Physical Weight loss / or gain	Activities / or dullness
A_1	200	196.40± 1.4	196.32± 1.1	Ź	±
B_1	400	198.88± 1.8	188.56 ± 4.7	Ź	+
C_1	600	200.58± 2.3	187.23 ± 3.4	ź	++
D_1	800	201.60± 3.0	168.10 ± 3.8	ź	++
E_1	000	198.48± 1.4	208.66 ± 1.5	ŷ	-
A_2	200	198.26± 1.2	190.12± 1.4	Ź	+
B_2	400	198.80± 0.4	183.24 ± 2.1	ź	++
C_2	600	199.65± 2.2	181.12 ± 4.2	ź	++
D_2	800	200.28± 3.4	176.24 ± 1.6	ź	++
E_2	000	200.44± 2.2	208.66 ± 2.6	ŷ	-

Key

ŷ	Ÿ	Slight increase in weight
Ź	Ÿ	Slight weight loss
ź	Ÿ	severe weight loss
+	Ÿ	Presence of features
±	Ÿ	Intermediate features
++	Ÿ	Marked presence of features
-	Ÿ	Absence of features

In the analyses, photomicrographs obtained from the control rats were compared with that for the groups treated with the crude extract of *Acalypha wilkesiana* (boiled decoction), while possible histological alterations between groups treated with different doses of the extract were also compared with the untreated group to establish the influence of dose related offences on the activity of the extract (Plate 5-81). Result of acute toxicity studies revealed that the crude extract of the leaf of *Acalypha wilkesiana* recorded no mortality rate at $L.D_{50}$ Ó 6, 344mg/kg b.w. (oral) and Ó 5, 766mg/kg b.w. (subcutaneous). Behavioral signs of acute toxicity were observed in experimental rats, especially rats of high dose treated animals in group C_1, D_1 and C_2, D_2 (600 and 800mg/kg b.w. in rats) respectively such as: Prolong sleep, dullness and reduced activities, while empirical measurements showed gross reduction in weight in all treated animals and were marked severely in cages C_2 and D_2 (600 and 800mg/kg b.w. in rats administered subcutaneously) as compared to rats in group C_1 and D_1 (600 and 800mg/kg b.w. oral administration), (Table 4.1).

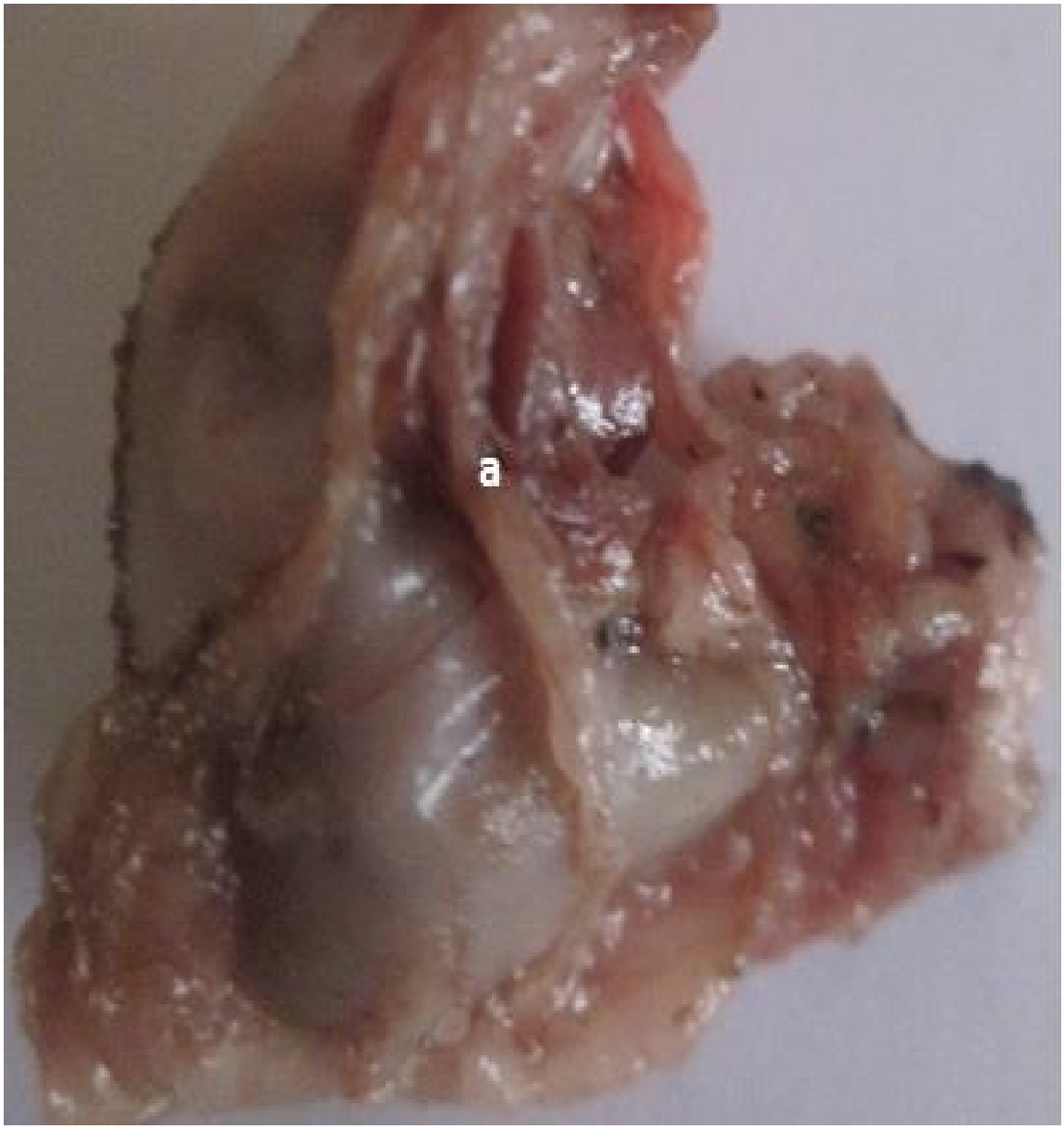

Plate 5: Gross examination of the ventral view of the stomach (Control) showing normal peritoneal folds, (Digital optic-shot: X5).

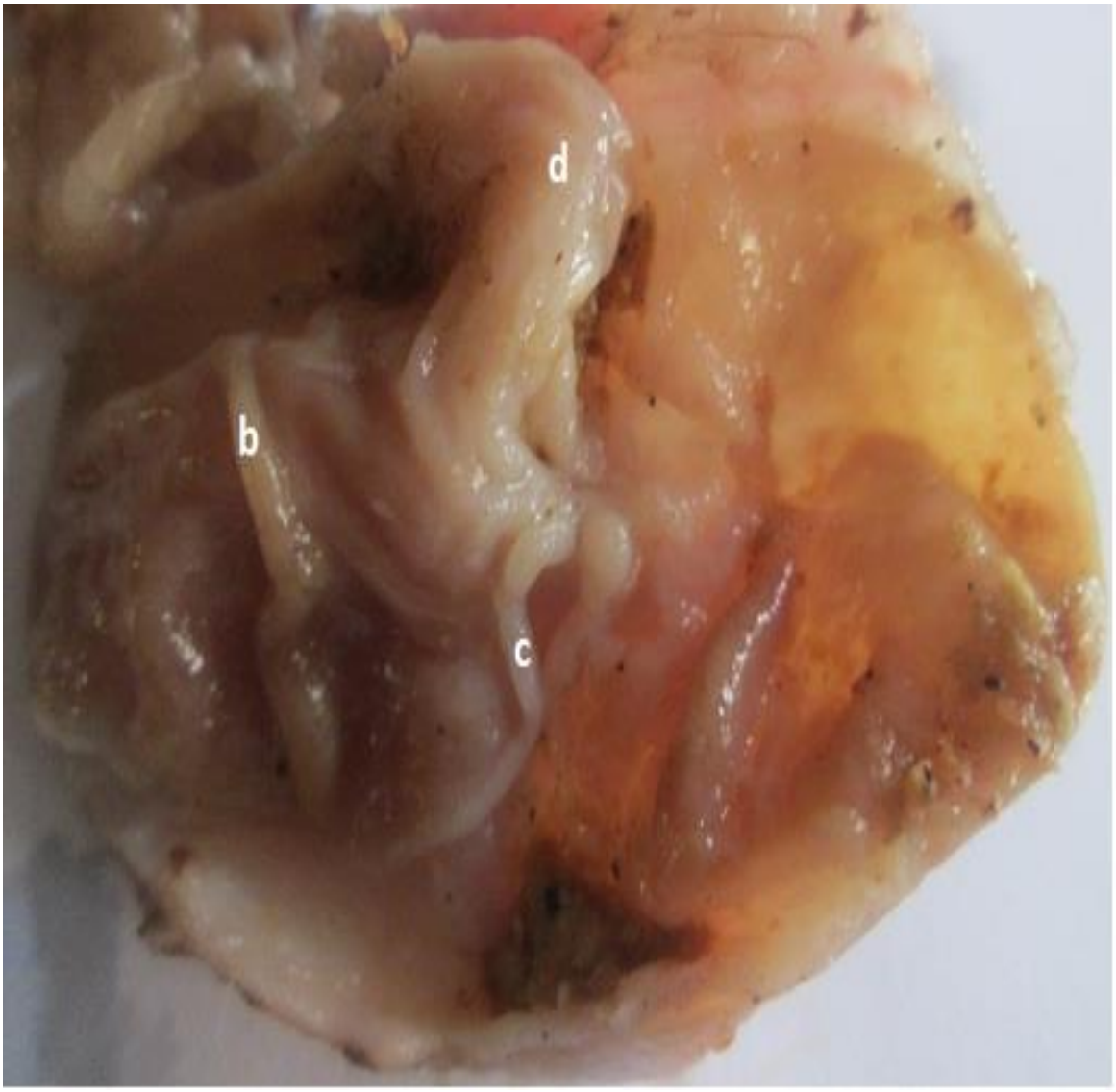

Plate 6: Gross examination of the ventral view of the cut surface of the stomach (Control) showing normal surrounding folds (b), peritoneal folds (c) and greater omentum (d) with consistent morphological pattern (Digital photo-shot: X5).

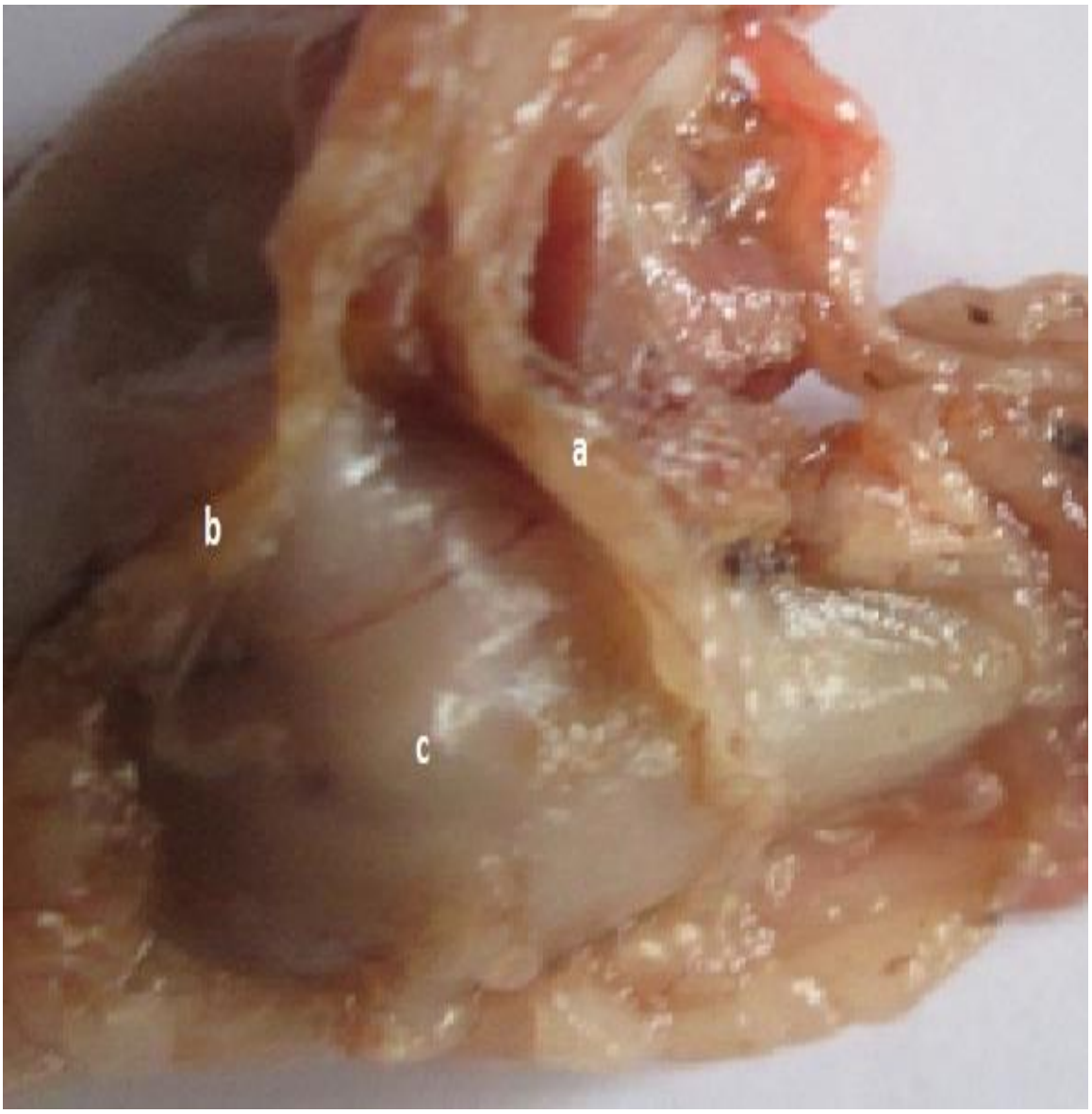

Plate 7: Gross examination of the stomach (ventral view) treated with high dose (800mg of *Acalypha wilkesiana* leaf extract). Showed normal morphological pattern of (a) = peritoneal folds, (b) = surrounding folds and (c) the Antrum. (Optic-shot: X5)

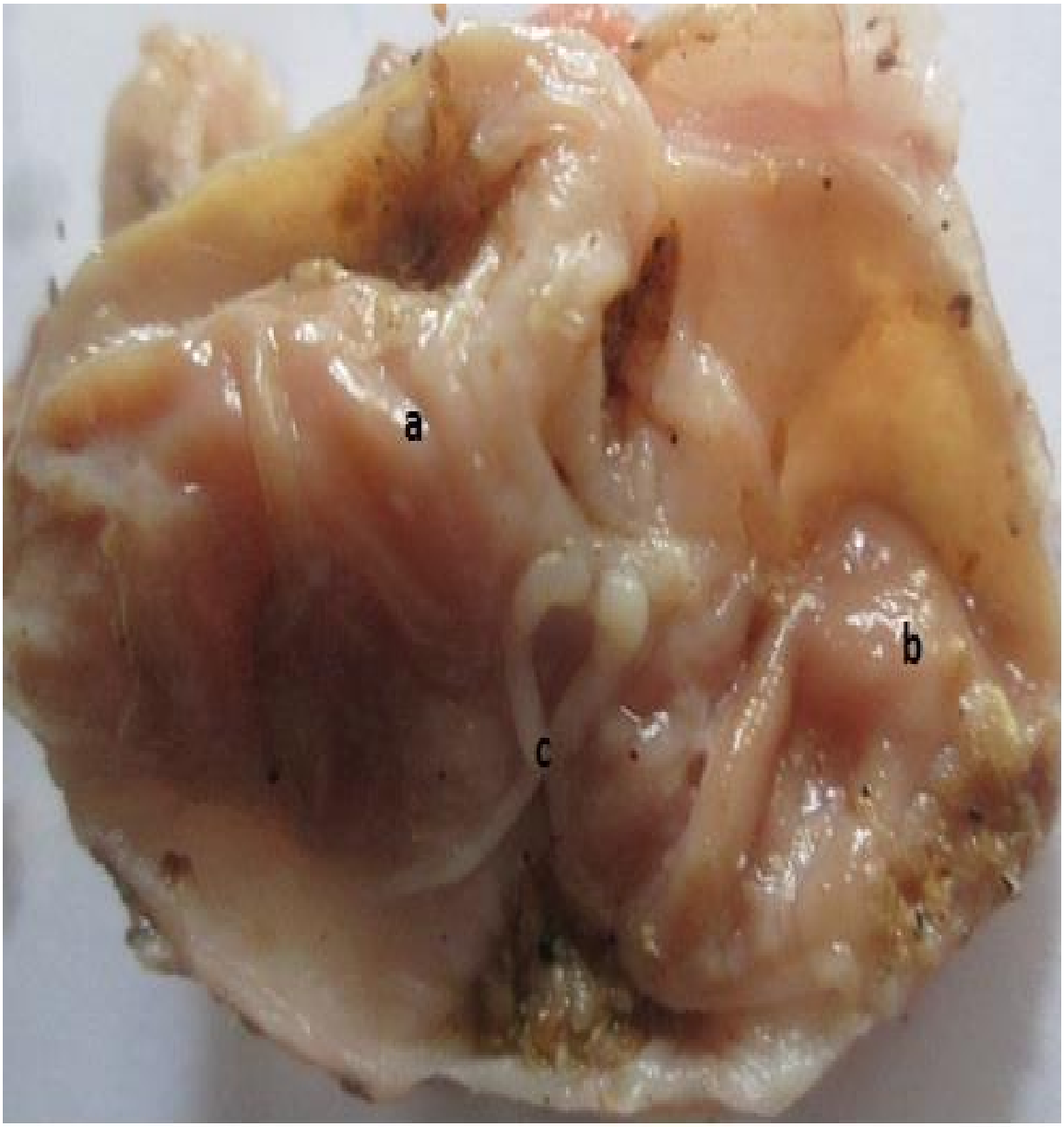

Plate 8: Gross examination of the ventral view of the stomach (cut surface) treated with high dose (800mg/kg b.w. oral administration of A*calypha wilkesiana* leaf extract) showing normal morphological architecture of the greater curvature (a), lesser curvature (b) and *incisura angularis* (c). (Digital optic-shot: X5)

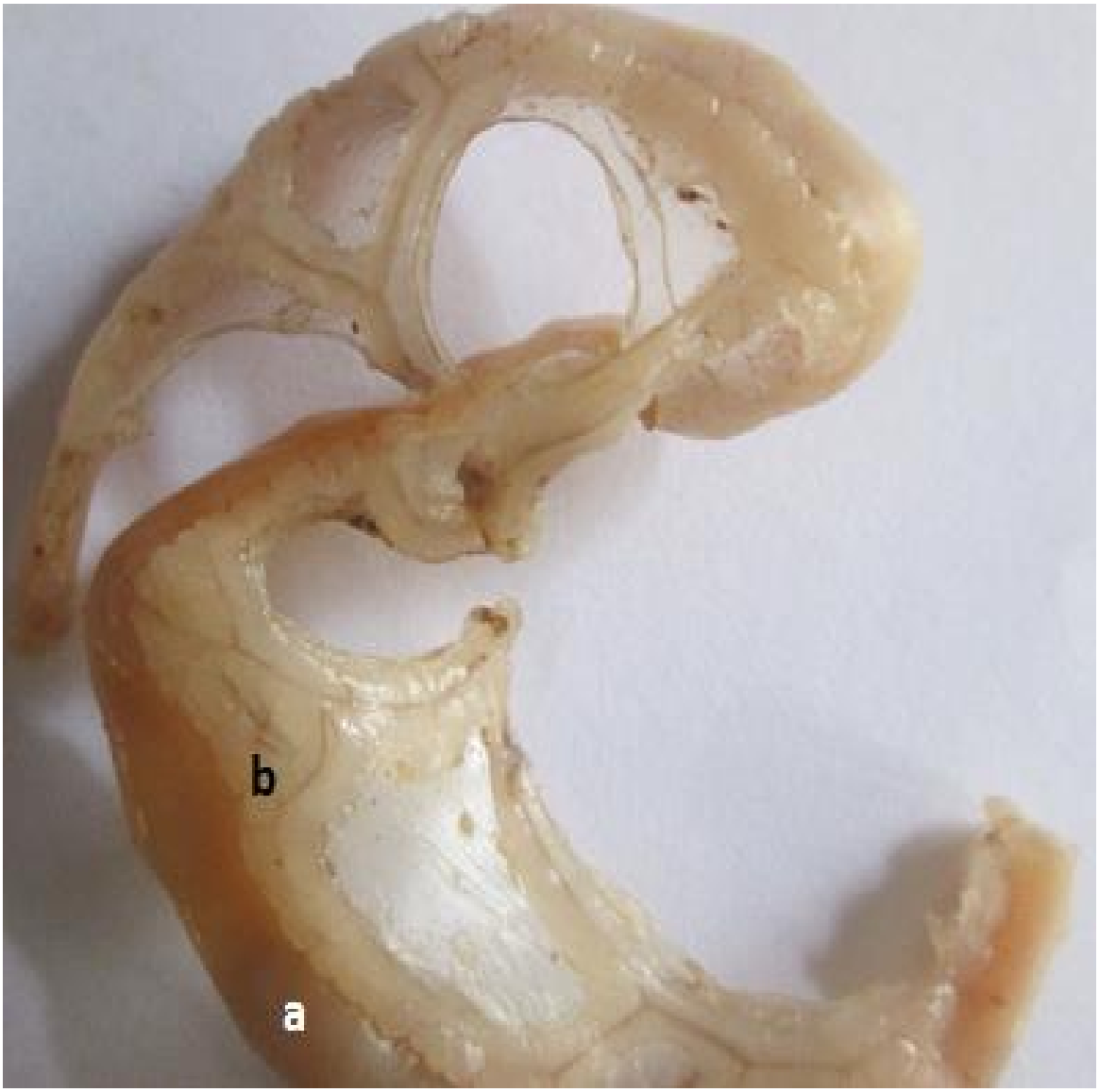

Plate 9: Gross examination of the dorsal view of the untreated skin (Control) excised from the thigh region showing normal morphological pattern of the dermal layer (a) and epidermal layer (b). (Digital optic-shot: X5)

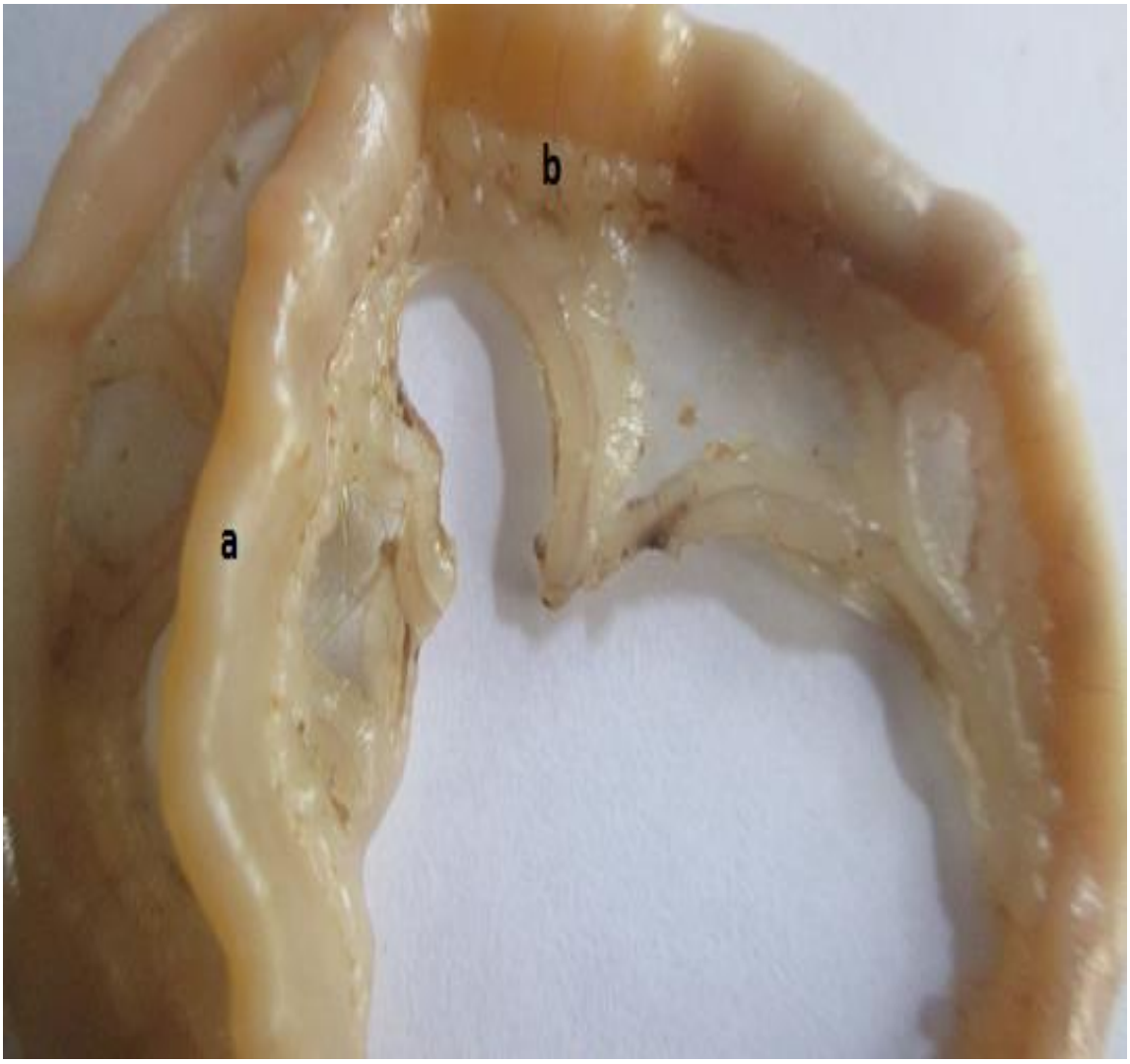

Plate 10: Gross examination of the ventral view of the skin treated with high dose (800mg of A*calypha wilkesiana* leaf extract- subcutaneous administration) excised from the thigh region showing normal morphology of the dermal layer (a) and epidermal layer (b). (Digital optic-shot: X5)

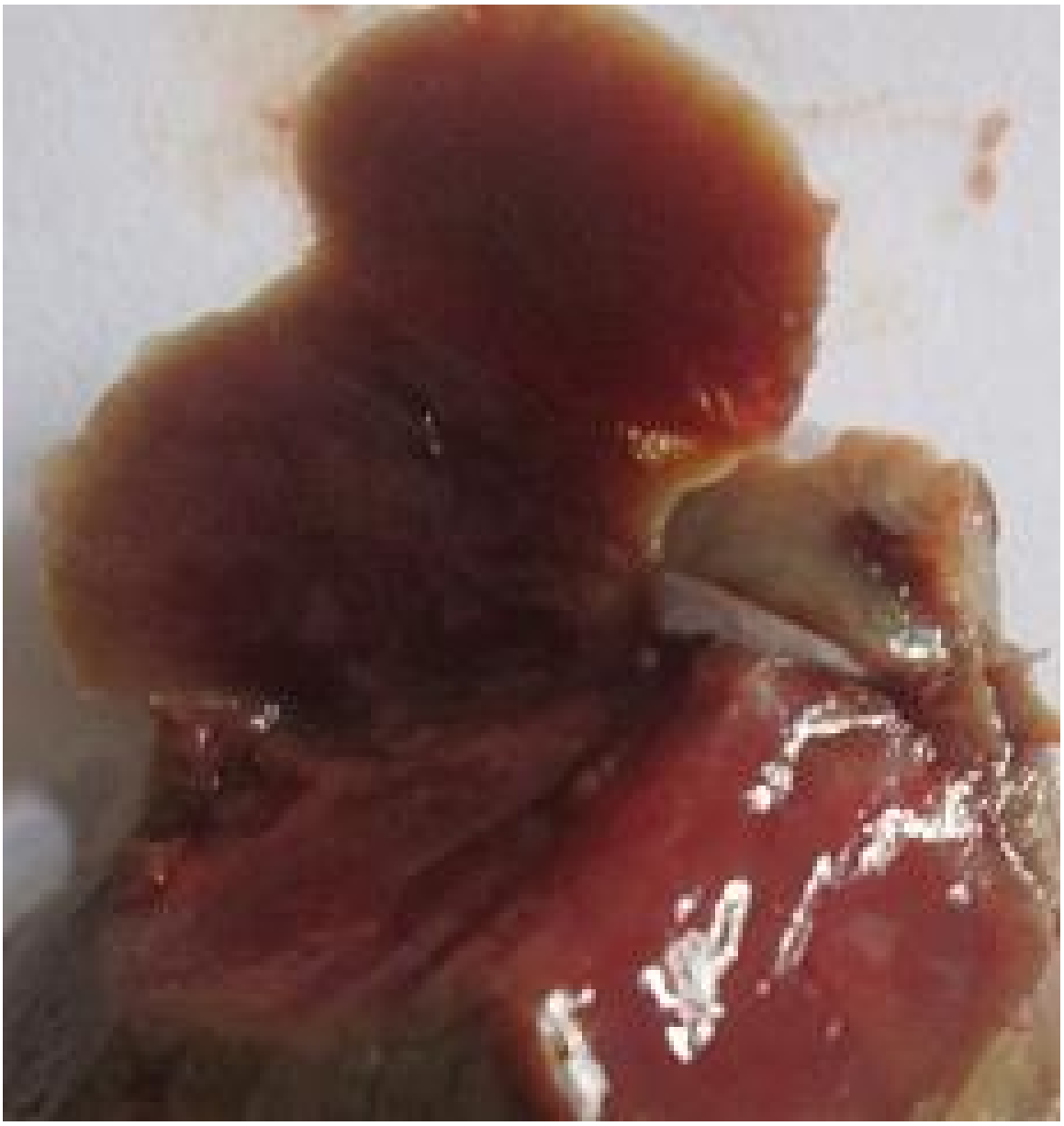

Plate 11: Gross examination of the mid ventral view of the cut surface of the liver from the caudate lobe (Control) showing smooth and normal morphological pattern (Digital optic-shot: X5).

Plate 12: Gross examination of the mid- ventral view of the cut surface of the liver from the caudate lobe treated with high dose (800mg/kg b.w. subcutaneous administration) of A*calypha wilkesiana* leaf extract showing normal morphological architecture of the cut surface without changing pattern. (Optic-shot: X5)

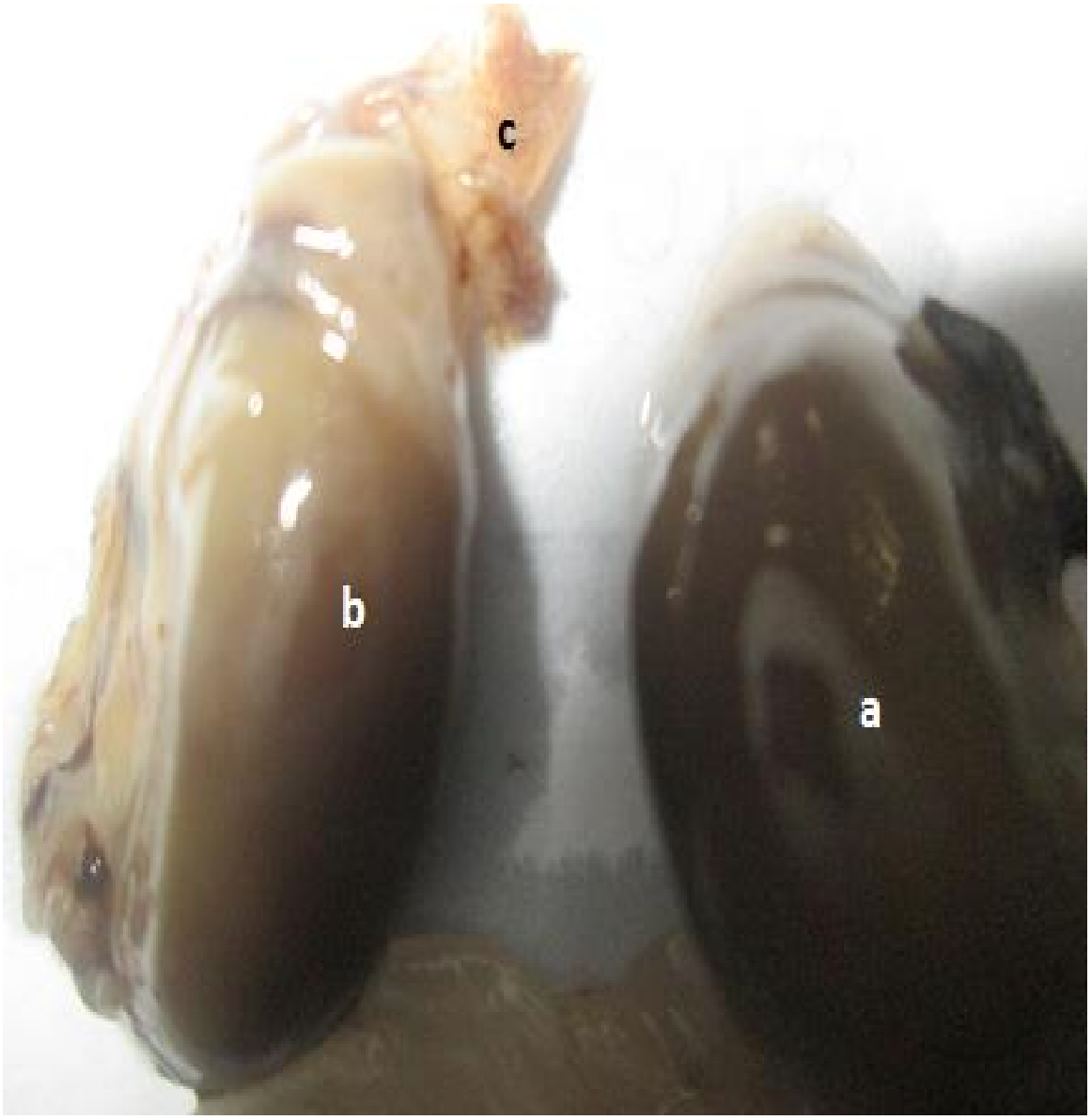

Plate 13: Gross examination of the ventral view of the left kidney (a) and the right kidney (b) from a control rat showing normal morphology of the kidney with the left slightly smaller than the right in size (Digital optic-shot: X5).

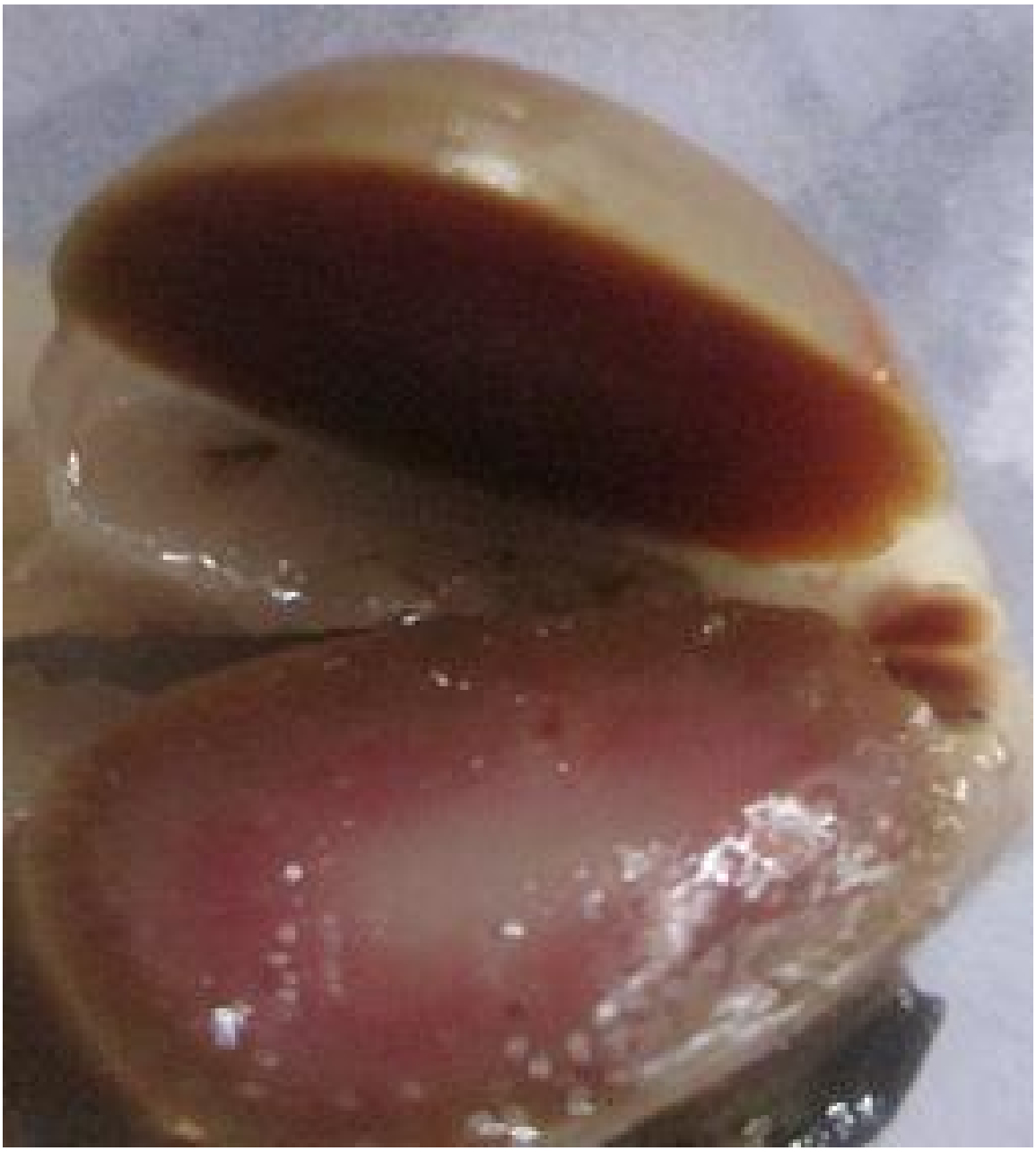

Plate 14: Gross examination of the lateral view of the cut surface of the right kidney from the control group (Digital optic-shot: X5).

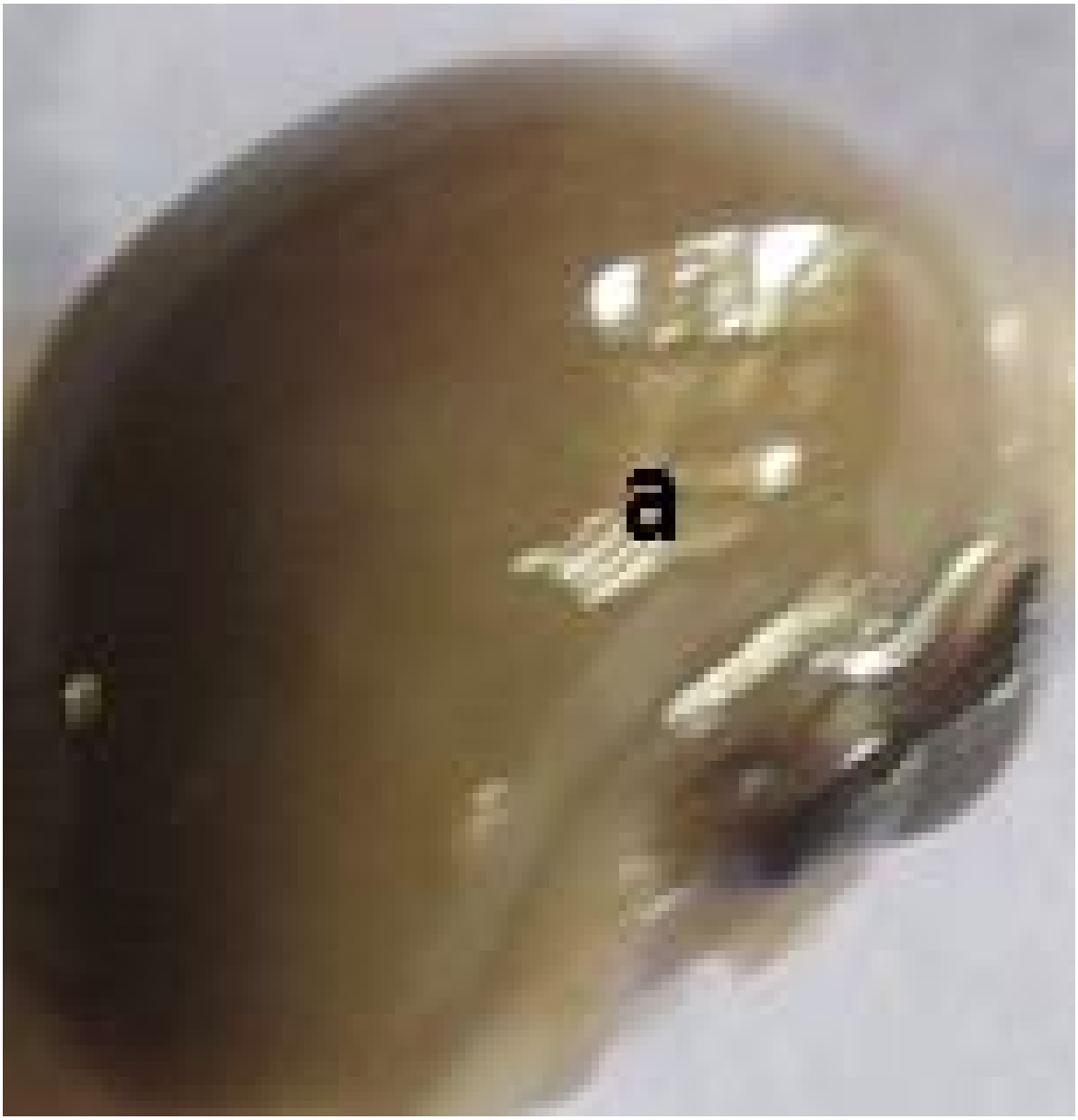

Plate 15: Gross examination of the lateral view of the right Kidney treated with high dose (800mg/kg b.w. oral administration of *Acalypha wilkesiana* leaf extract) showing normal morphological pattern of the right kidney. (Digital optic-shot: X5)

Plate 16: Gross examination of the ventral view of the left Kidney treated with high dose (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* leaf extract) showing normal morphological pattern of the left kidney. (Digital optic-shot: X5)

Plate 17: Gross examination of the ventral view of the left Kidney treated with high dose (800mg/kg b.w. Oral Administration of *Acalypha wilkesiana* leaf extract) showing smooth and normal morphological pattern of the cut surface of the left kidney. (Digital optic-shot: X5)

Plate 18: Gross examination of the dorsal view of the left Kidney treated with high dose (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* leaf extract) showing smooth and normal morphological pattern of the cut surface of the left kidney. (Digital optic-shot: X5)

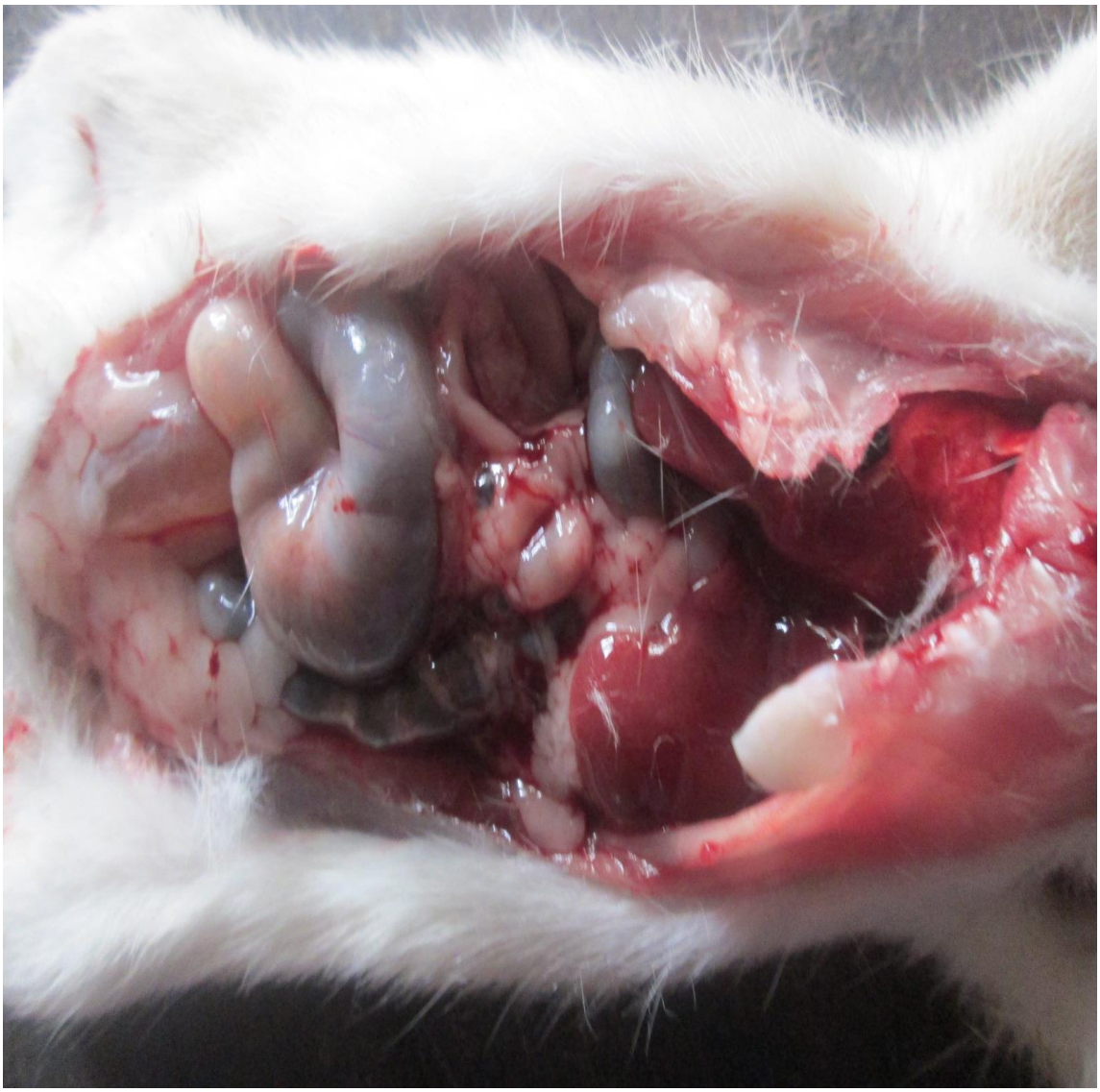

Plate 19: Ventral view of the contents of the abdominal and thoracic cavities (Optic-shot X5)

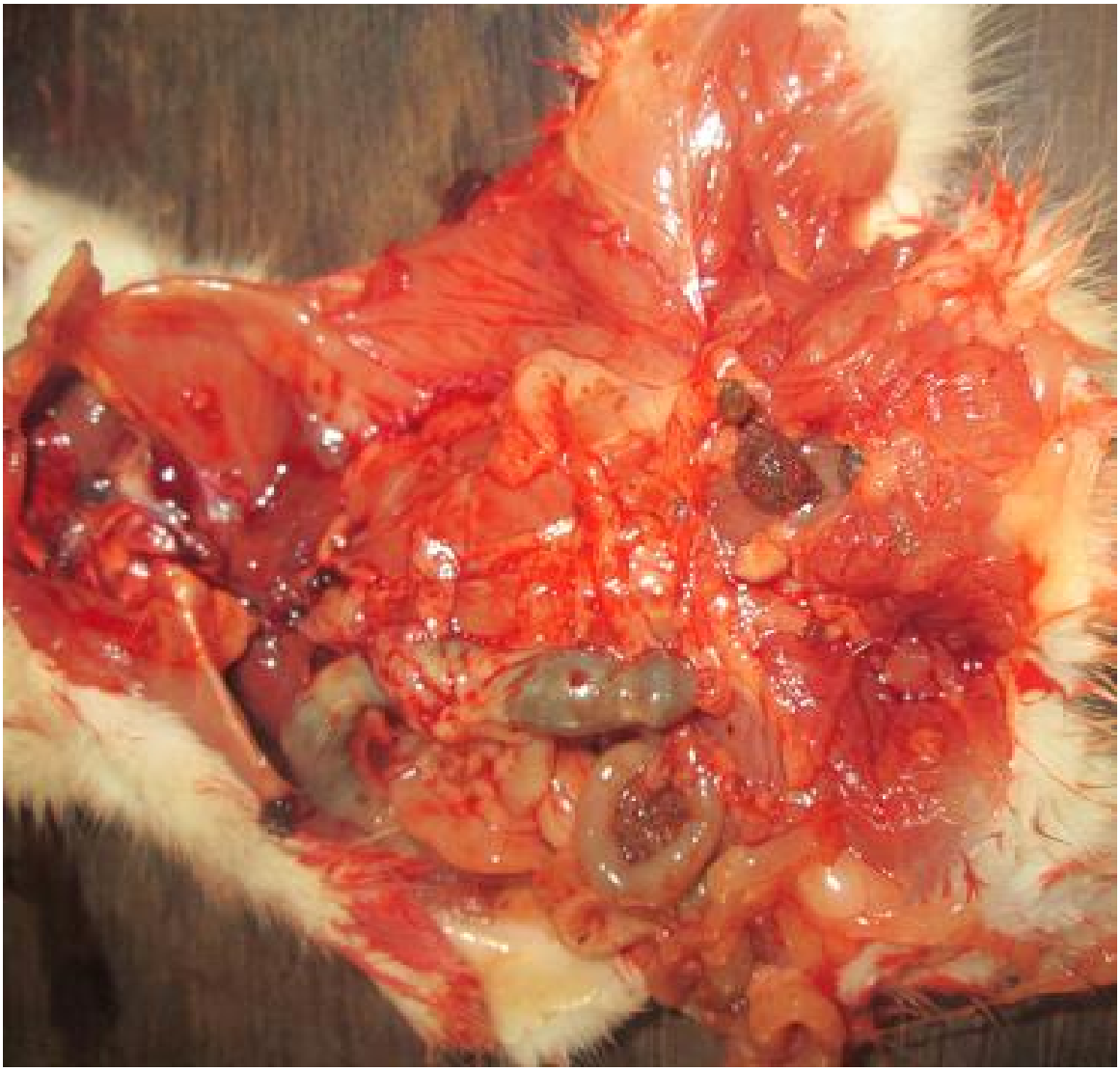

Plate 20: Ventral view of the abdomen following excision of organs of interest (Optic-shot X5)

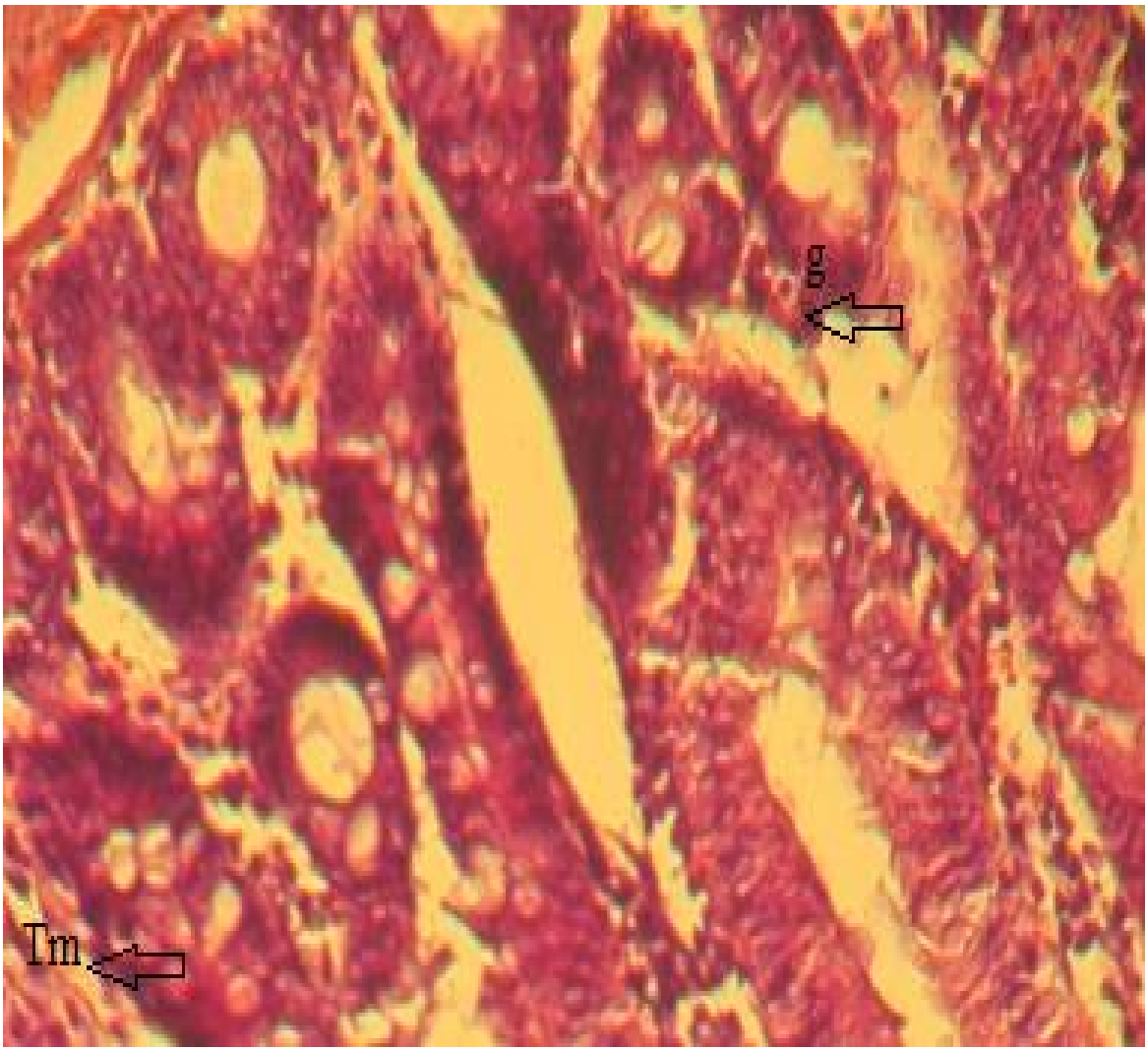

Plate 21: Section of control ratôs oesophagus showing normal histopathological pattern of the oesophagus revealing the oesophageal glands (g) and Tunica muscularis (Tm). Stain uptake: Mayerôs H&E X400 magnification.

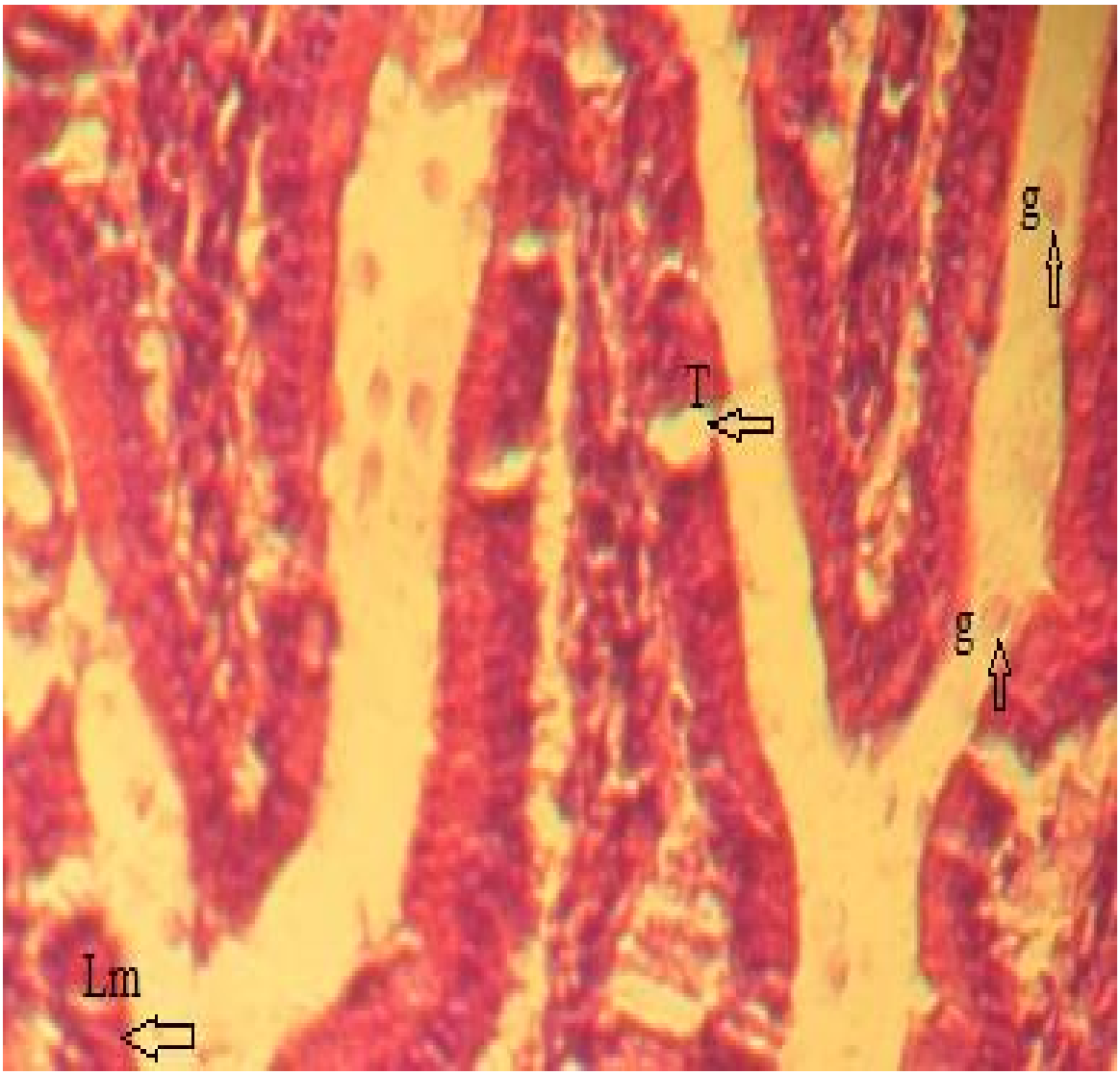

Plate 22: Oesophageal section of high dose treated rats C_1 (600mg/kg b.w. oral administration) showing normal oesophageal glands (g), distended tubules (T) and lamina epithelialis mucosae (Lm). Stain uptake: Mayerôs H&E X 400 magnification

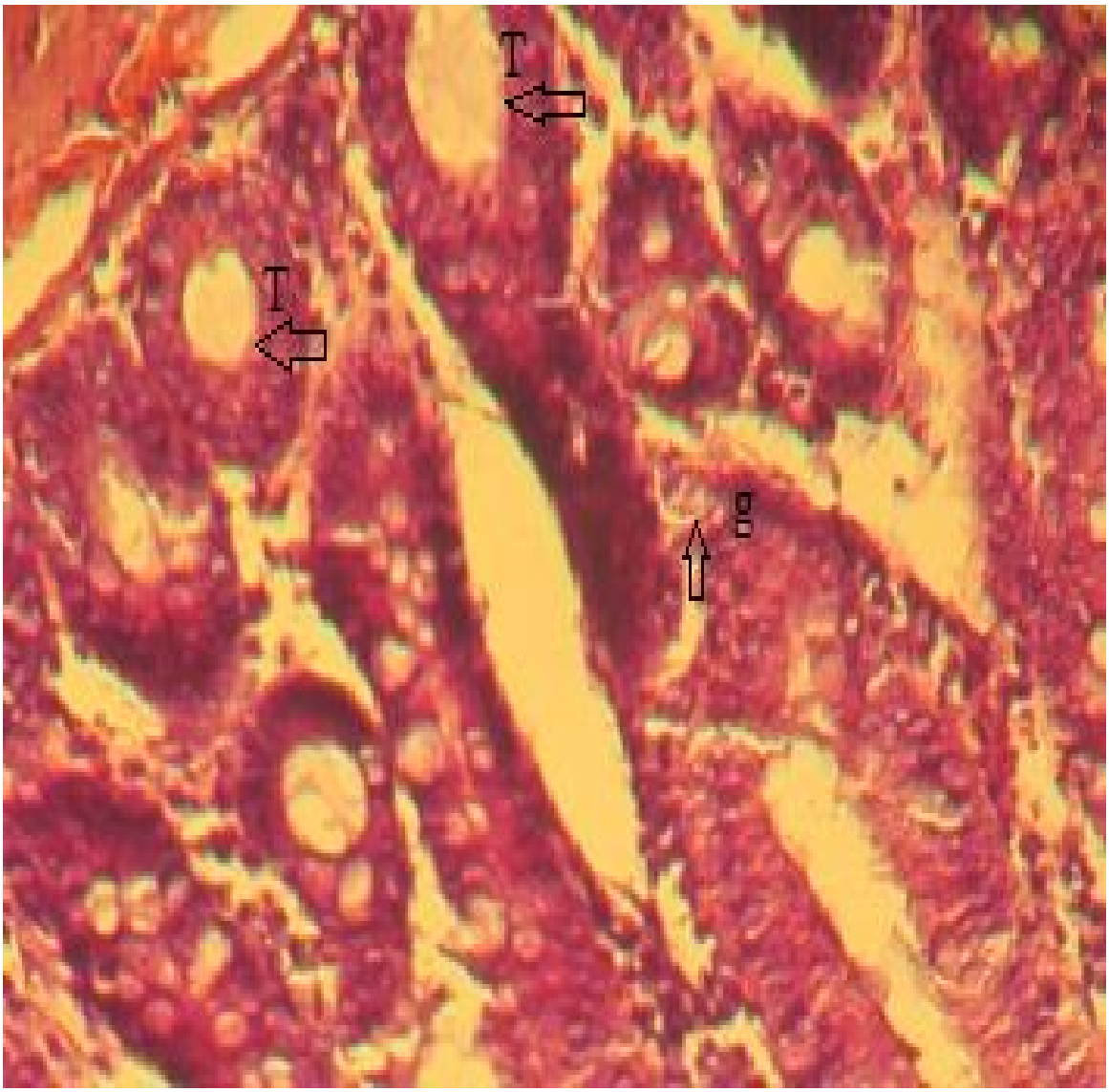

Plate 23: Oesophageal section from the high dose treated rats D_1 (800mg/kg b.w.) showing normal oesophageal glands (g) and distended tubule (T). Stain uptake: PAS X400 magnification.

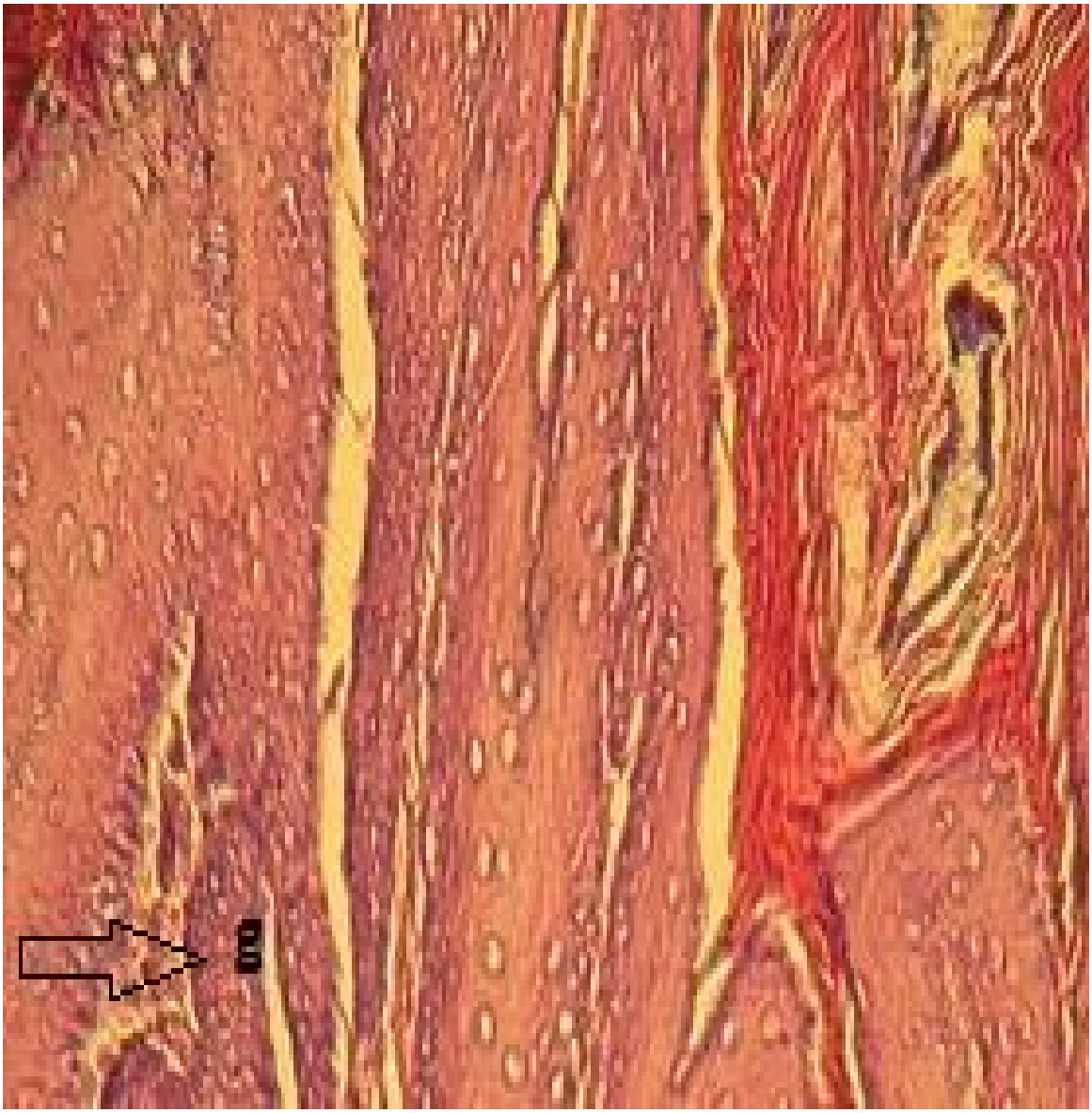

Plate 24: Section of the control ratôs stomach taken from the corpus region, showing the gastric glands (g) without changing pattern, Stain uptake: Mayerôs H&E X400 magnification.

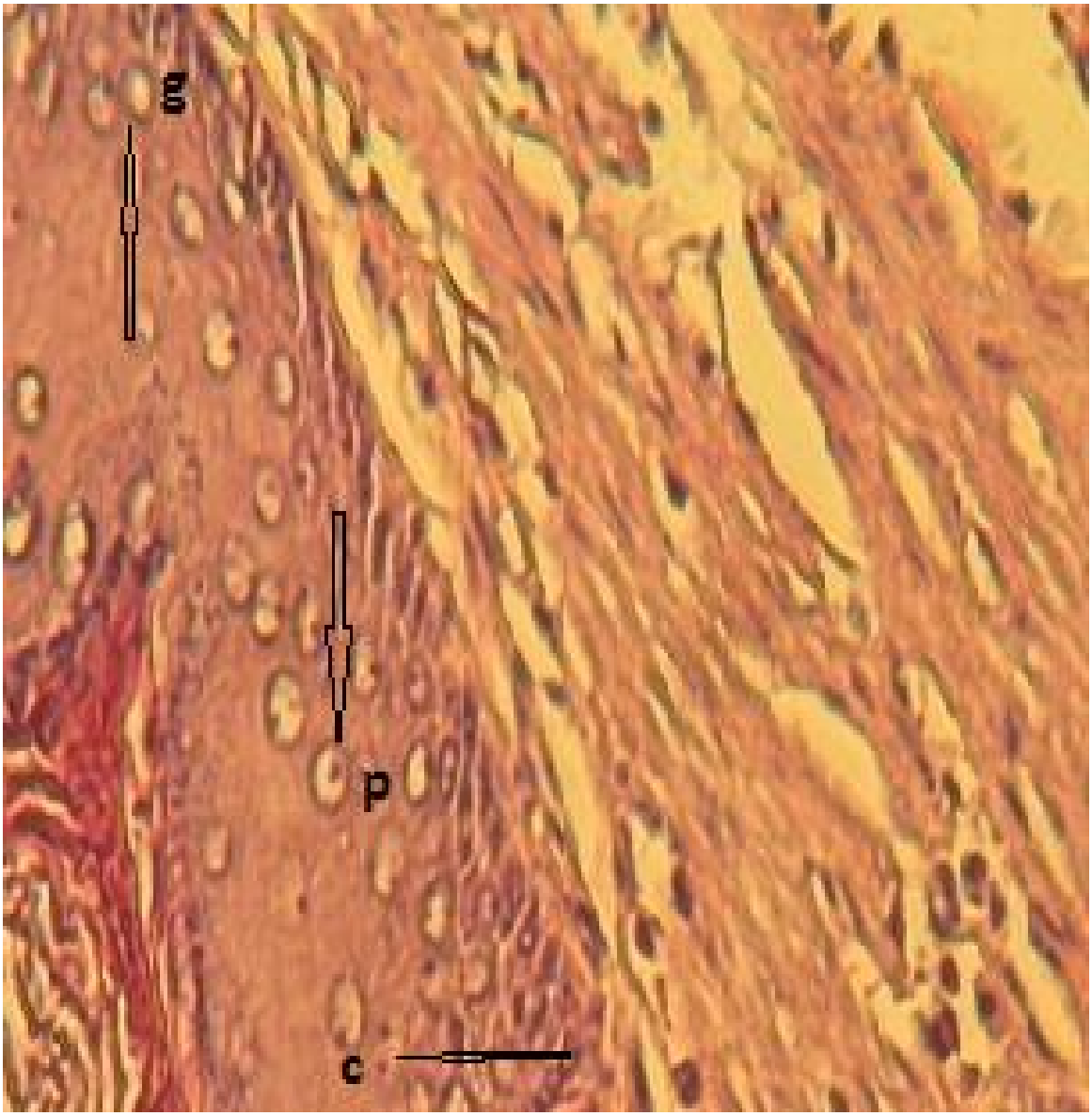

Plate 25: Section of the treated ratôs stomach C_1 (600mg/kg b.w. - oral administration) showing normal histology of the pyloric portion of the stomach containing the chief cells (c), parietal cell (p) and the gastric pit (g). Stain uptake: Mayerôs H&E X400 magnification.

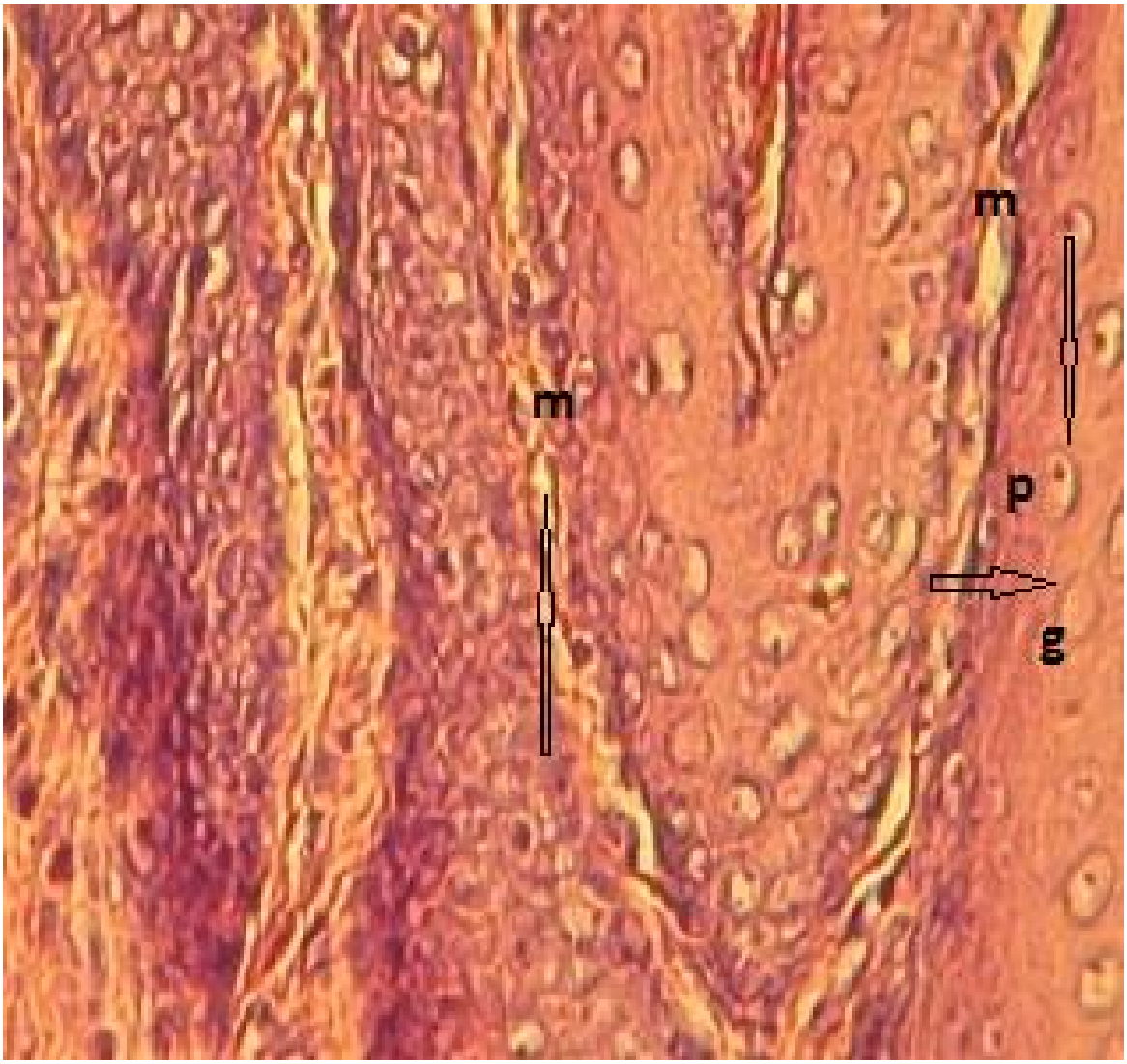

Plate 26: Section of the treated ratôs stomach C_1 (800mg/kg b.w. - oral administration) showing normal histology of the pyloric portion of the stomach with the sub-mucosal tissue (m), parietal cell (p) and the gastric pit (g). Stain uptake: Mayerôs H&E X400 magnification.

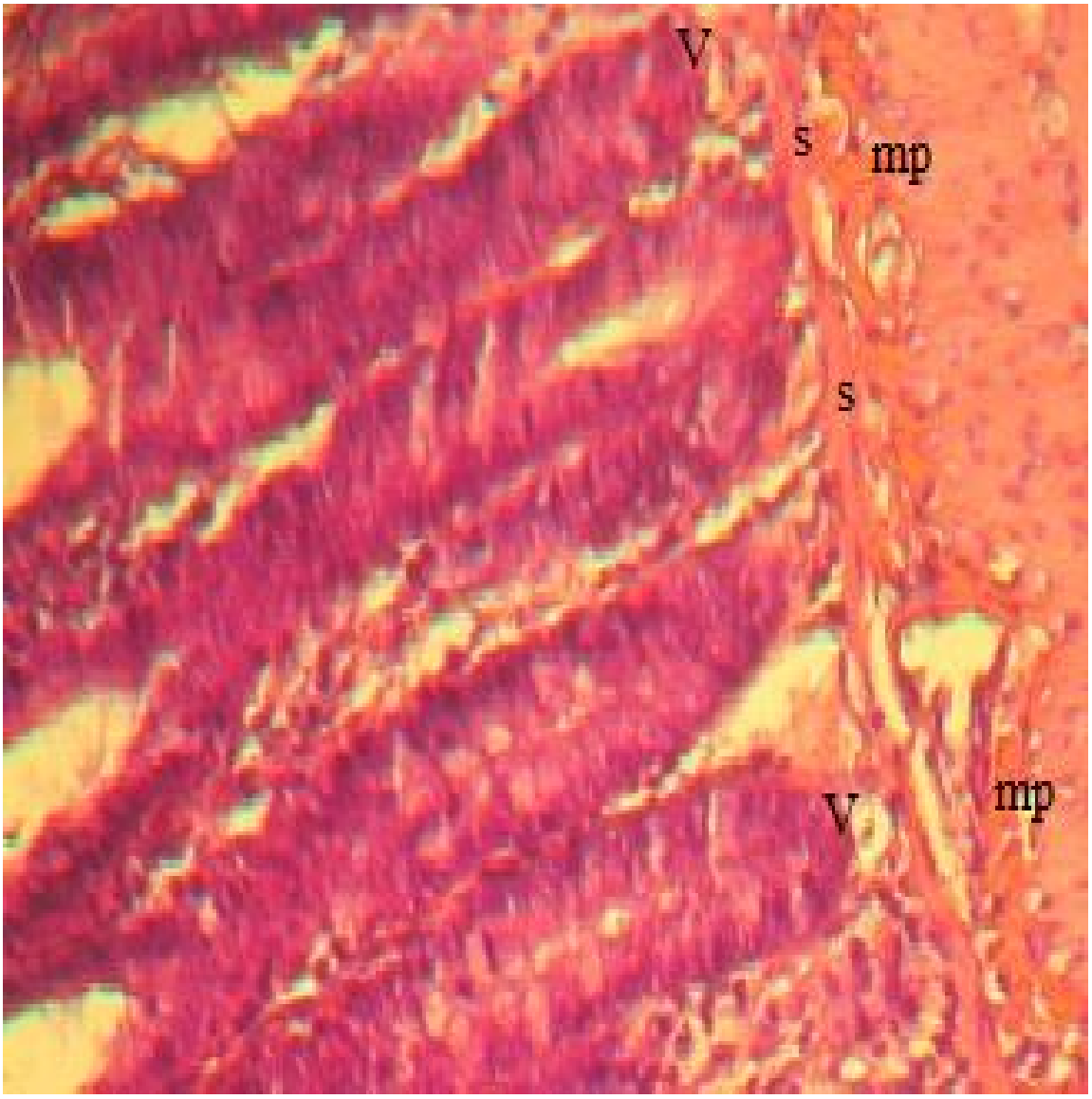

Plate 27: Section of the untreated ratôs small intestine- duodenum (control group) showing the intestinal villi (v), serosa (s) and muscularis propria (mp). Stain uptake: Coleôs H&E. X400 magnification.

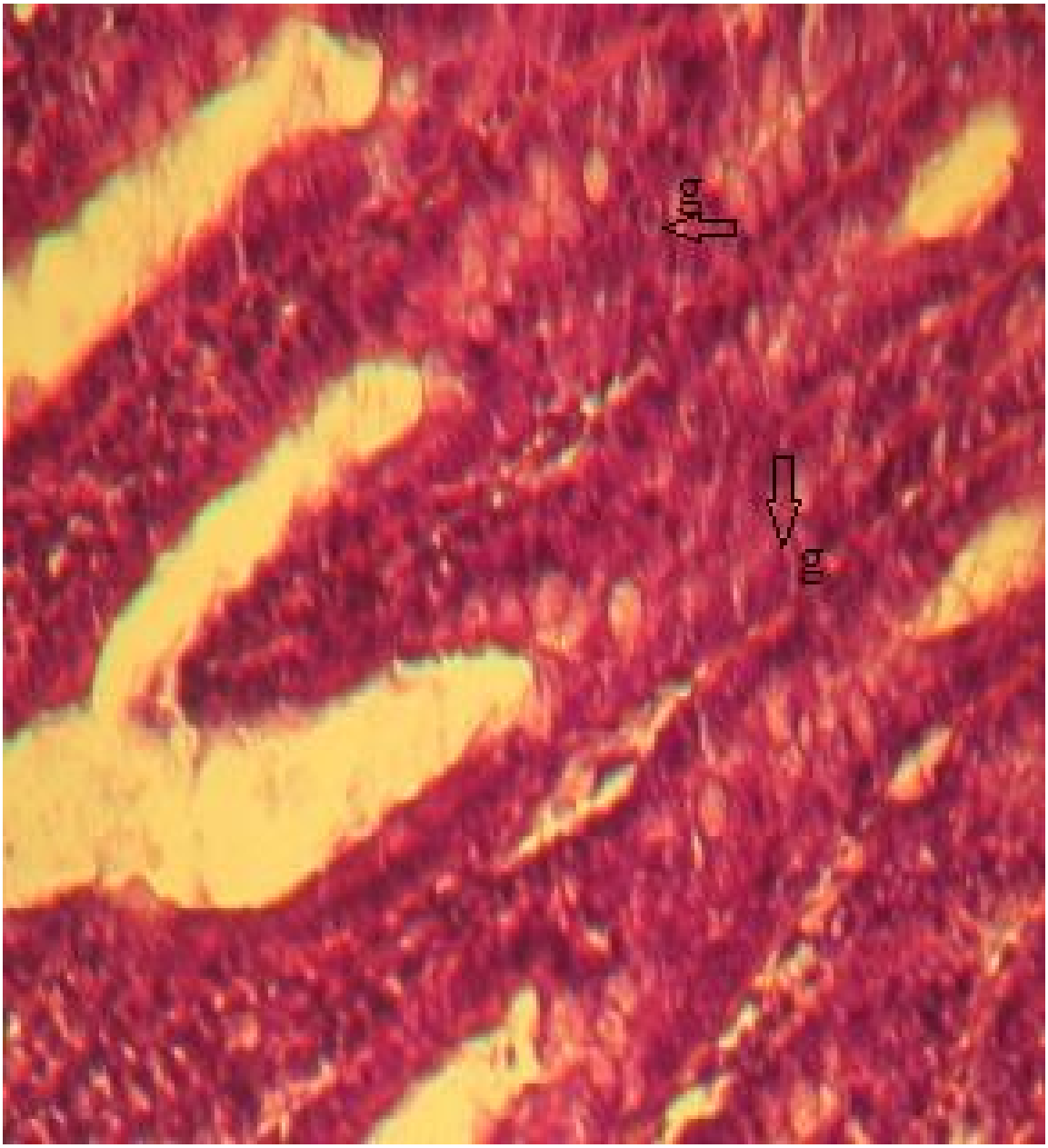

Plate 28: Section of the untreated small intestine- Duodenum (control) group E showing the duodenal glands (g), Stain uptake: PAS. X400 magnification

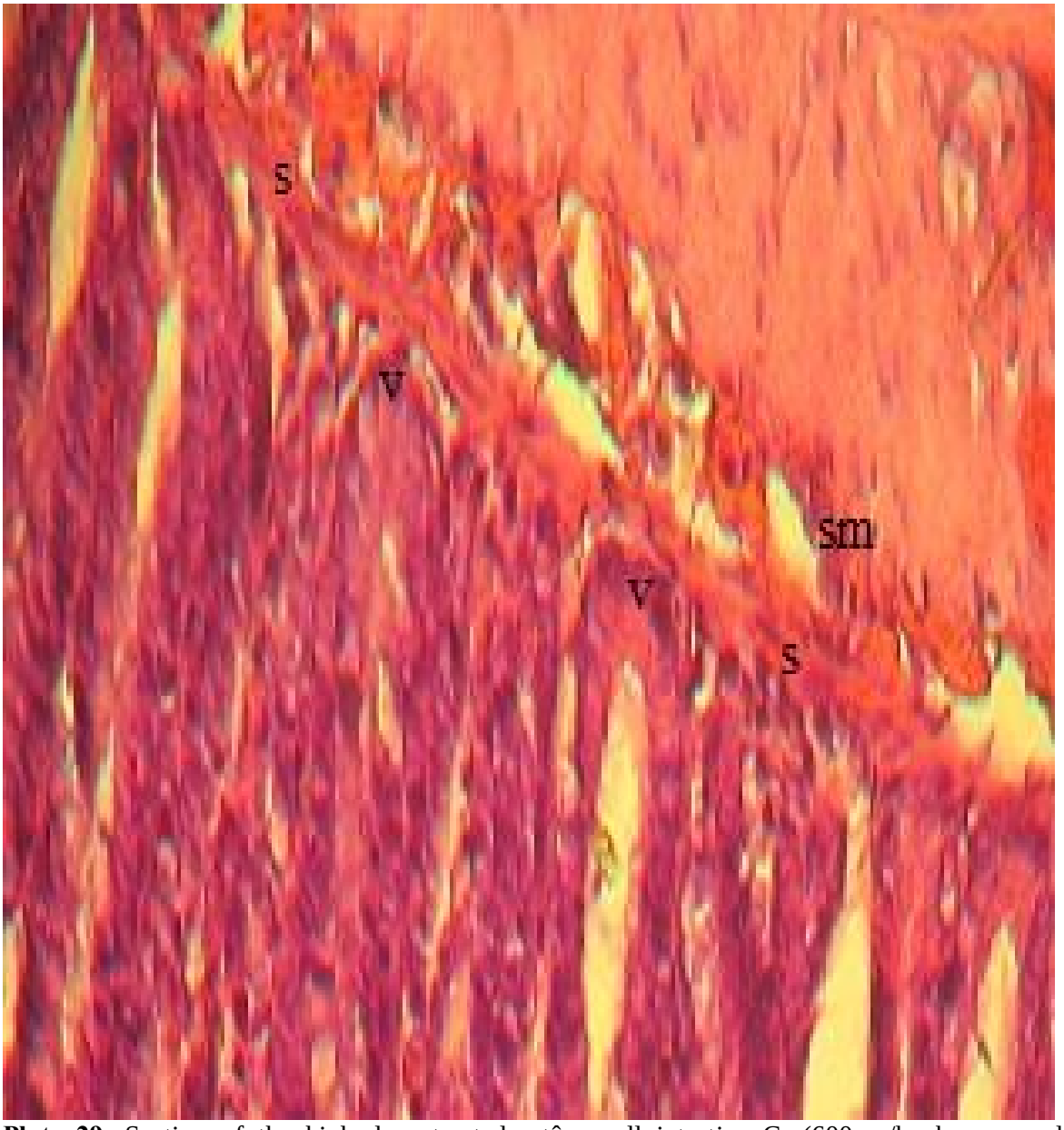

Plate 29: Section of the high dose treated ratôs small intestine C_1 (600mg/kg b.w. - oral administration) showing normal histological pattern of the small intestine- duodenum, with the intestinal villi (V), serosa (s), and submucosal tissue (sm). Stain uptake: Coleôs H&E X400 magnification

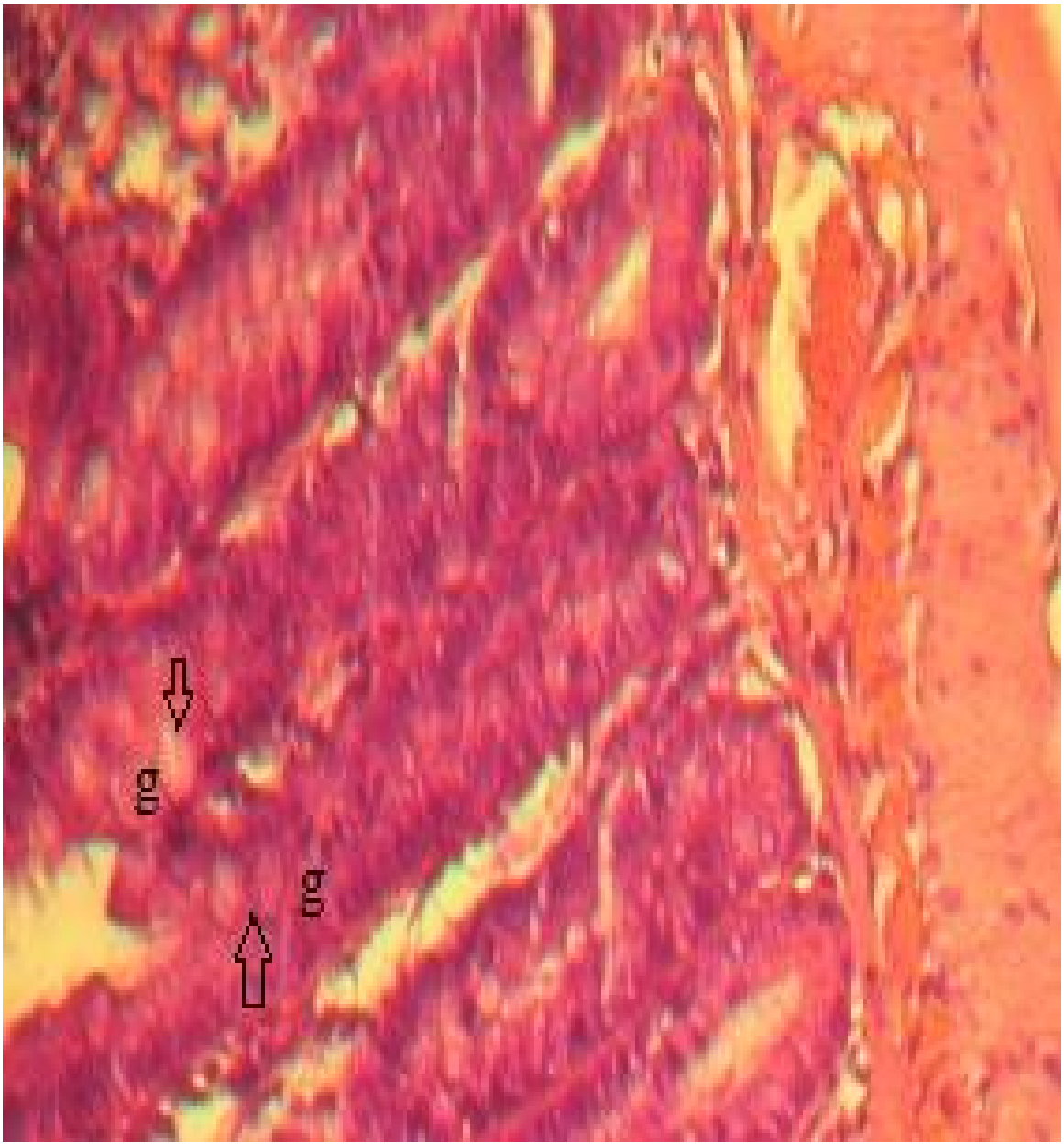

Plate 30: Section of the high dose treated ratôs small intestine D_1 (800mg/kg b.w. - oral administration) showing normal histological pattern of the small intestine- Jejunum, with goblet cell (G). Stain uptake: Mayerôs H&E X400 magnification

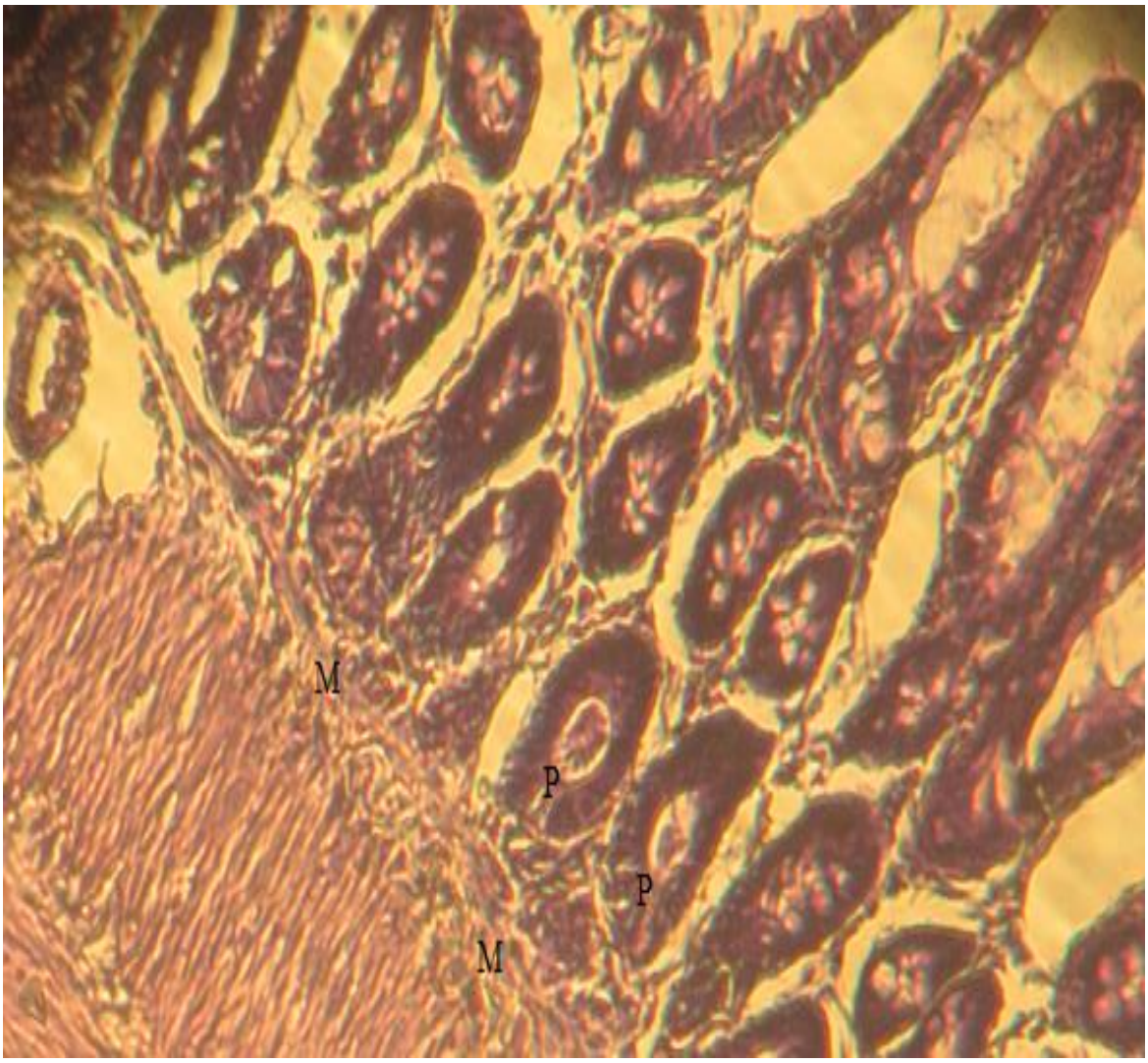

Plate 31: Section of the high dose treated ratôs small intestine C_1 (600mg/kg b.w. - oral administration) showing normal histopathological pattern of the small intestine- ileum, with the paneth cell (P) and muscular layer (M). Stain uptake: PAS X400 magnification

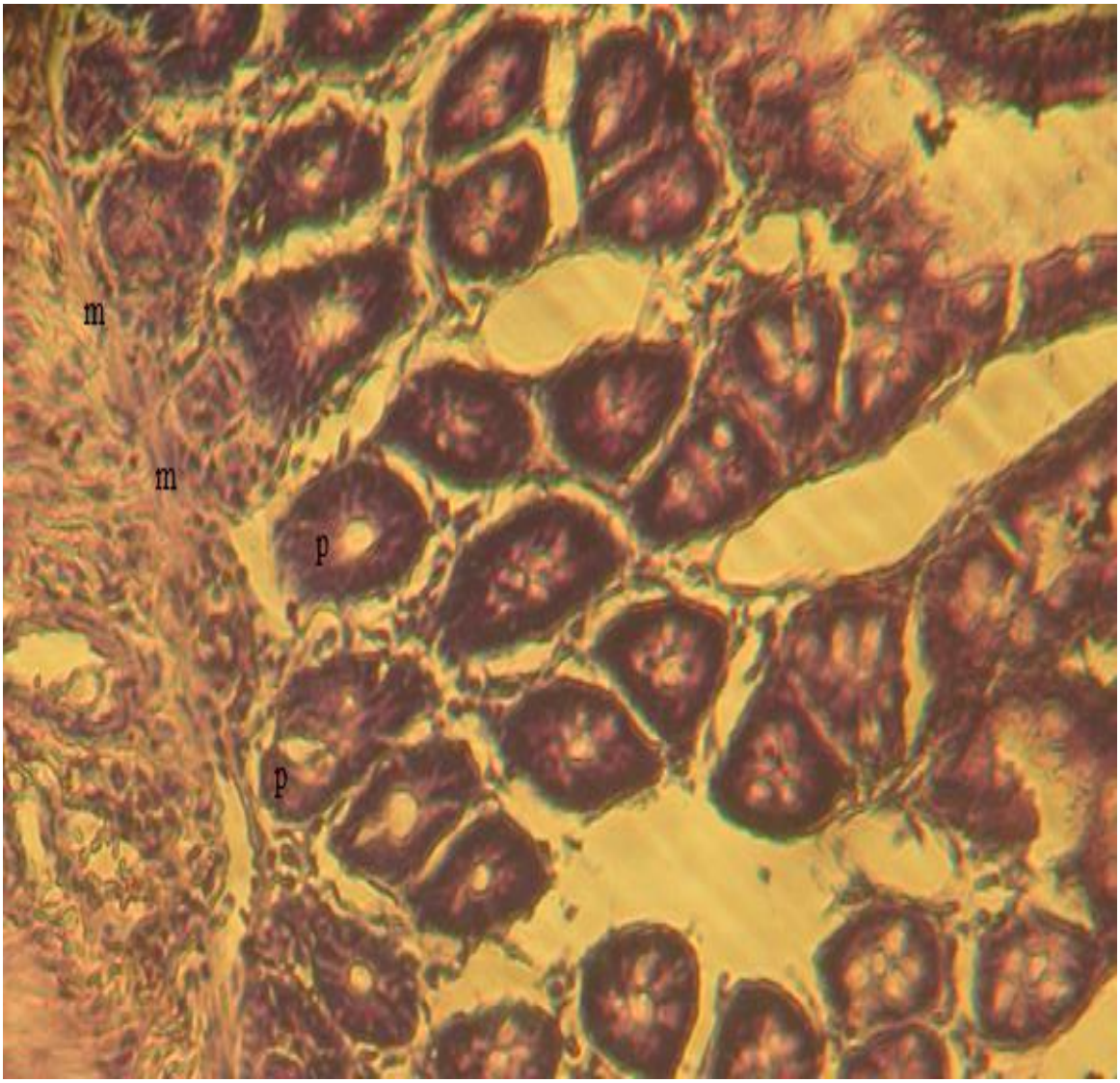

Plate 32: Section of the high dose treated ratôs small intestine D_1 (800mg/kg b.w. - oral administration) showing normal histopathological pattern of the small intestine- ileum, revealing the paneth cell (p) and muscular layer (m)Stain uptake: PAS X400 magnification.

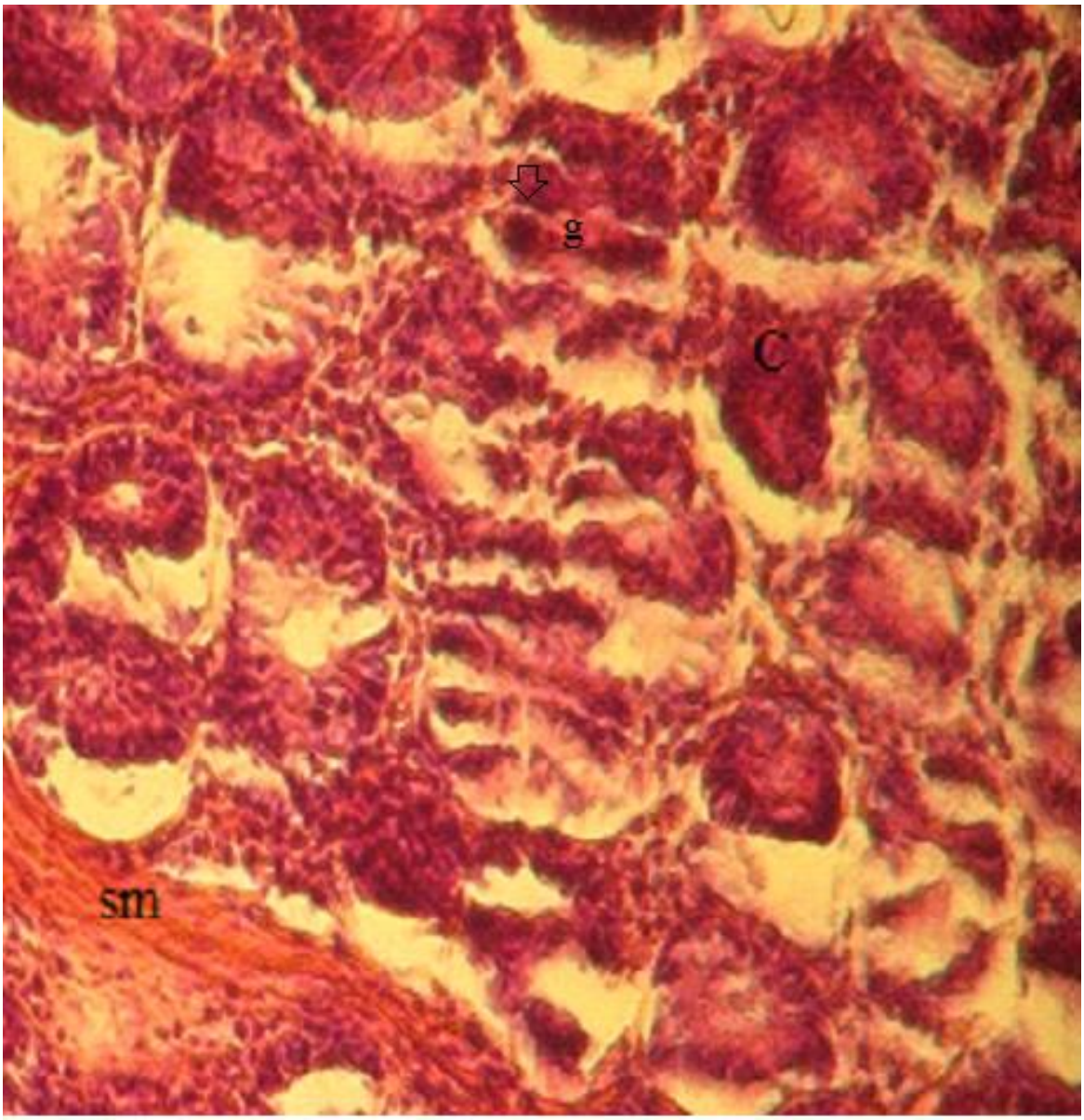

Plate 33: Section of the untreated large intestine- colon (control) group E showing the goblet cells (g), submucosa (sm) and Crypt (C). Stain uptake: PAS. X400 magnification.

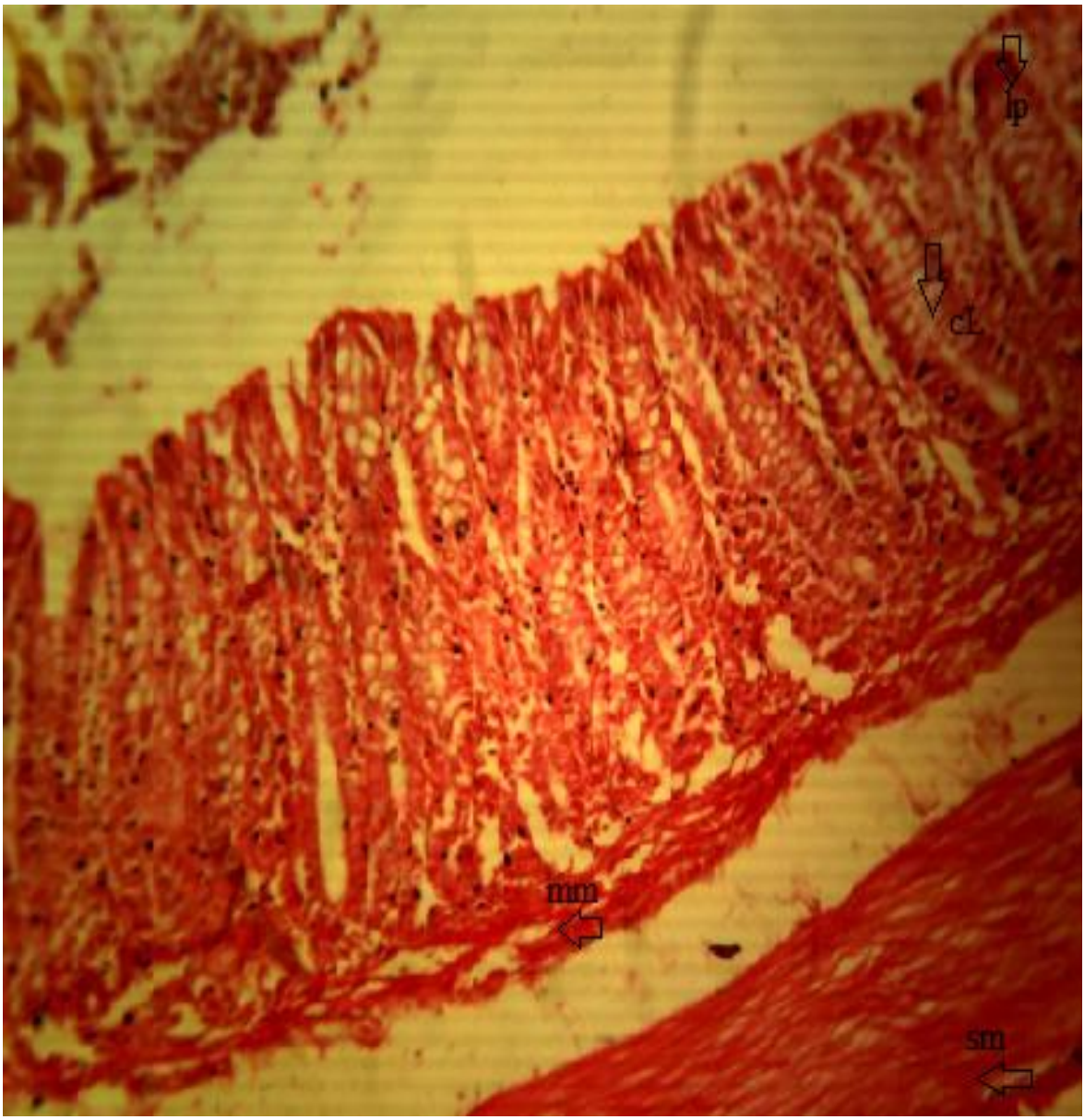

Plate 34: Section of the large intestine-colon of the high dose treated rats C_1 (600mg/kg b.w. - oral administration) showing normal histopatholological pattern of the Crypt of Lieberkuhn (CL), submucosa (sm), Muscularis mucosae (mm) and Lamina propria mucosae (Lp). Stain uptake: Mayerôs H&E X400 magnification.

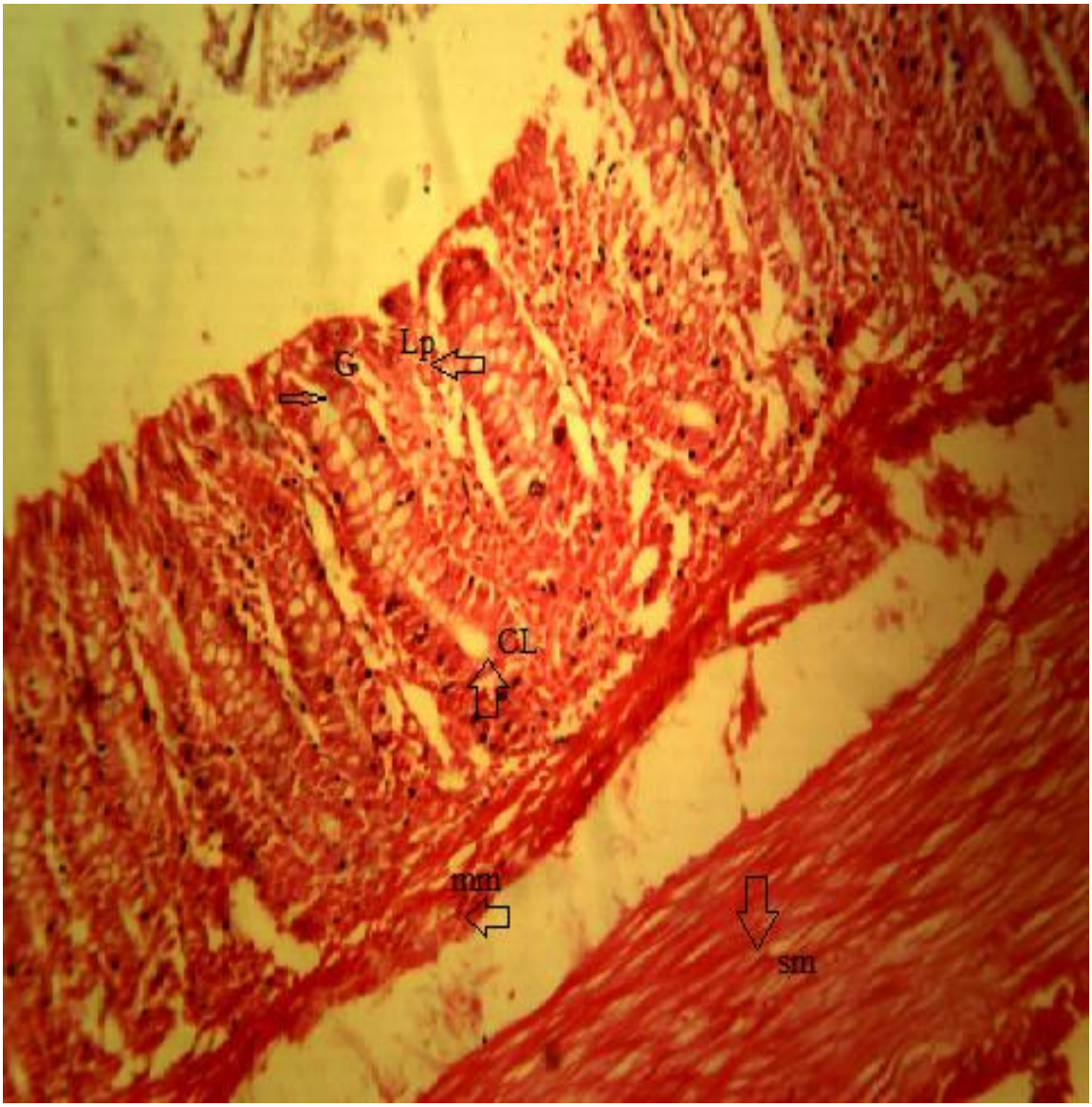

Plate 35: Section of the large intestine-colon of the high dose treated rats D_1 (800mg/kg b.w. - oral administration) showing normal histopatholological pattern of the Goblet cells (g), Crypt of Lieberkuhn (CL), submucosa (sm), Muscularis mucosae (mm) and Lamina propria (Lp). Stain uptake: Mayerôs H&E X400 magnification.

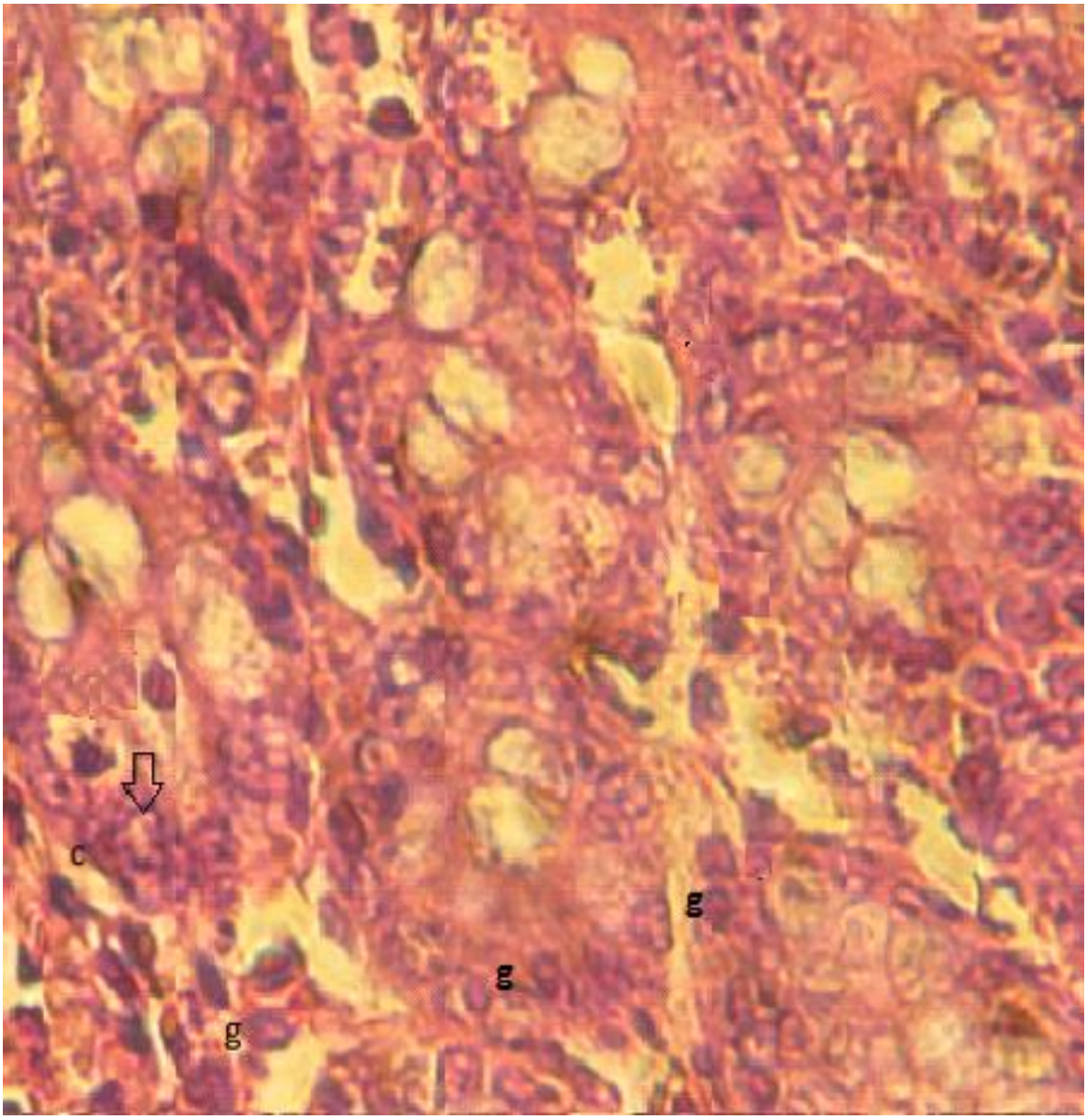

Plate 36: Section of untreated ratôs rectum (control) group showing normal histopathological pattern of the Goblet cell (g) and Crypts (C). Stain uptake: Coleôs. H&E X400 magnification

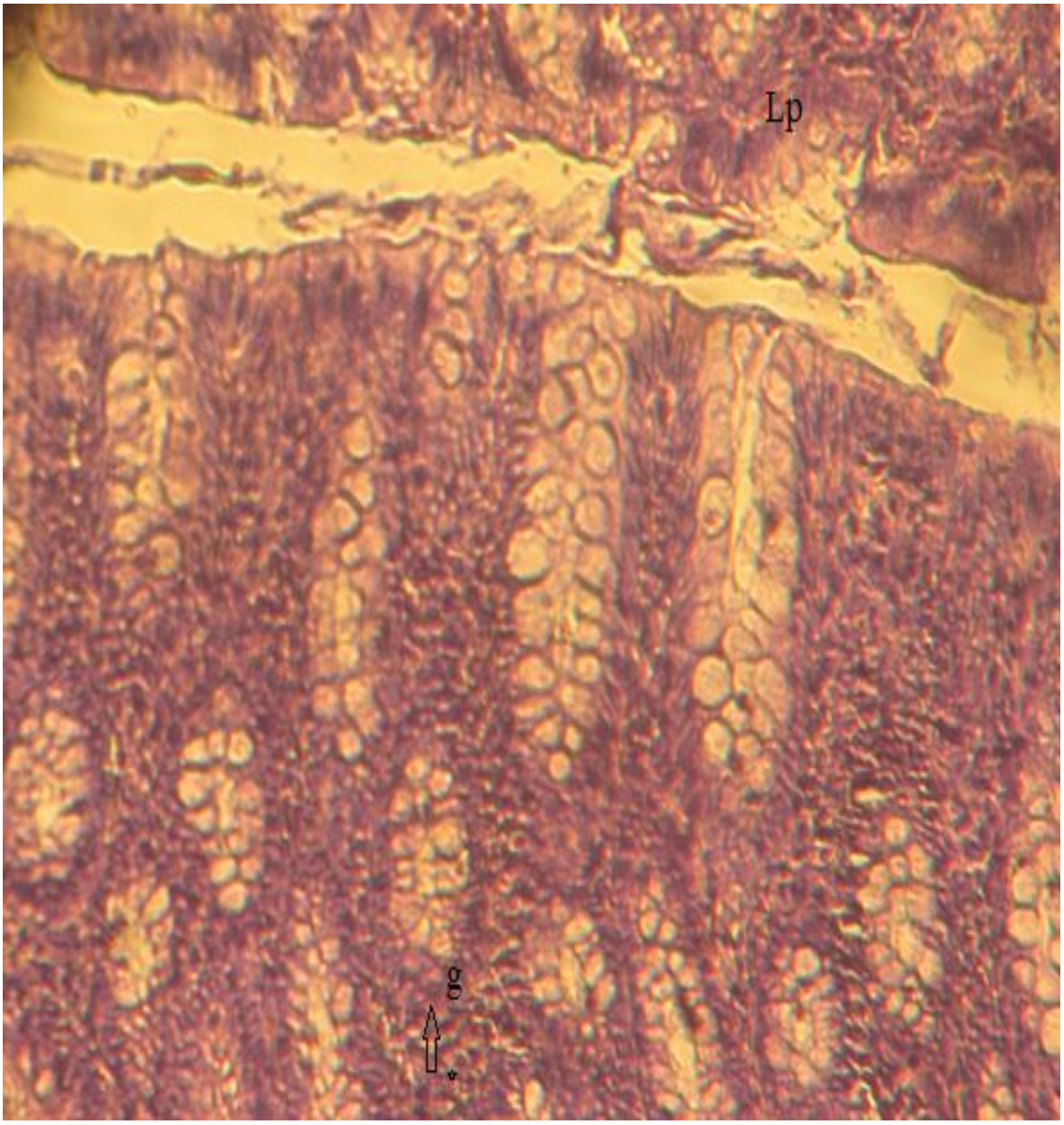

Plate 37: Section of the untreated ratôs rectum (control) group showing normal histopathological pattern of the lamina propria mucosae (Lp) and Goblet cell (g). Stain uptake: PAS. X400 magnification

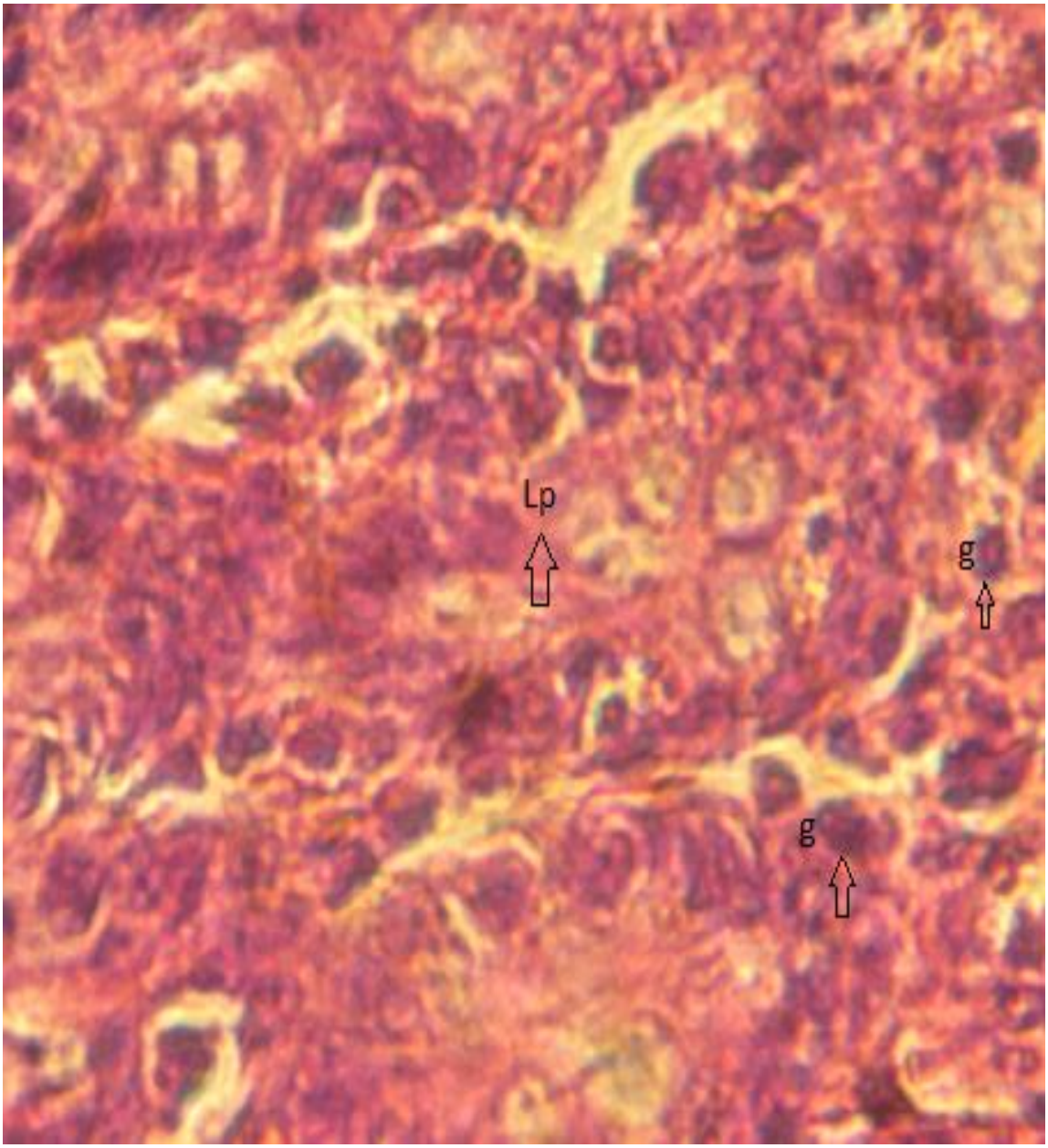

Plate 38: Section of high dose treated ratôs rectum C_1 (600mg/kg b.w. - oral administration). Showing normal histopathological pattern of the goblet cell and lamina propria mucosae (Lp). Stain uptake: Coleôs H&E X400 magnification

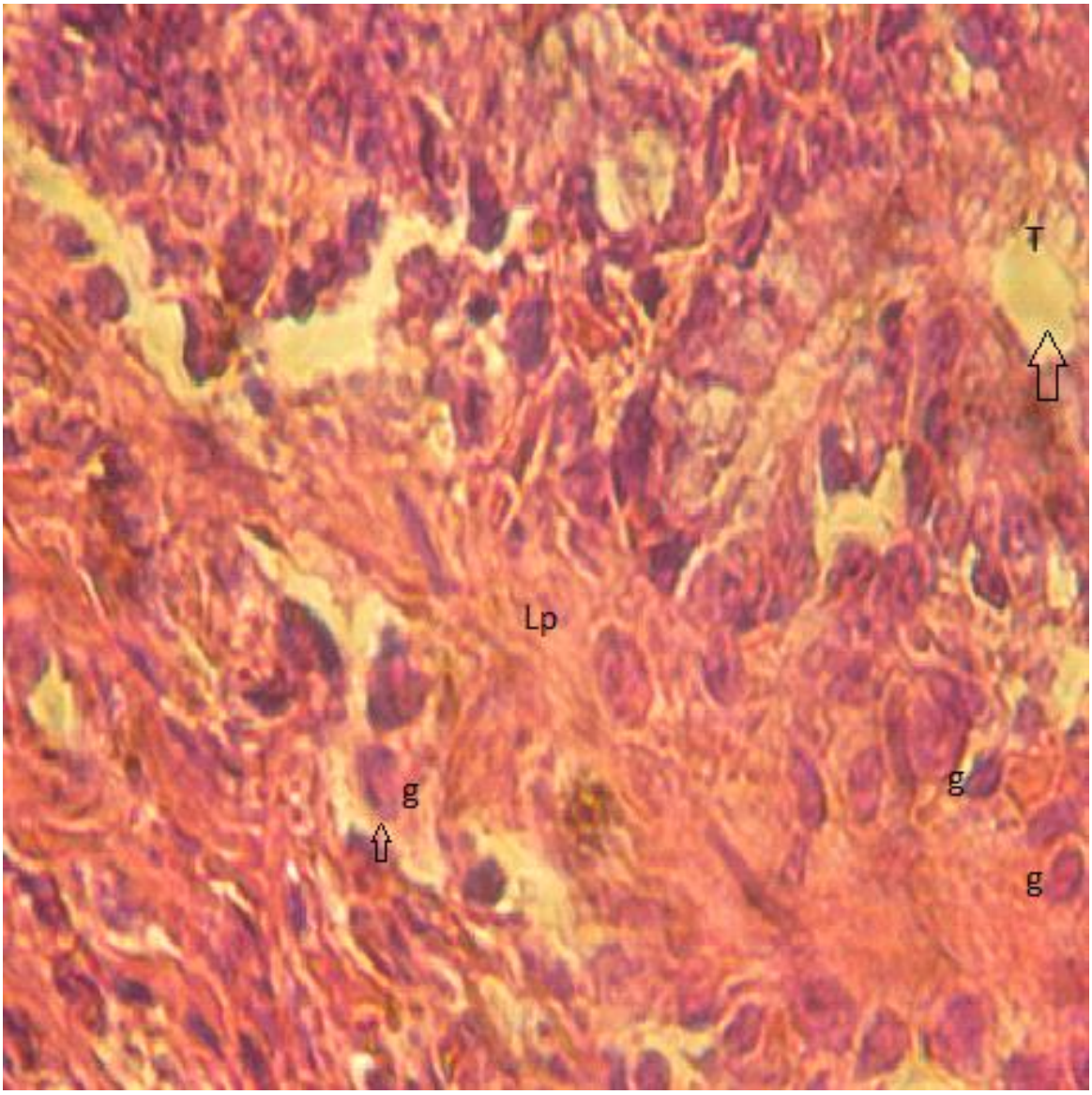

Plate 39: Section of high dose treated ratôs rectum D_1 (800mg/kg b.w. - oral administration). Showing normal histopathological pattern of the distended tube, goblet cell and lamina propria mucosae (Lp) Stain uptake: Coleôs H&E X400 magnification

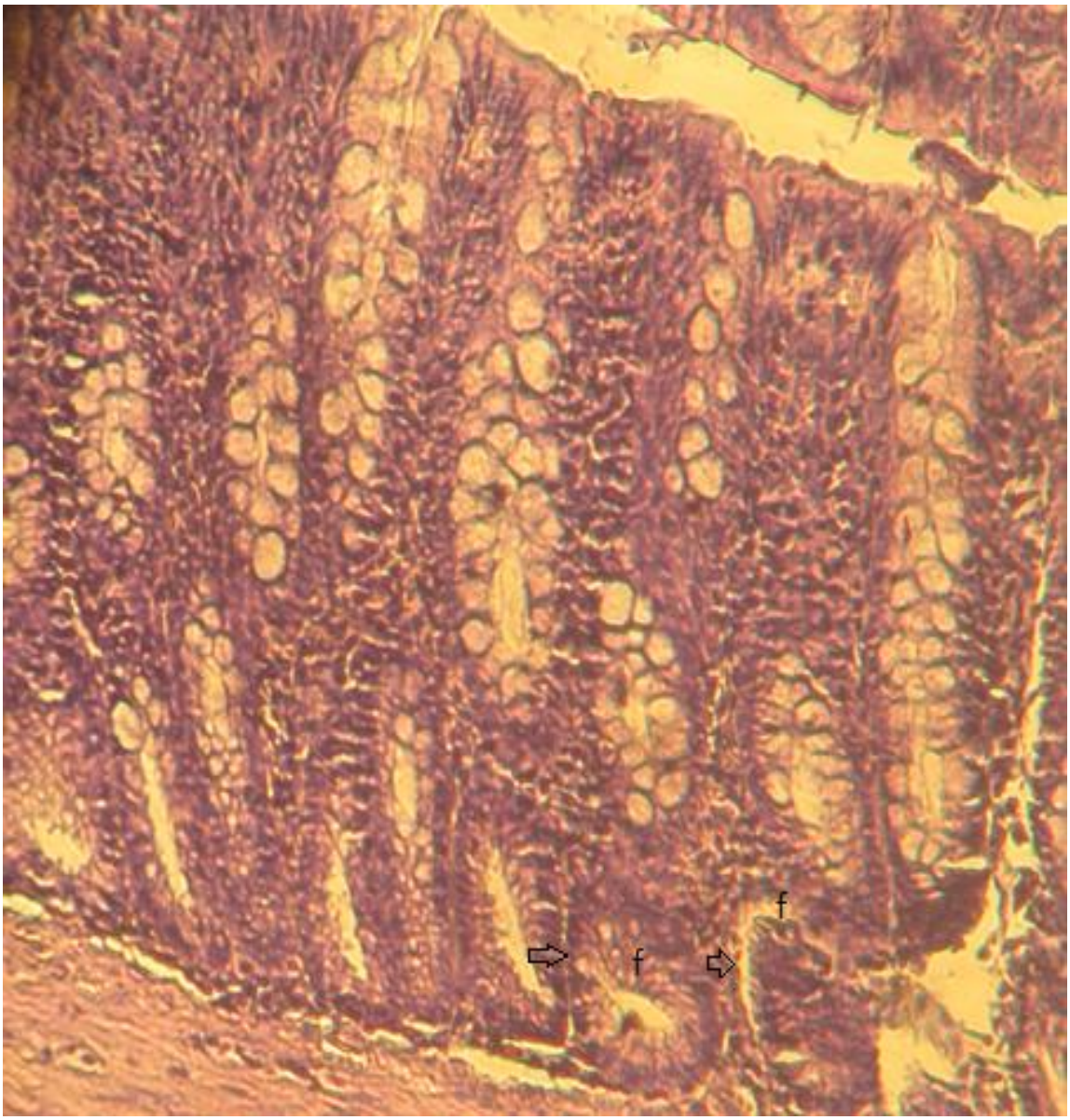

Plate 40: Section of high dose treated ratôs rectum C_1 (600mg/kg b.w. - oral administration) showing normal histopathological pattern of the rectum with normal rectal folds (f), stain uptake: PAS X400 magnification.

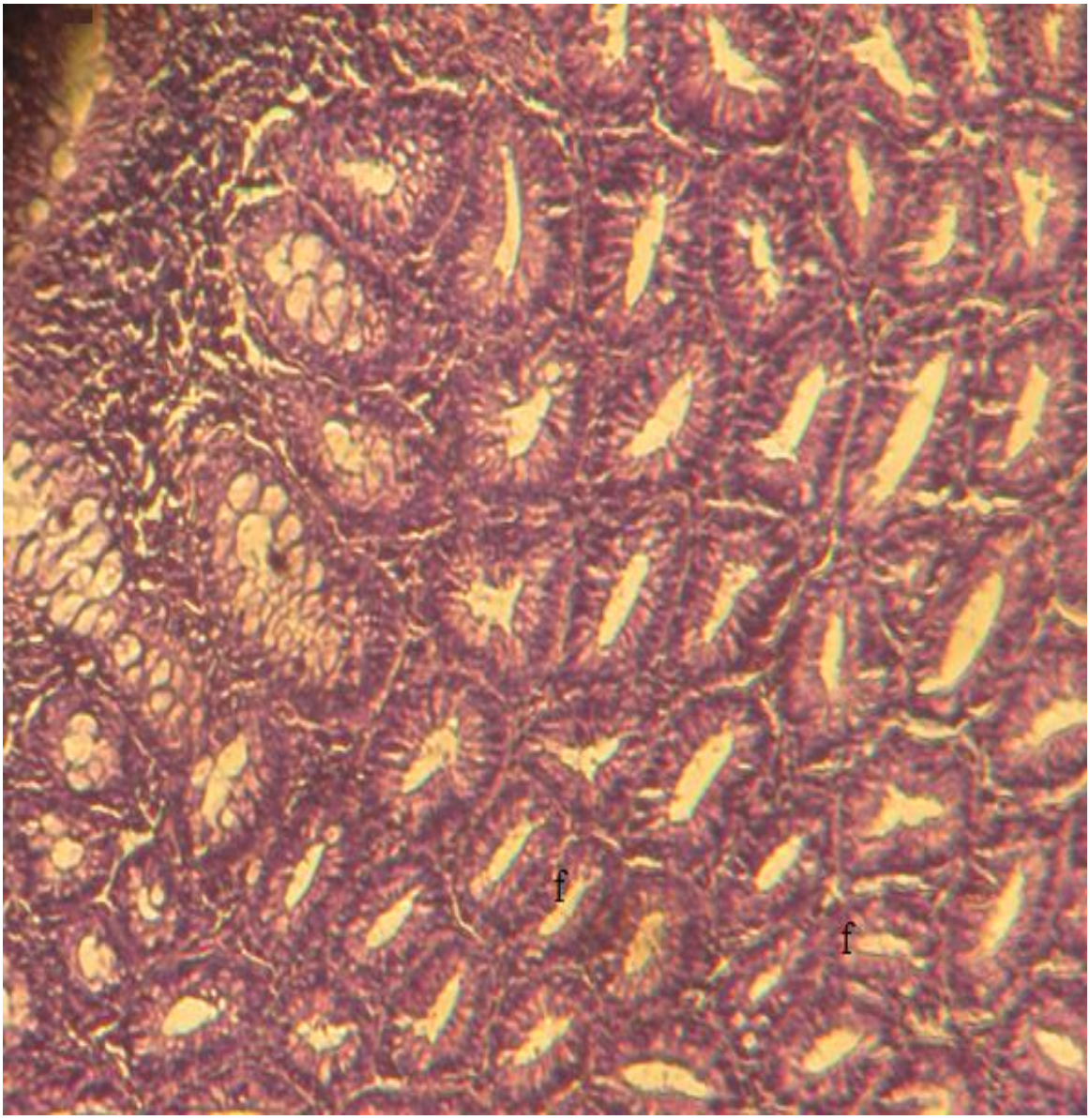

Plate 41: Section of the high dose treated ratôs rectum D_1 (800mg/kg b.w. - oral administration) showing normal histopathological pattern of the rectum with normal rectal folds (f). Stain uptake: PAS X400 magnification.

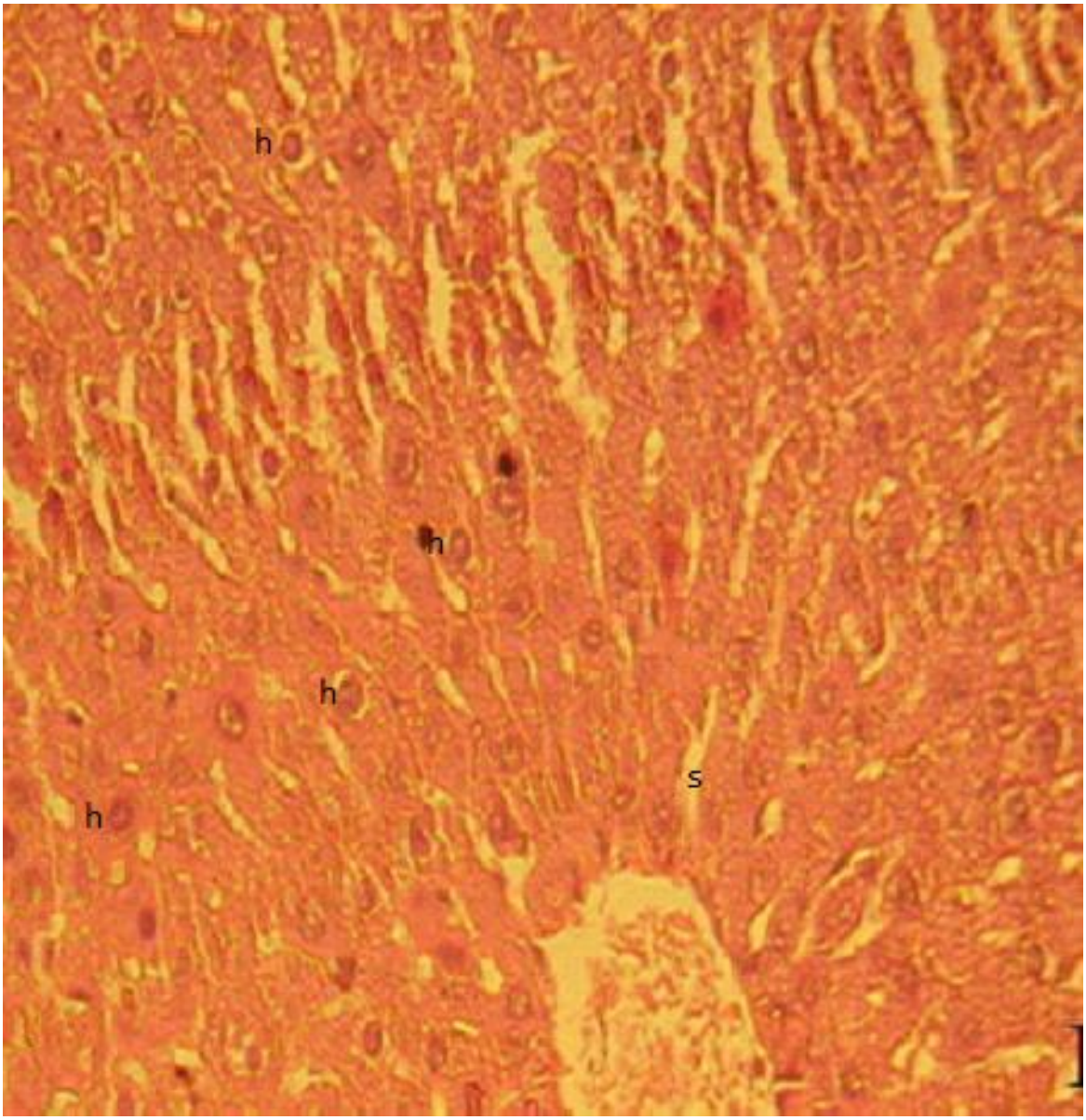

Plate 42: Section of the untreated ratôs liver (control), showing normal histopathological pattern of the liver, revealing the hepatocyte (h) and the sinusoid (s), stain uptake: Mayerôs H&E X400 magnification.

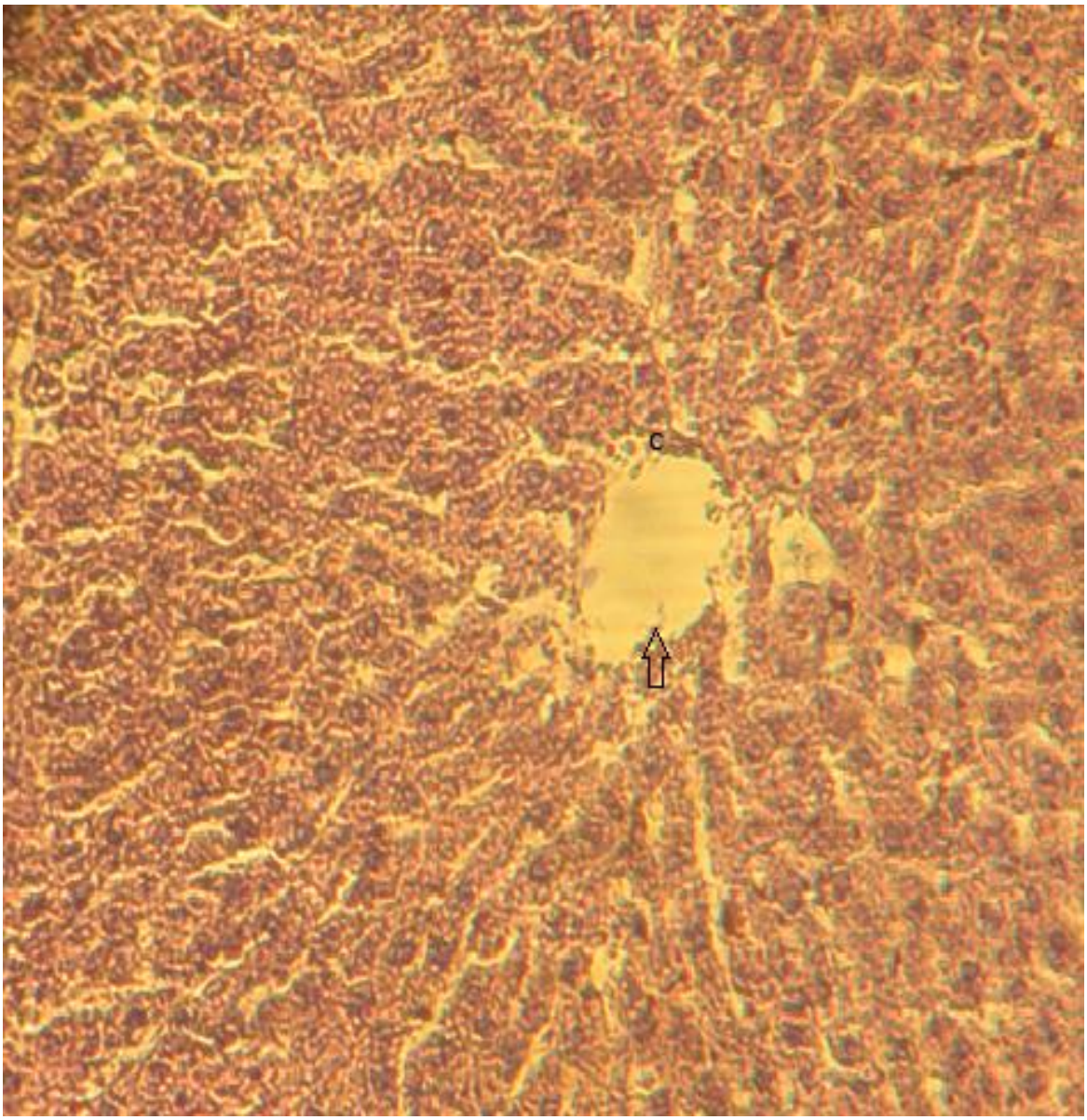

Plate 43: Section of the untreated ratôs liver (control), showing normal histopathological pattern of the liver, revealing the central vein (c), and stain uptake: PAS X400 magnification.

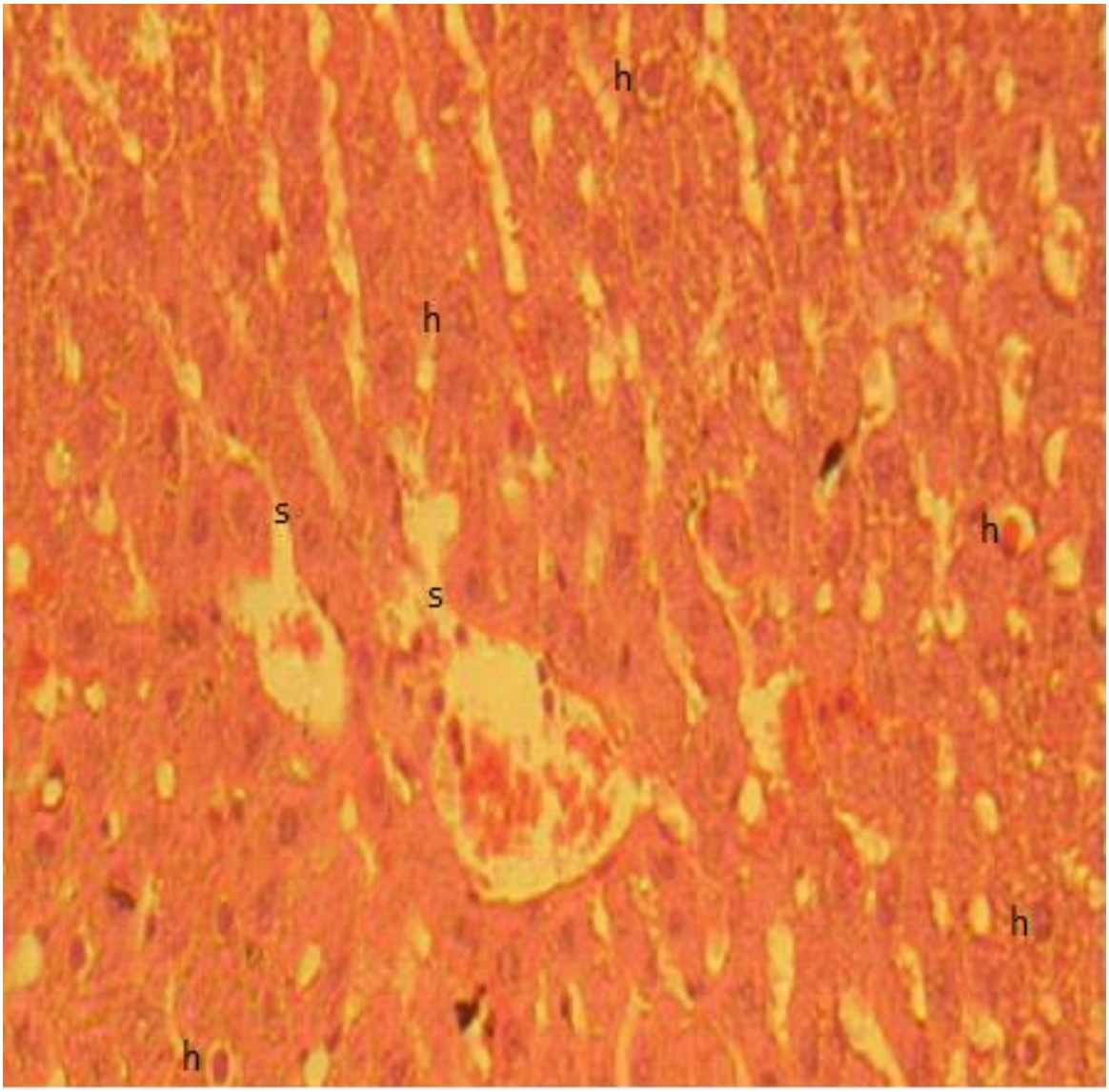

Plate 44: Section of the treated ratôs liver with high dose C_1 (600mg/kg b.w. oral administration), showing normal histological pattern of the Liver, revealing the hepatocyte (h) and the sinusoid (s), stain uptake: H&E X400 magnification.

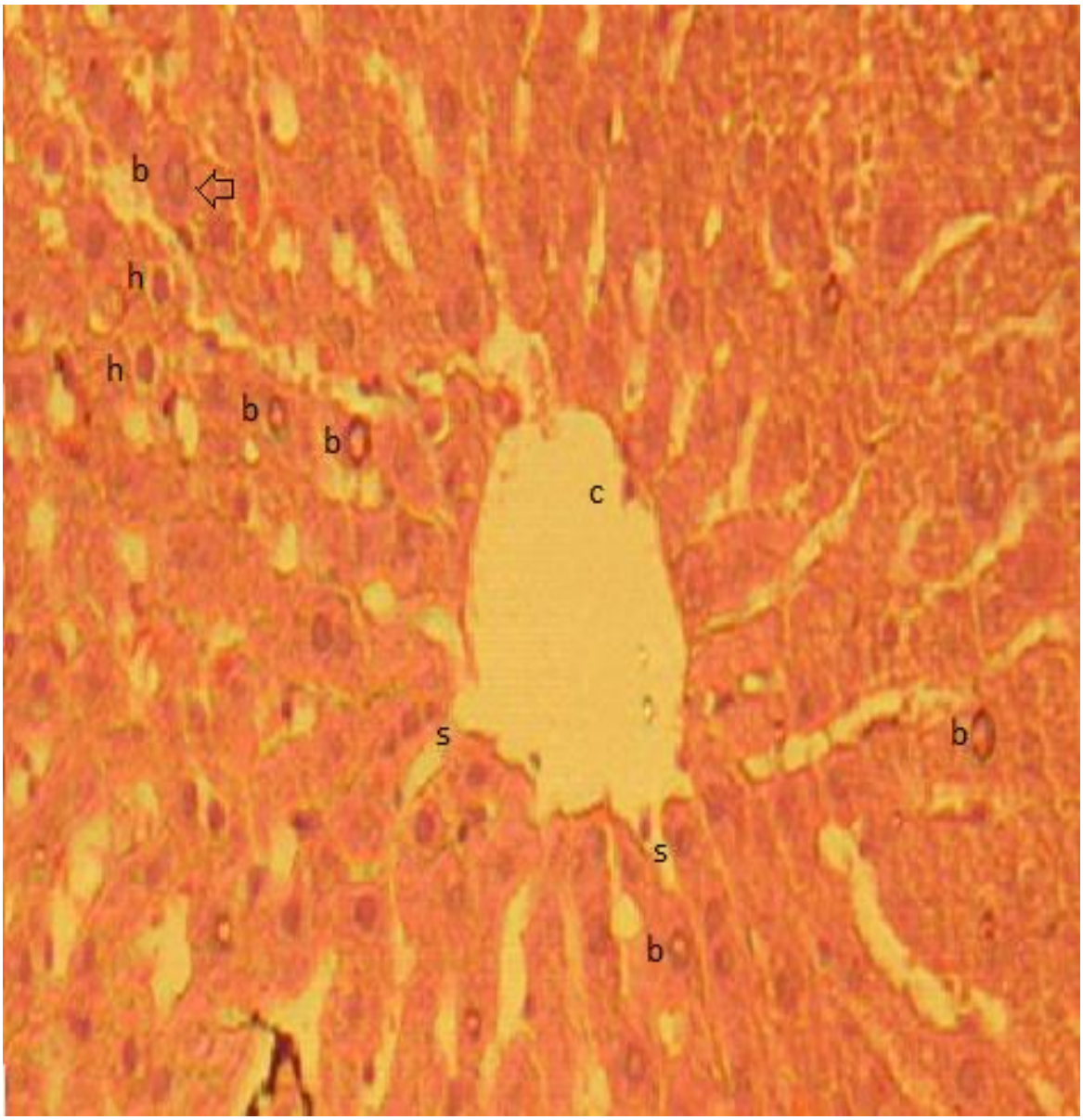

Plate 45: Section of treated ratôs liver with high dose D_1 (800mg/kg b.w. oral administration of *Acalypha wilkesiana* crude leaf extract) showing normal histopathological pattern of the liver, with the sinusoid (s), central vein (c), bile duct (b) and the hepatocyte (h). Stain uptake- Mayerôs H&E X400 magnification.

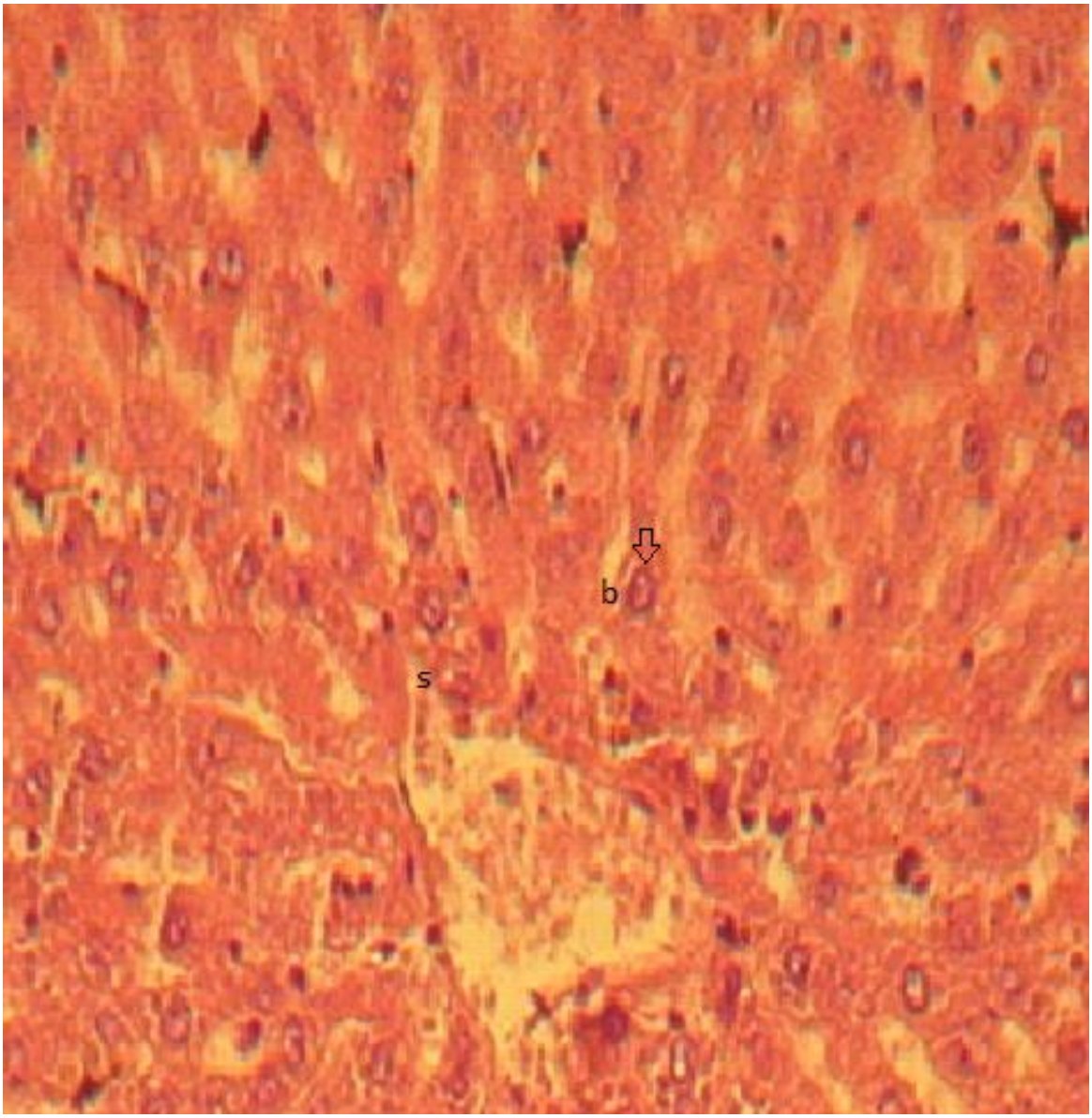

Plate 46: Section of treated ratôs liver with high dose C_2 (600mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) showing normal histopathological pattern of the liver, with the sinusoid (s) and the bile duct (b). Stain uptake- Mayerôs H&E X400 magnification.

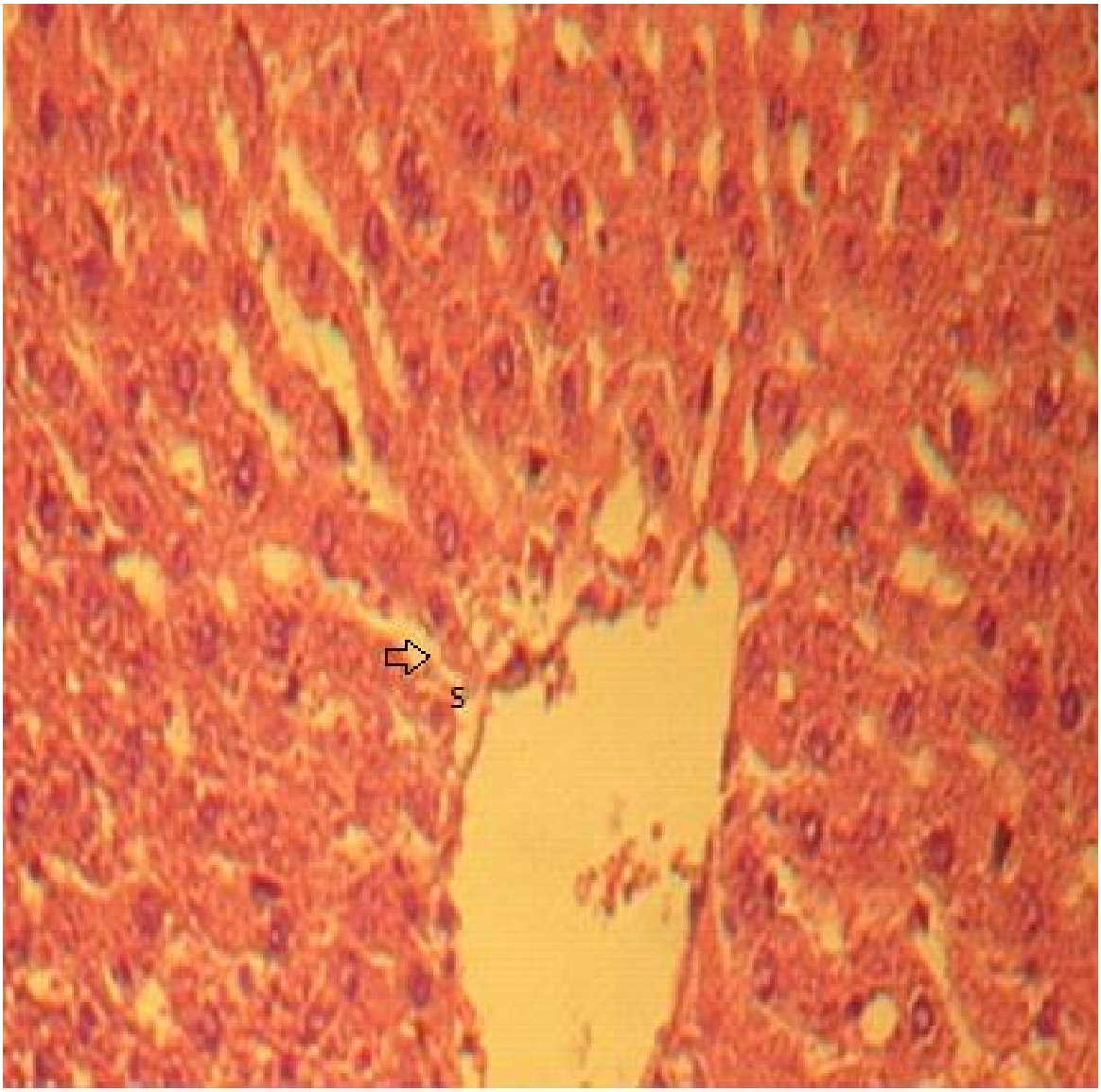

Plate 47: Section of treated ratôs liver with high dose D_2 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) showing normal histopathological pattern of the liver, with the sinusoid (s), stain uptake- Mayerôs H&E X400 magnification.

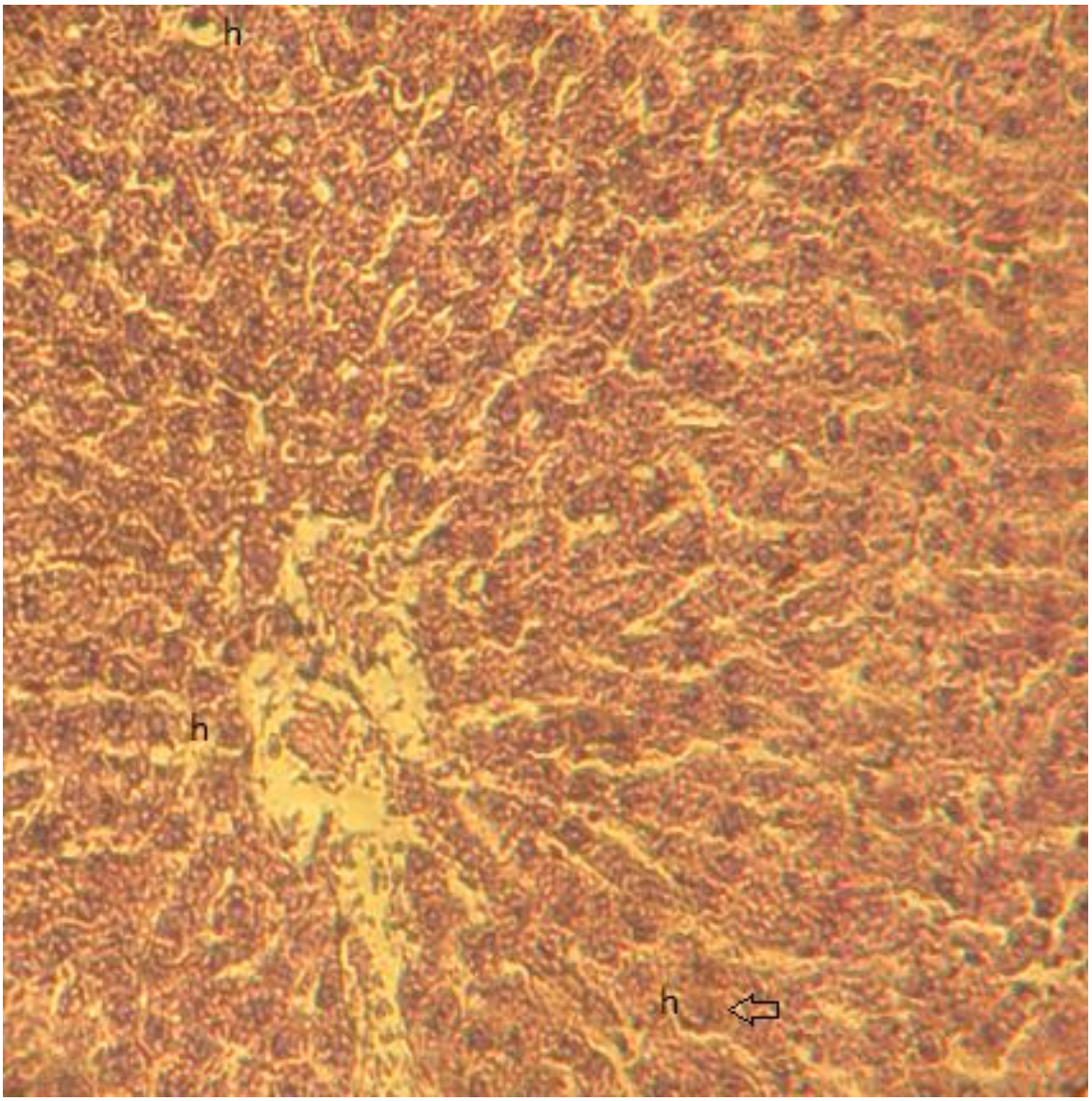

Plate 48: Section of treated ratôs liver with high dose D_1 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) showing normal histopathological pattern of the liver, revealing the hepatocyte (h), stain uptake- PAS X400 magnification.

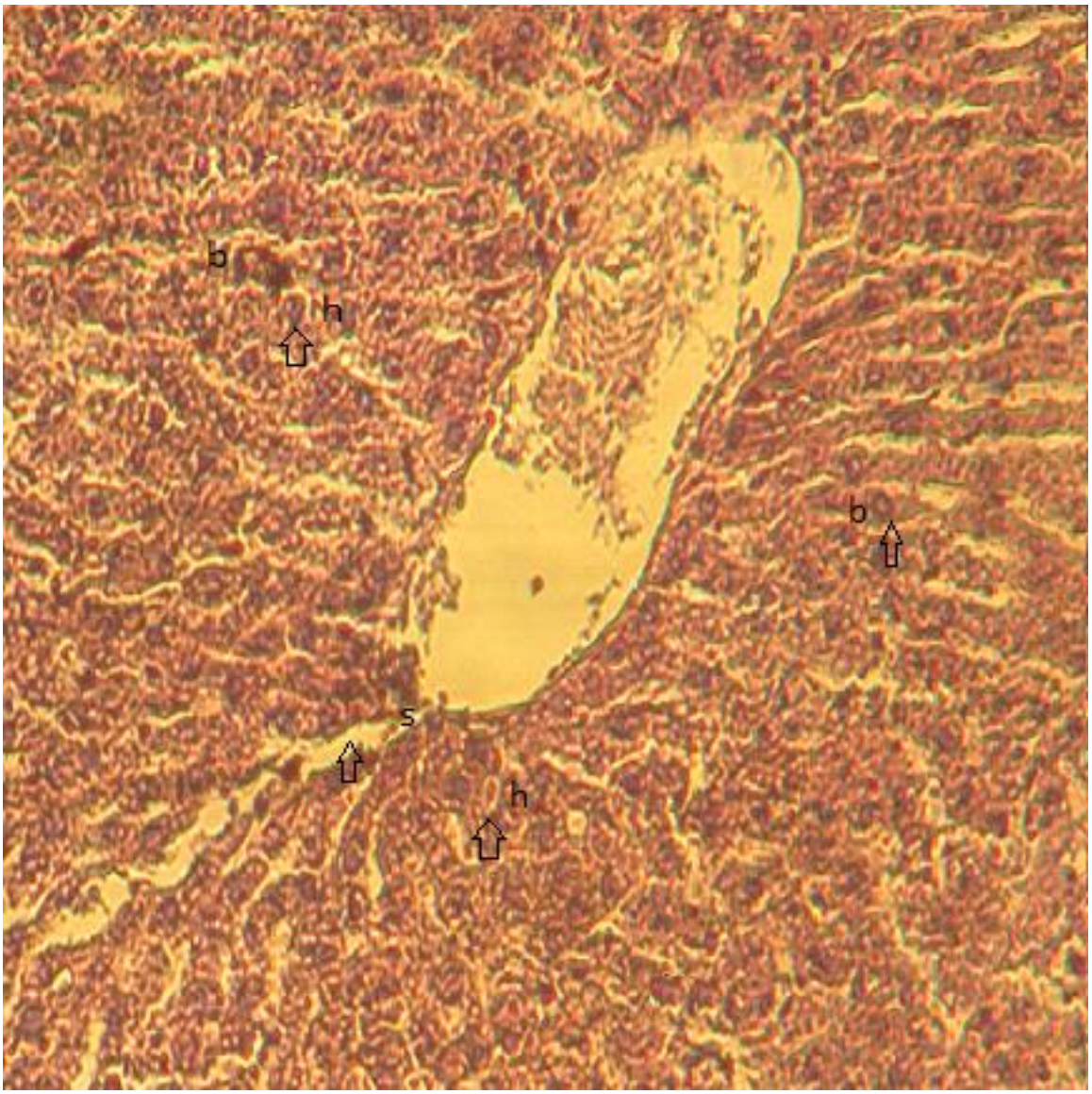

Plate 49: Section of treated ratôs liver with high dose D_2 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) showing normal histopathological pattern of the Liver, revealing the hepatocyte (h), stain uptake- PAS X400 magnification.

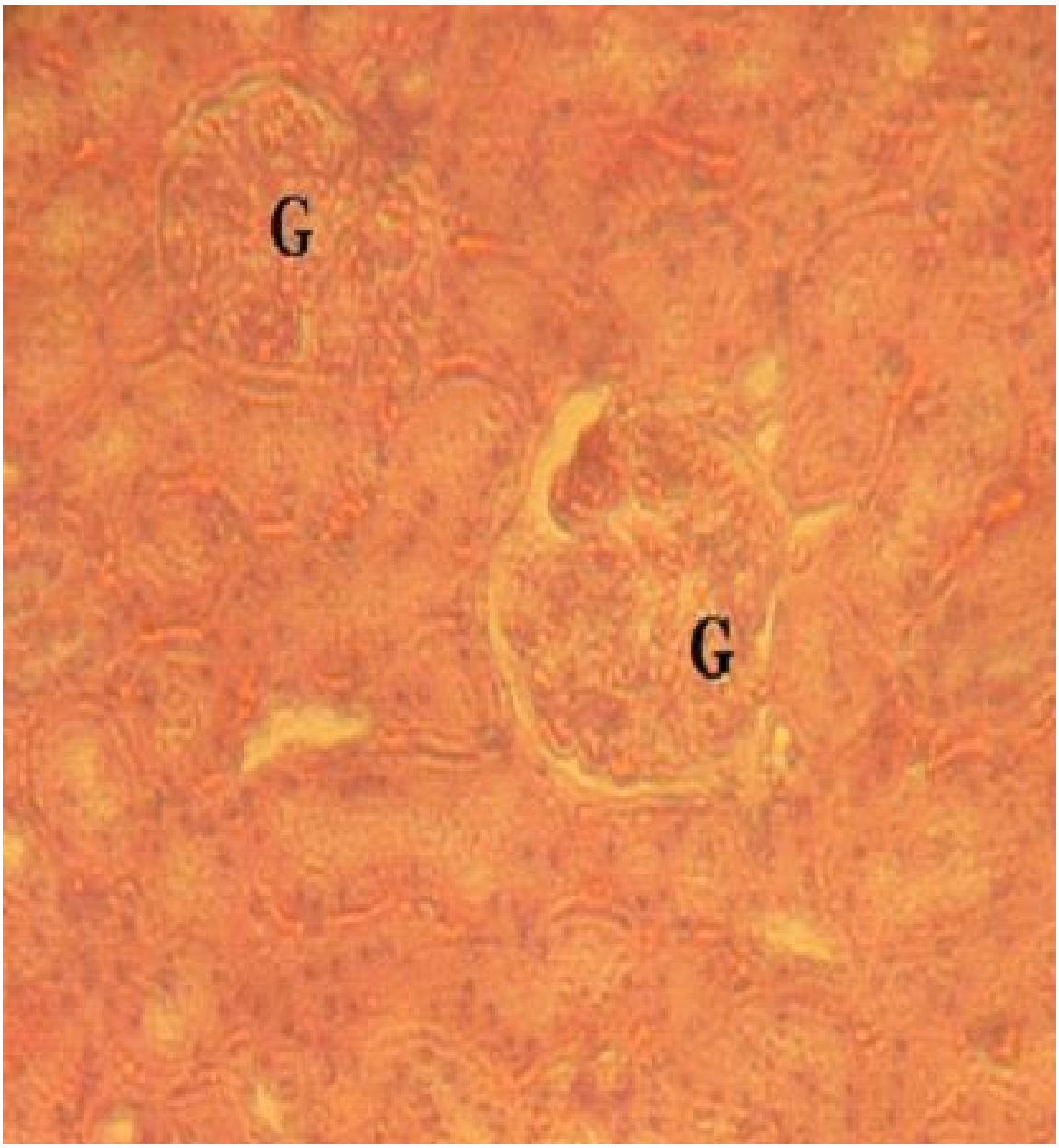

Plate 50: Section of the untreated ratôs kidney (Cortex) group E_1 and E_2, showing normal histopathological pattern of the Glomerulus (G). Stain uptake: Mayerôs H&E X400 magnification.

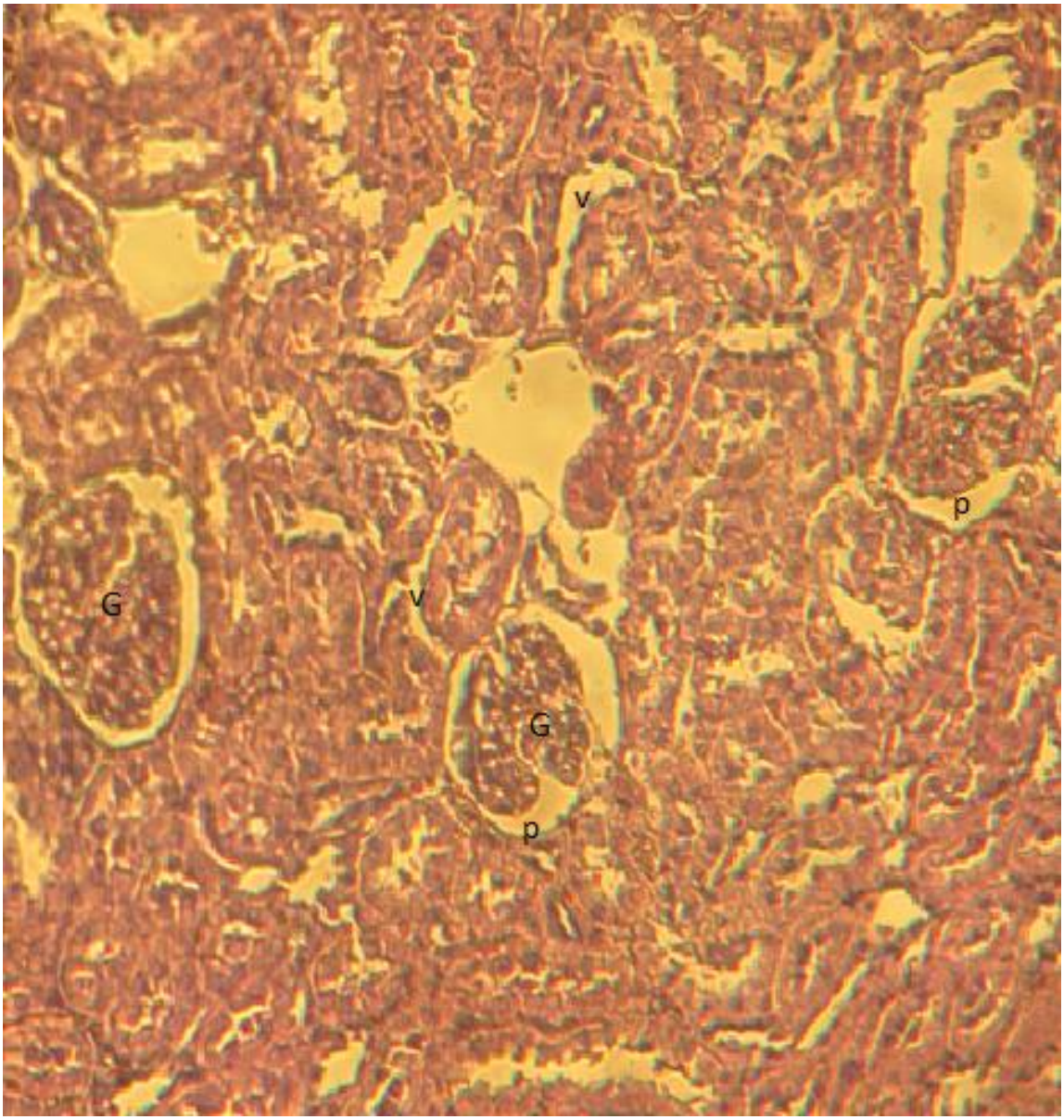

Plate 51: Section of the untreated ratôs kidney (Cortex) group E_1 and E_2, showing normal Histopathological pattern of the kidney, revealing the glomerulus (G), parietal layer (p) and the visceral layer (v), stain uptake: PAS X400 magnification.

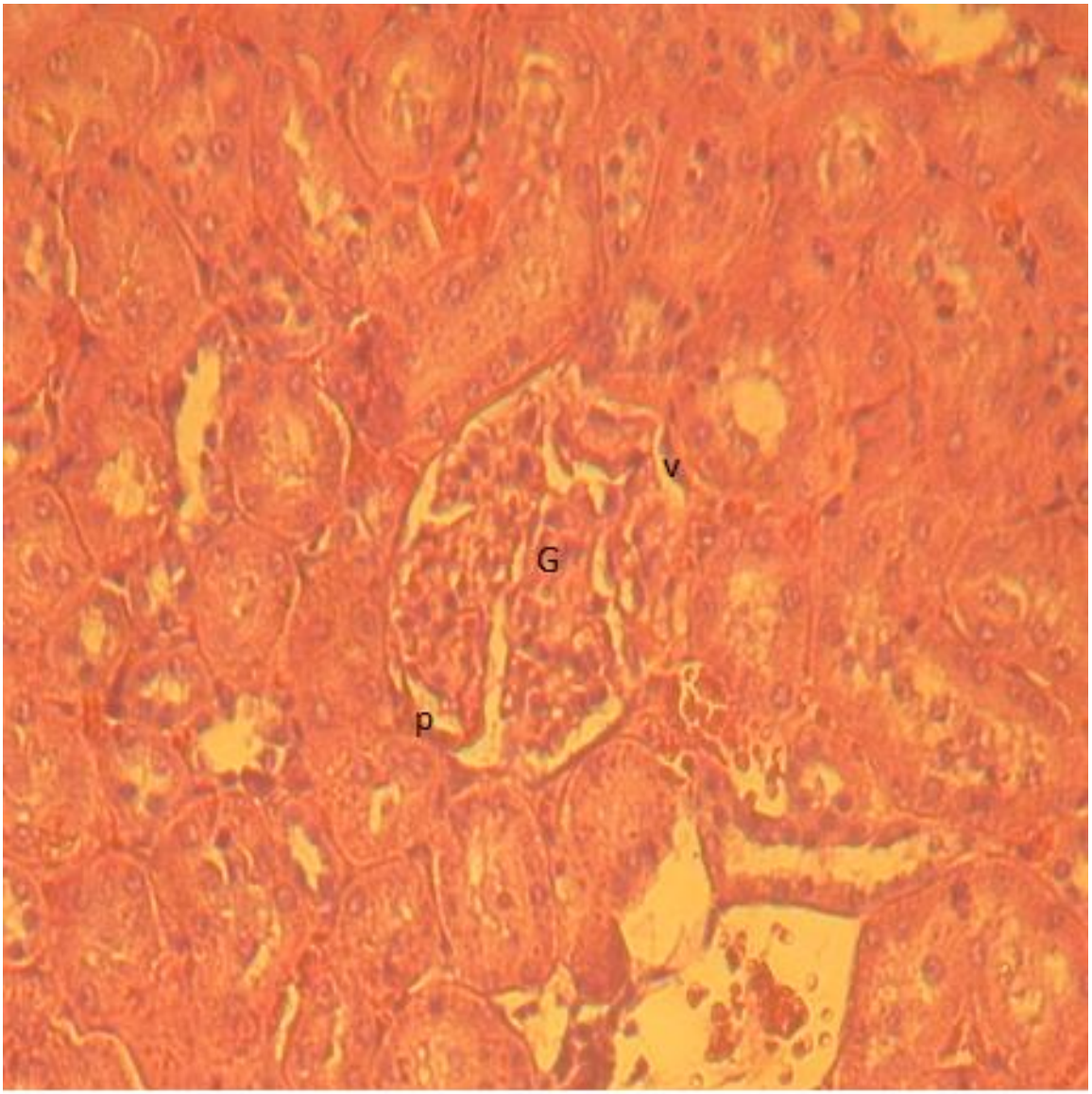

Plate 52: Section of treated ratôs kidney (cortex) with high dose C_1 (600mg/kg b.w. oral administration of *Acalypha wilkesiana* extract) showing normal histopathological pattern of the Kidney (Cortex) revealing the glomerulus (G), parietal layer (p) and the visceral layer (v). Stain uptake: Mayerôs H&E X400 magnification

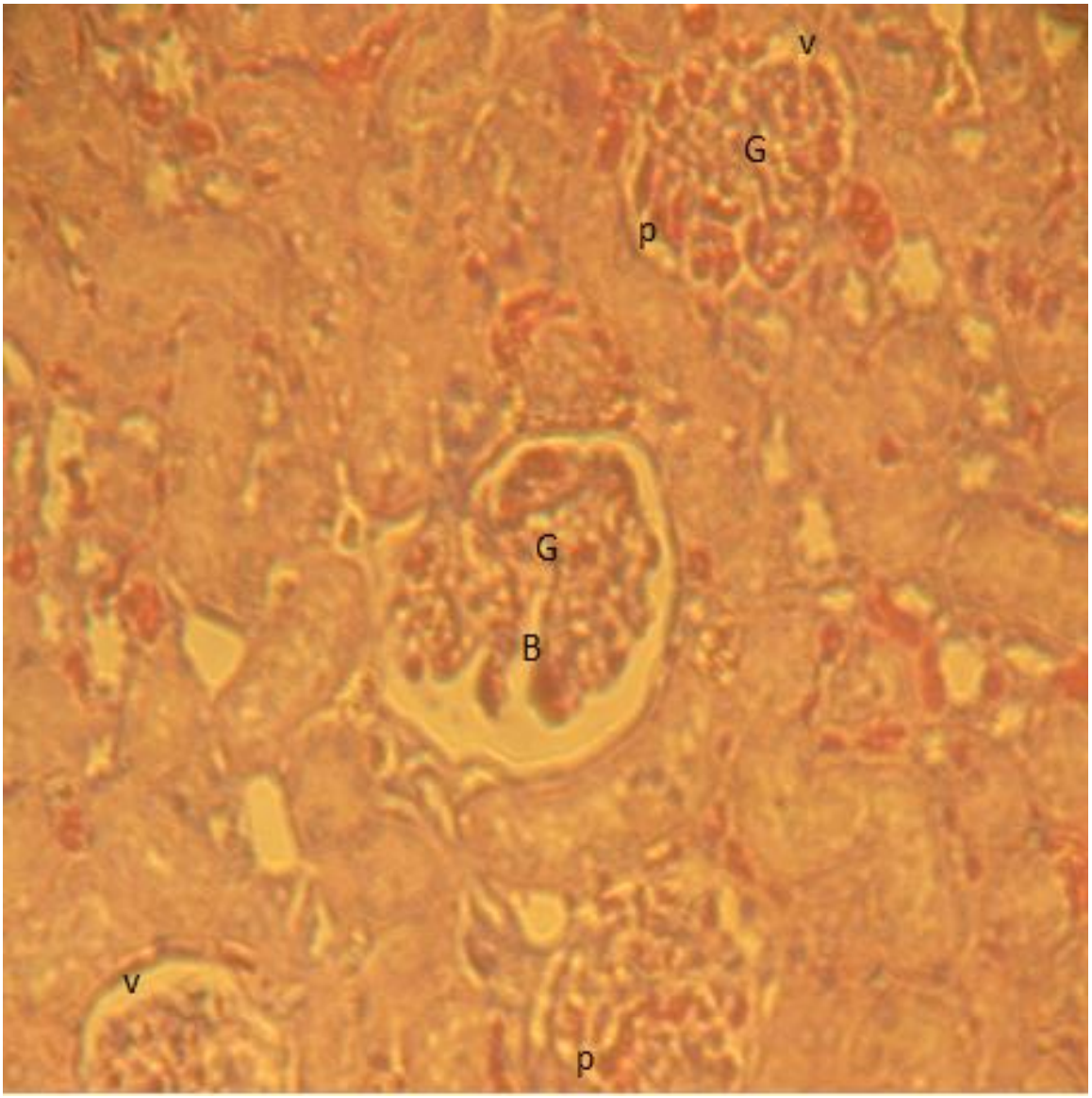

Plate 53: Section of treated ratôs kidney (cortex) with high dose D_1 (800mg/kg b.w. oral administration of *Acalypha wilkesiana* extract) showing normal histopathological pattern of the Kidney (Cortex) revealing the glomerulus (G), bowmanôs space (B), parietal layer (p) and the visceral layer (v), stain uptake: Mayerôs H&E X400 magnification.

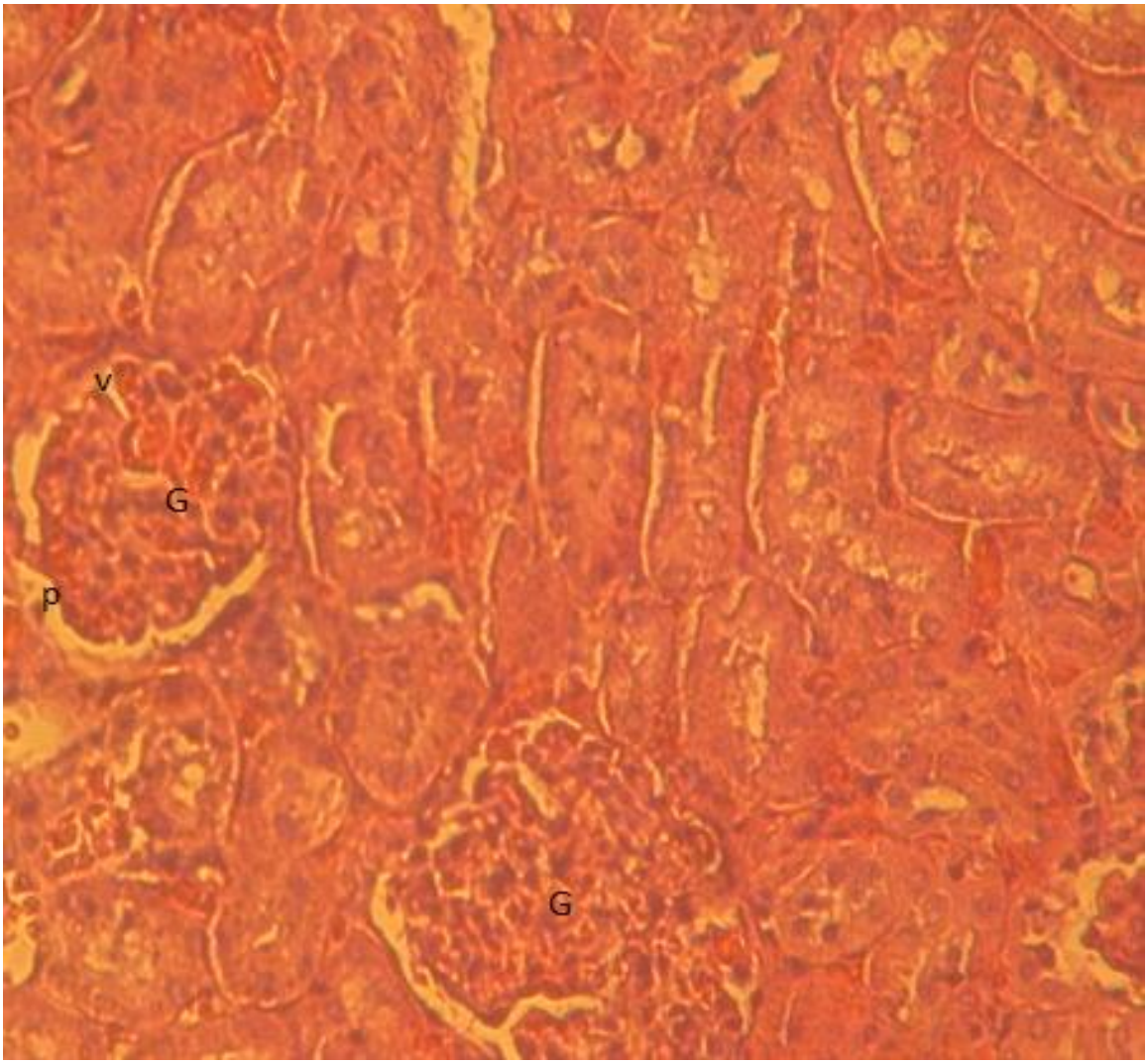

Plate 54: Section of treated ratôs kidney (cortex) with high dose C_2 (600mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* extract) showing normal histopathological pattern of the Kidney (Cortex) revealing the glomerulus (G), parietal layer (p) and the visceral layer (v), stain uptake: Mayerôs H&E X400 magnification.

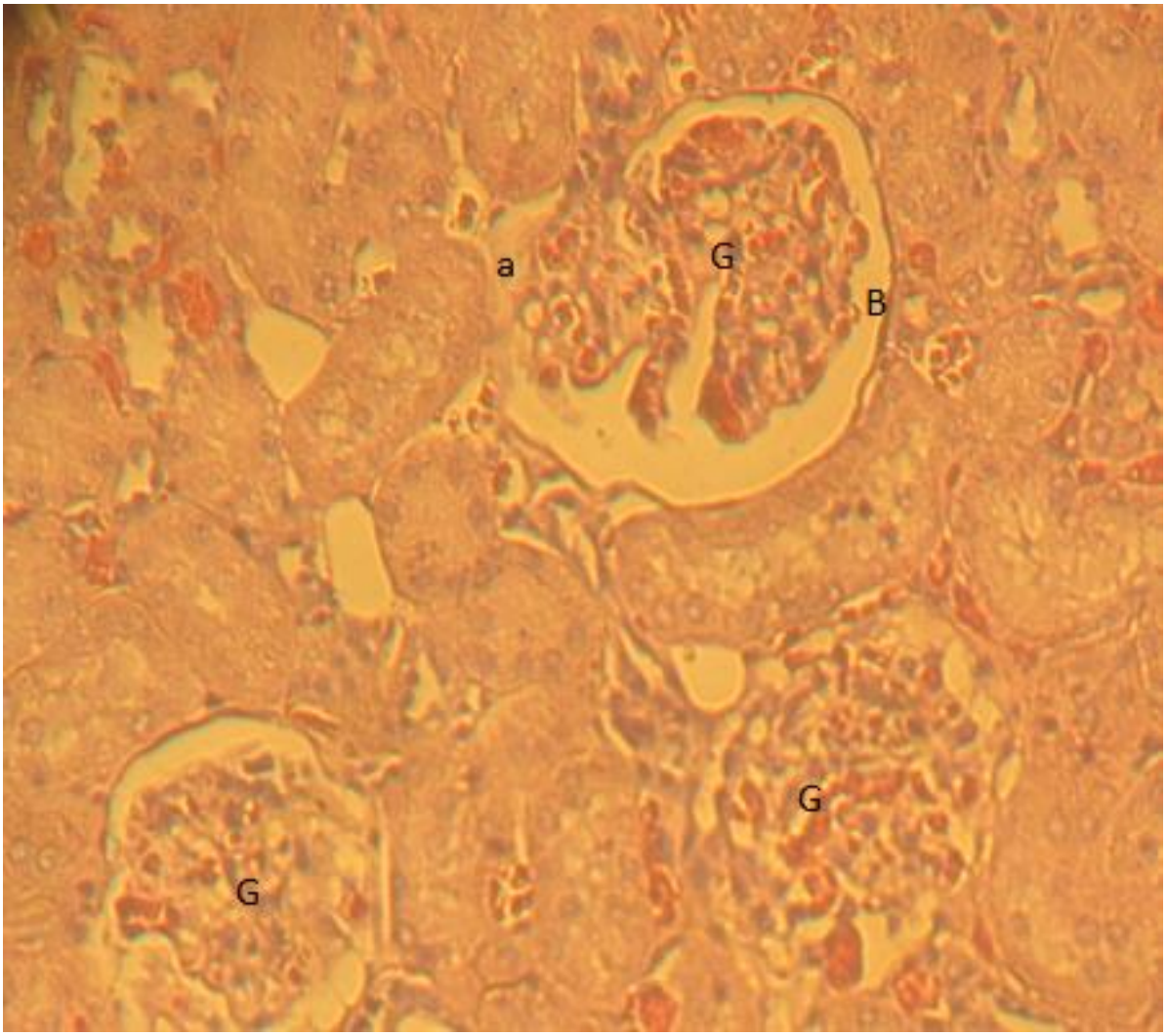

Plate 55: Section of treated ratôs right kidney (cortex) with high dose D_2 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* extract) showing normal histopathological pattern of the Kidney (Cortex) revealing the glomerulus (G), Bowmanôs space (B) and afferent glomerular arteriole (a). Stain uptake: Mayerôs H&E X400 magnification.

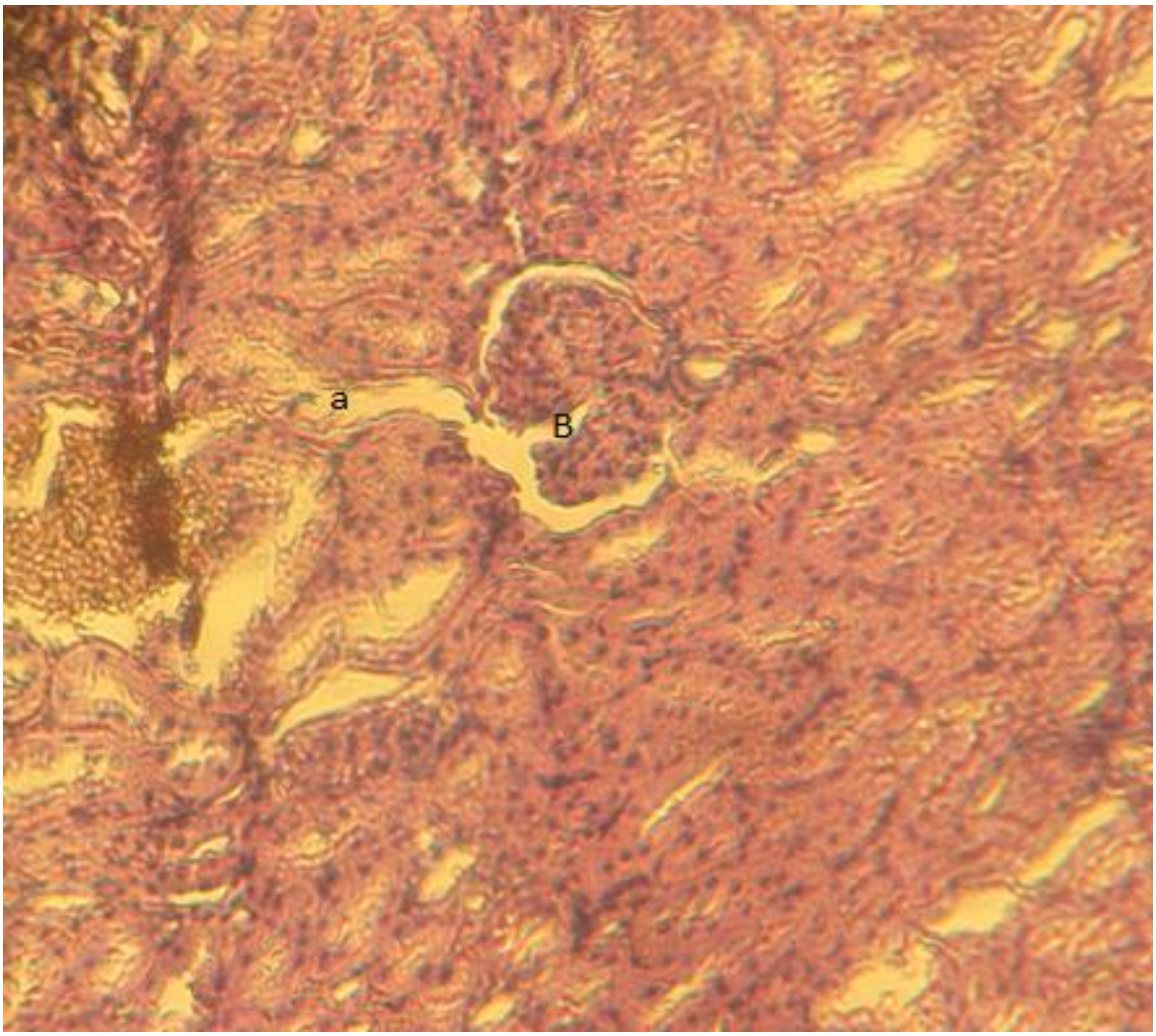

Plate 56: Section of treated ratôs right kidney (cortex) with high dose D_1 (800mg/kg b.w. oral administration of *Acalypha wilkesiana* extract) showing normal histopathological pattern of the Kidney (Cortex) revealing the Bowmanôs space (B) and afferent glomerular arteriole (a). Stain uptake: PAS X400 magnification.

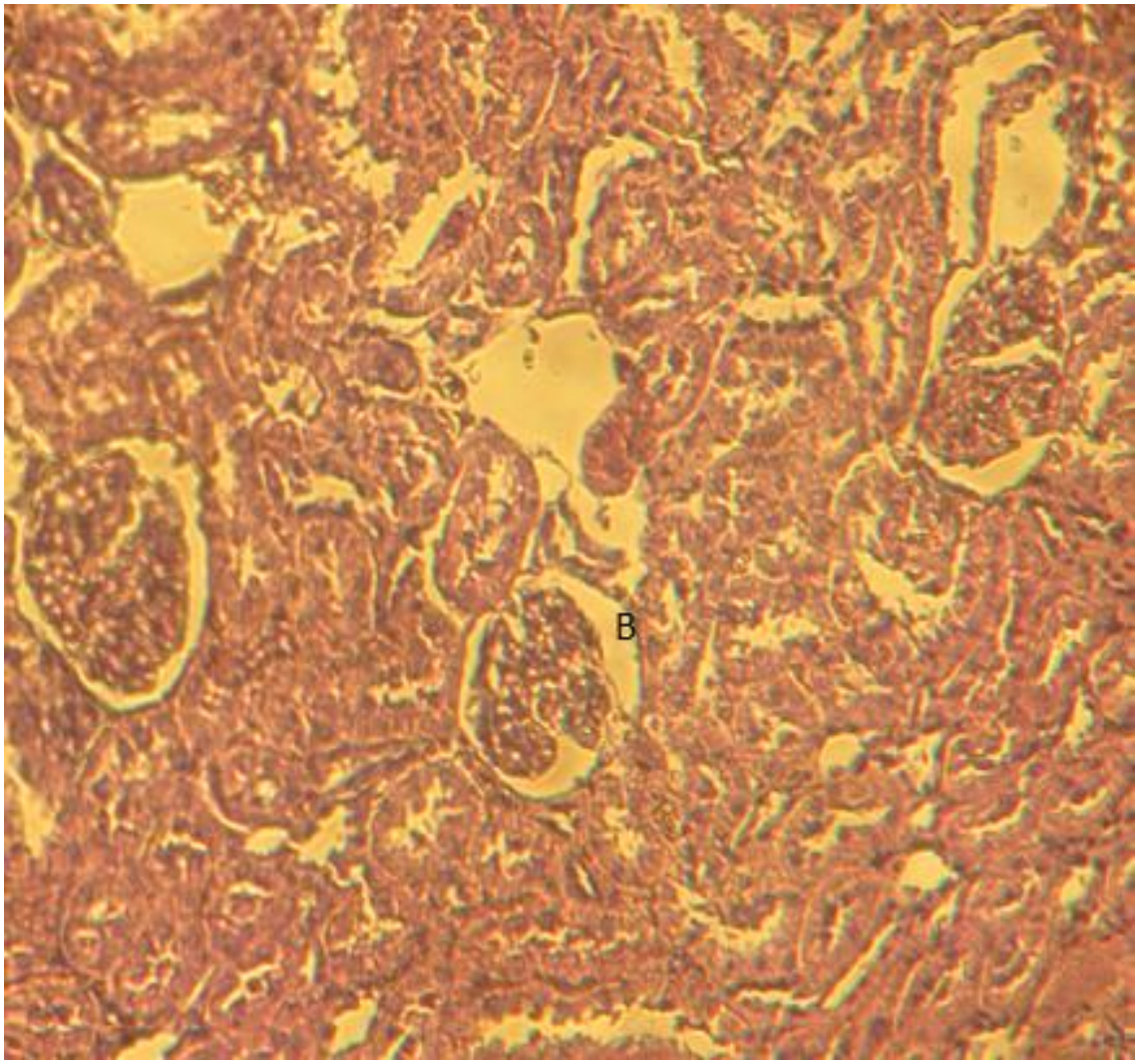

Plate 57: Section of treated ratôs right kidney (cortex) with high dose D_2 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* extract) showing normal histopathological pattern of the Kidney (Cortex) revealing the Bowmanôs space (B). Stain uptake: PAS X400 magnification.

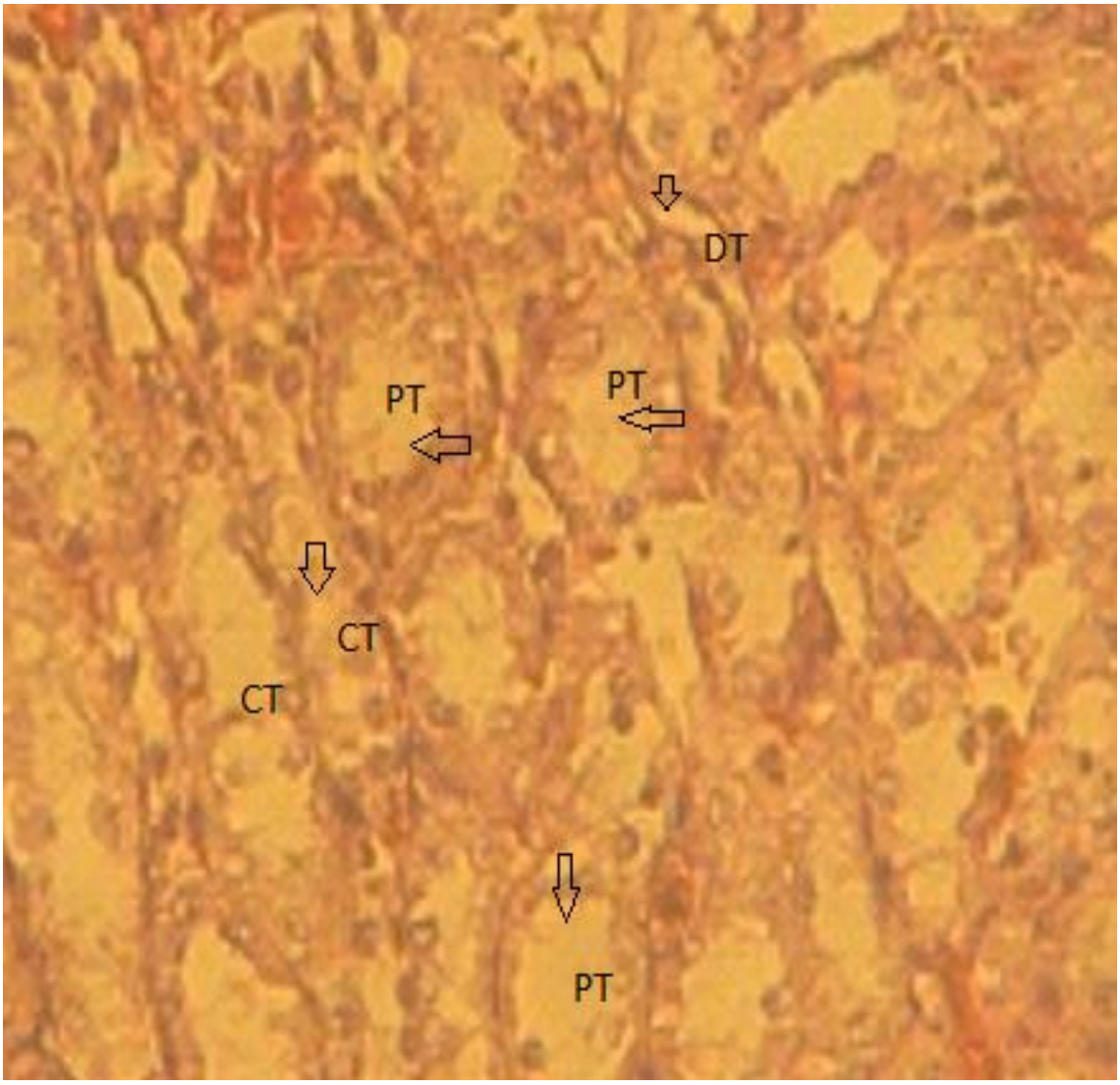

Plate 58: Section of the untreated ratôs kidney (Medulla) group E_1 and E_2, showing normal histopathological pattern of the kidney (medulla), while revealing the proximal tubules (PT), collecting tubules (CT) and the distal tubule (DT). Stain uptake: Mayerôs H&E X400 magnification.

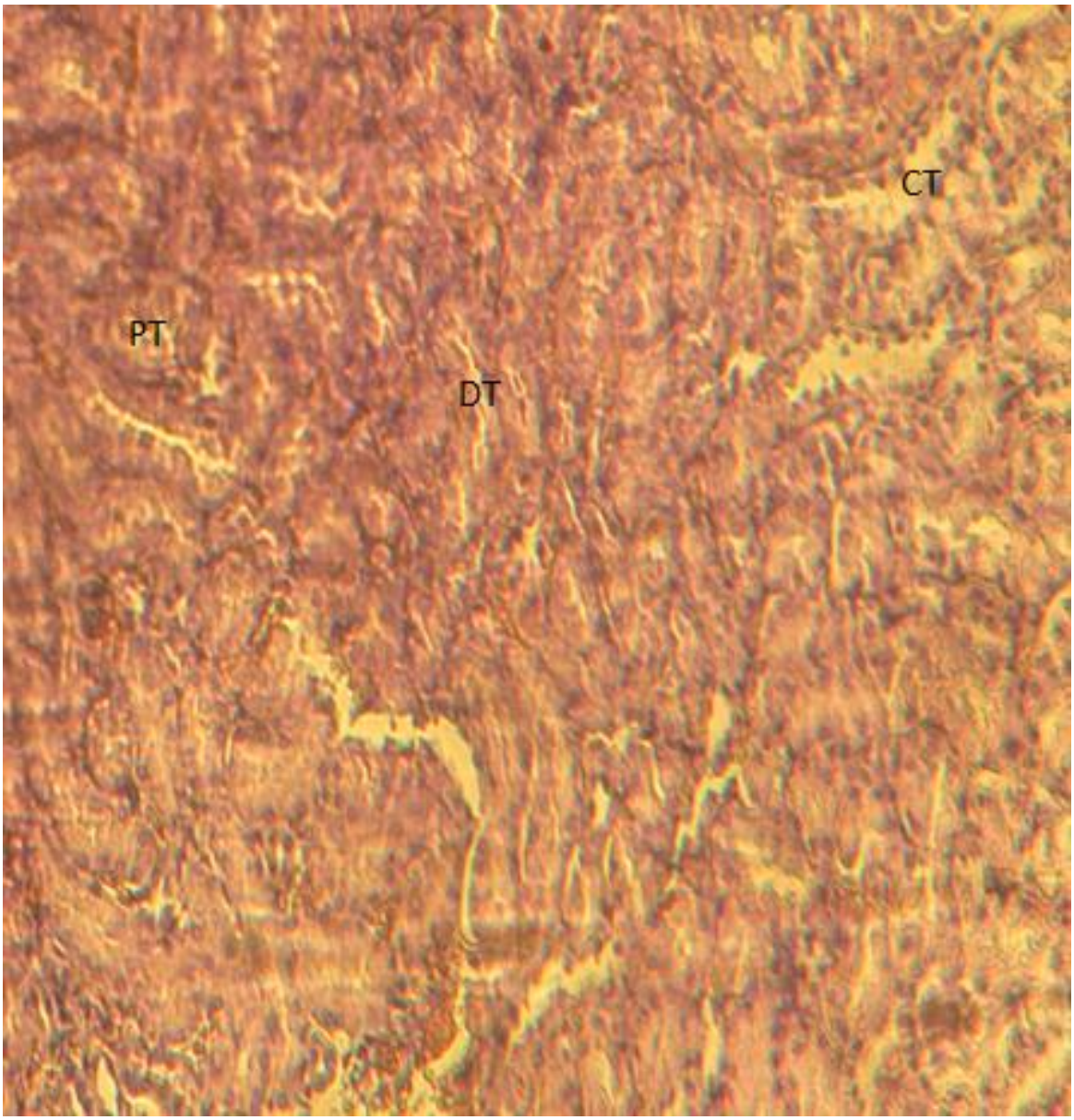

Plate 59: Section of untreated ratôs right kidney (Medulla) group E_1 and E_2, showing normal histopathological pattern of the kidney (medulla), while revealing the proximal tubules (PT), collecting tubules (CT) and the distal tubule (DT). Stain uptake: PAS X400 magnification.

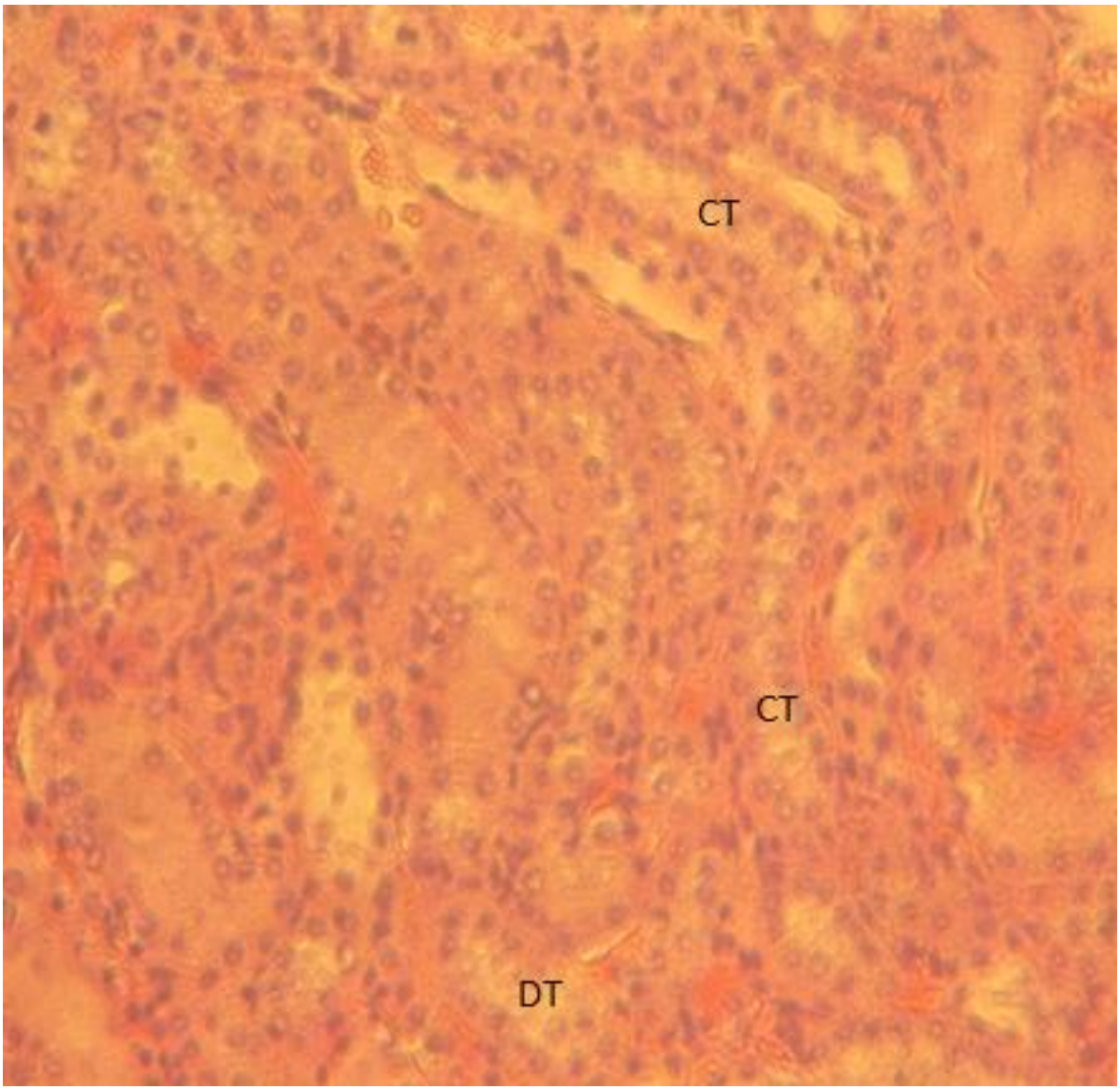

Plate 60: Section of treated ratôs kidney (medulla) with high dose C_1 (600mg/kg b.w. oral administration of *Acalypha wilkesiana* leaf extract) showing normal histopathological pattern of the kidney (medulla) revealing the distal tubule (DT) and the collecting tubule (CT). Stain uptake: Mayerôs H&E X400 magnification.

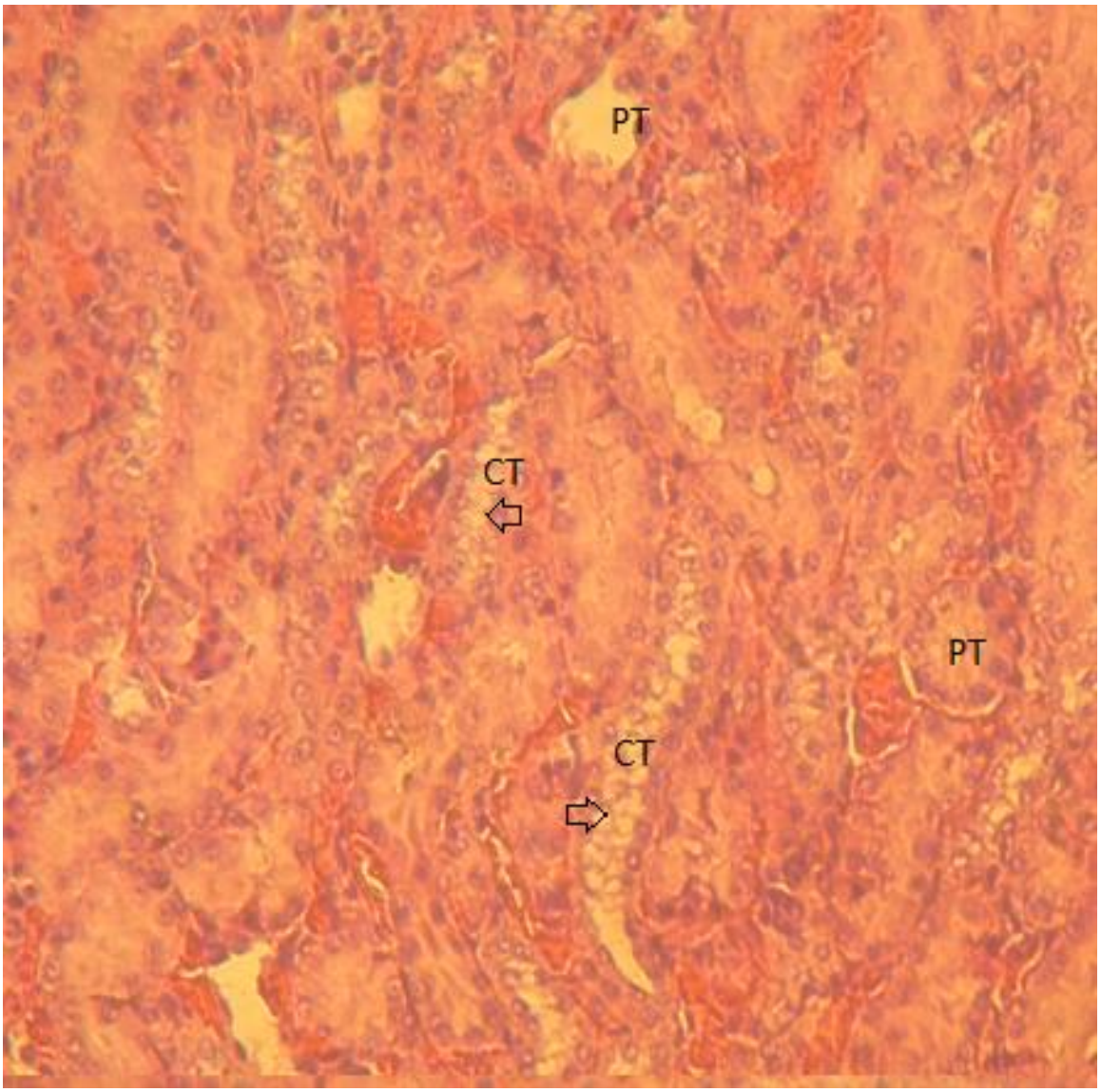

Plate 61: Section of treated ratôs kidney (medulla) with high dose D_1 (800mg/kg b.w. oral administration of *Acalypha wilkesiana* leaf extract) showing normal histopathological pattern of the kidney (medulla) revealing the distal tubule (DT) and the collecting tubule (CT). Stain uptake: Mayerôs H&E X400 magnification.

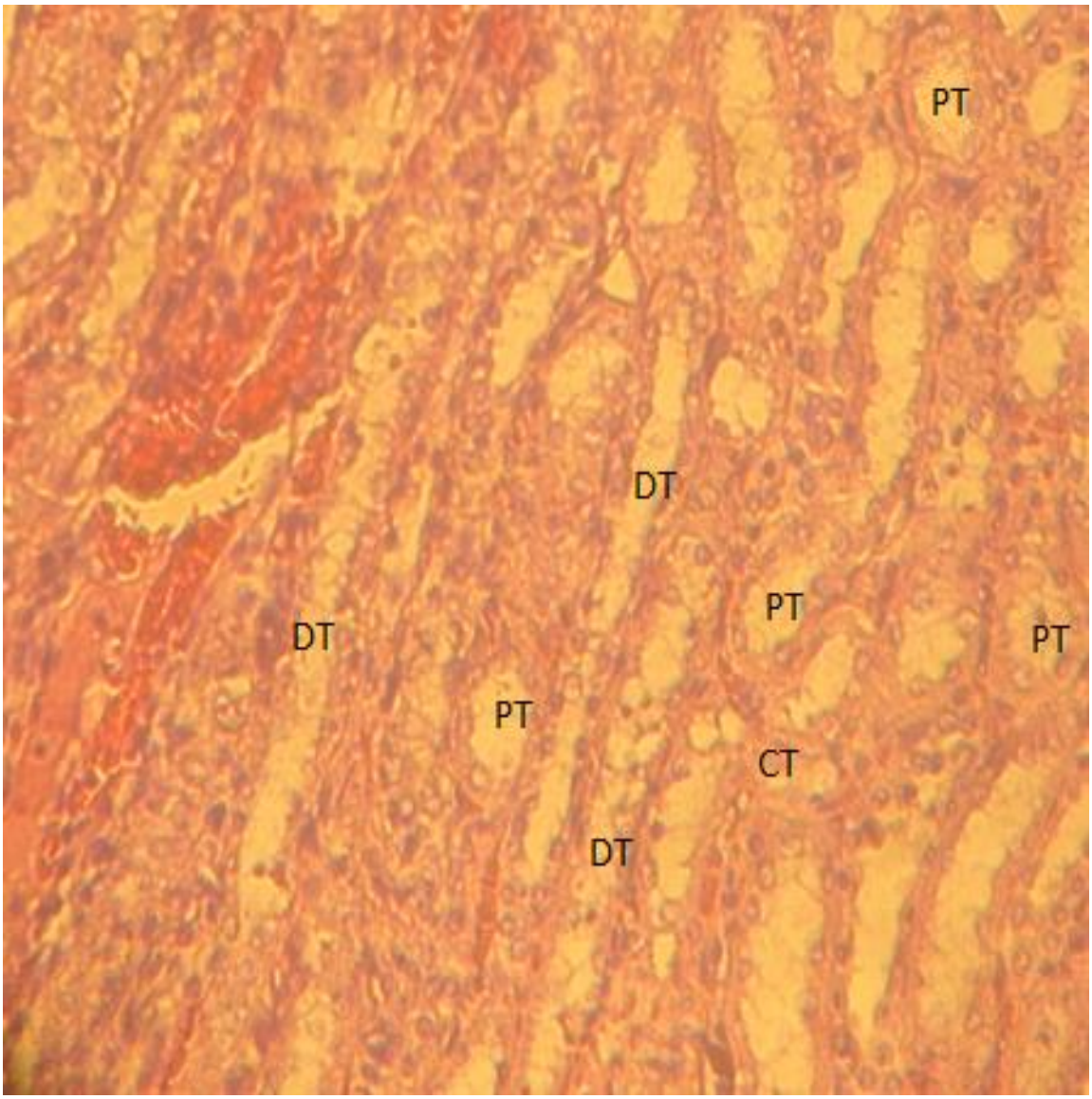

Plate 62: Section of treated ratôs kidney (medulla) with high dose C_2 (600mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* leaf extract) showing normal histopathological pattern of the kidney (medulla) revealing the distal tubule (DT), proximal tubule and the collecting tubule (CT). Stain uptake: Mayerôs H&E X400 magnification.

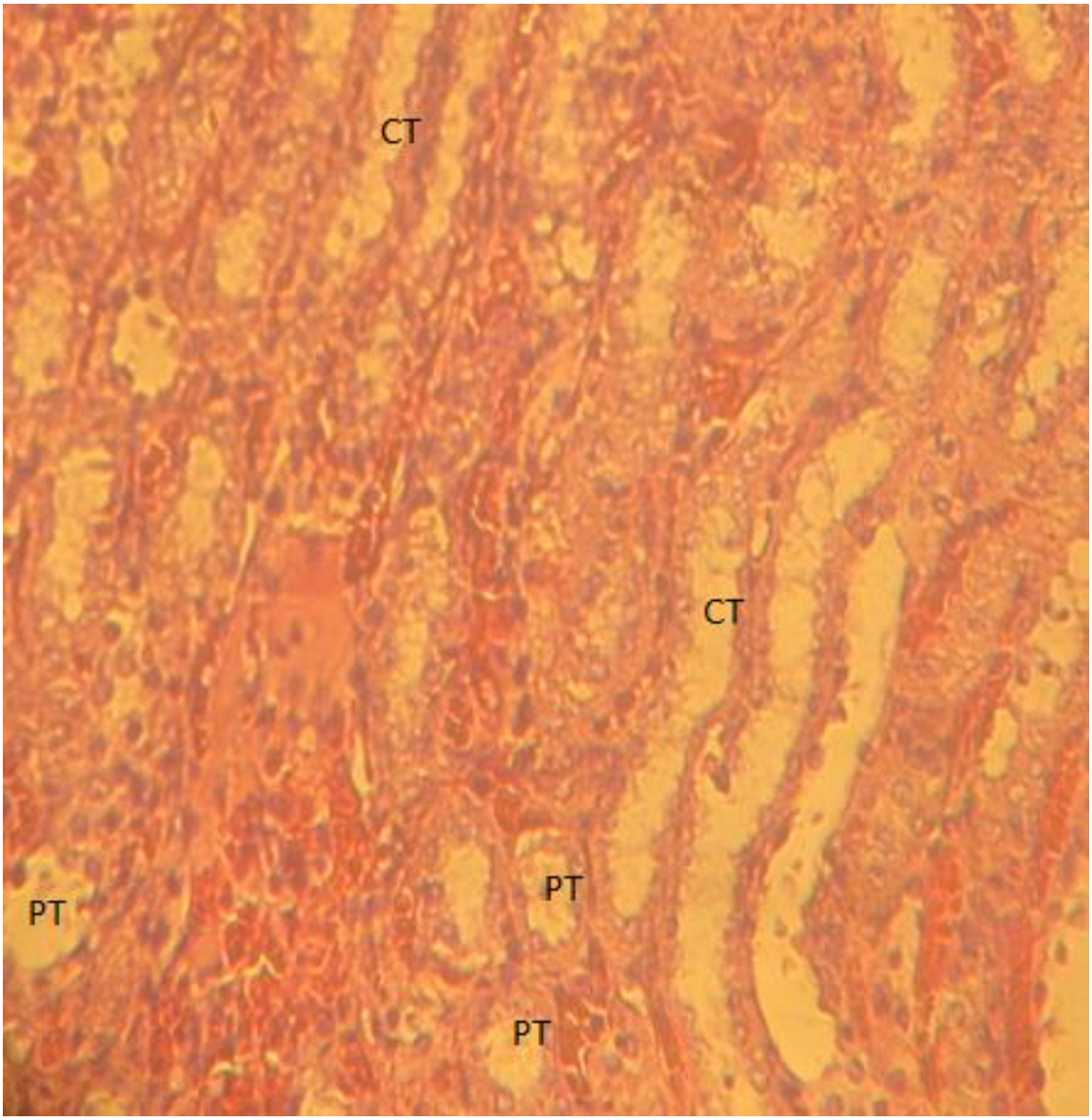

Plate 63: Section of treated ratôs kidney (medulla) with high dose D_2 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* leaf extract) showing normal histopathological pattern of the kidney (medulla), with the proximal tubule (PT) and the collecting tubule (CT). Stain uptake: Mayerôs H&E X400 magnification.

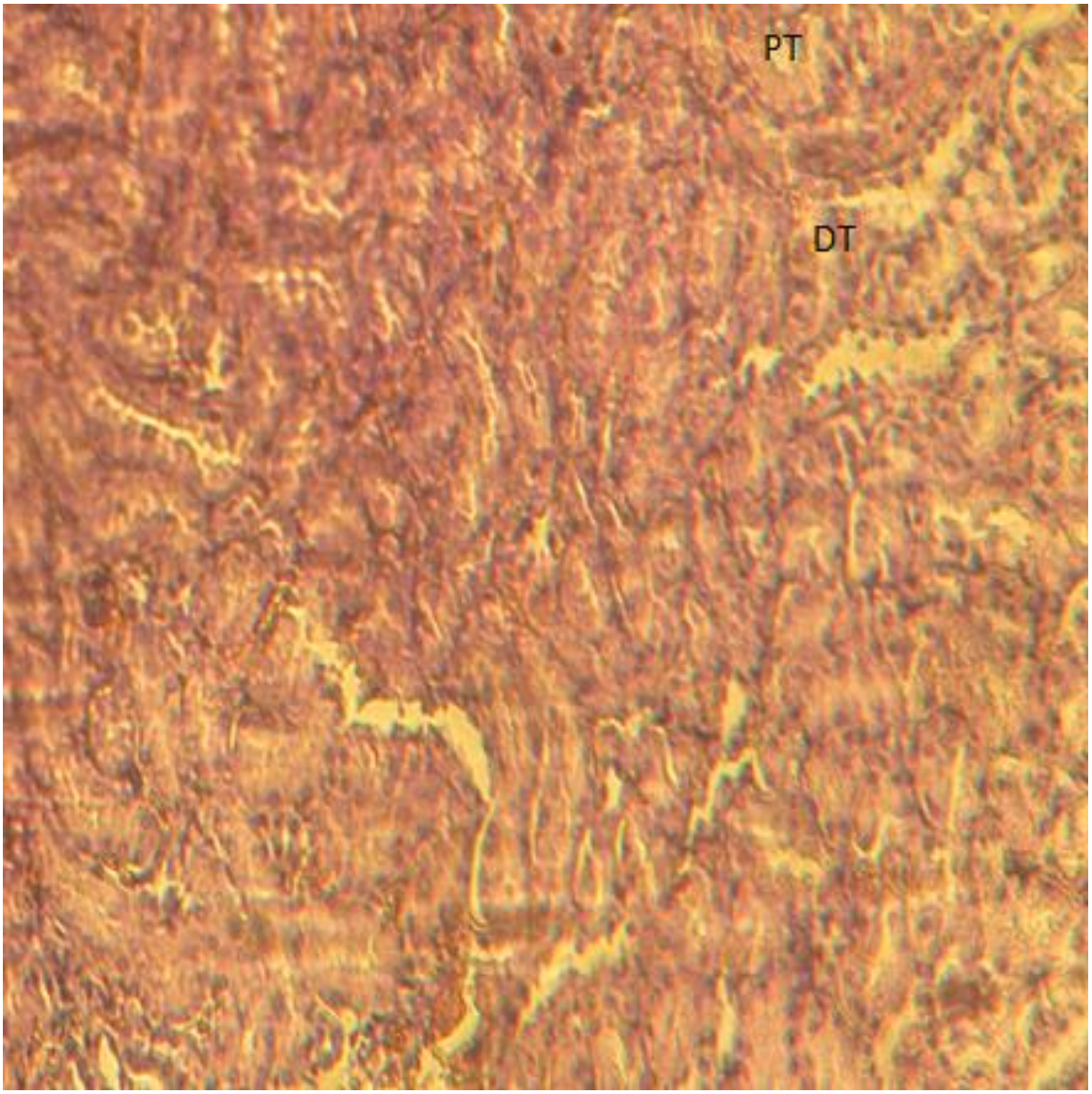

Plate 64: Section of treated ratôs kidney (medulla) with high dose D_1 (800mg/kg b.w. oral administration of *Acalypha wilkesiana* leaf extract) showing normal histopathological pattern of the kidney (medulla) revealing the proximal tubule (PT) and the distal tubule (DT). Stain uptake: PAS X400 magnification.

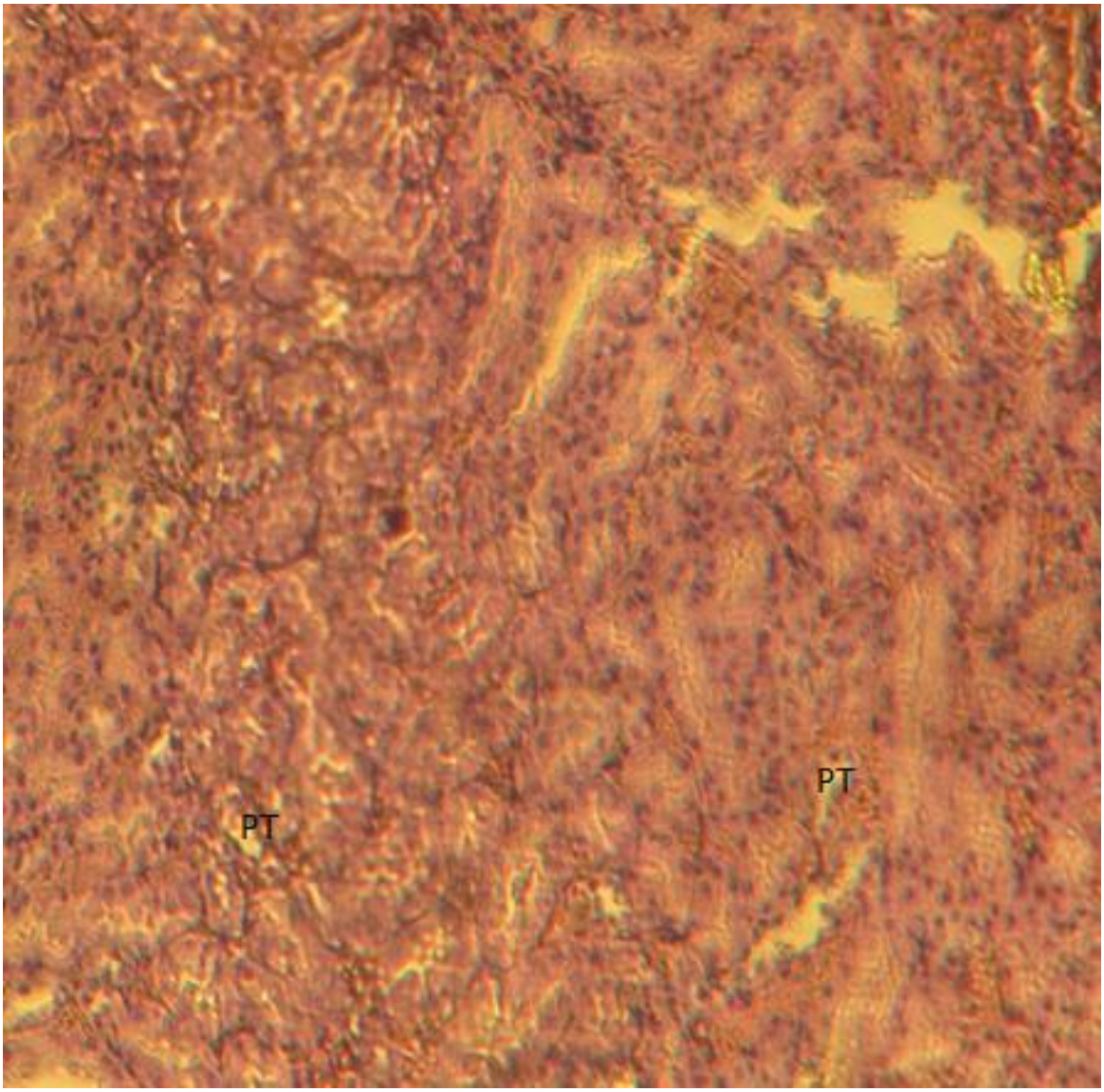

Plate 65: Section of treated ratôs right kidney (medulla) with high dose D_2 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* leaf extract) showing normal histopathological pattern of the kidney (medulla) revealing the proximal tubule (PT) and the distal tubule (DT). Stain uptake: PAS X400 magnification.

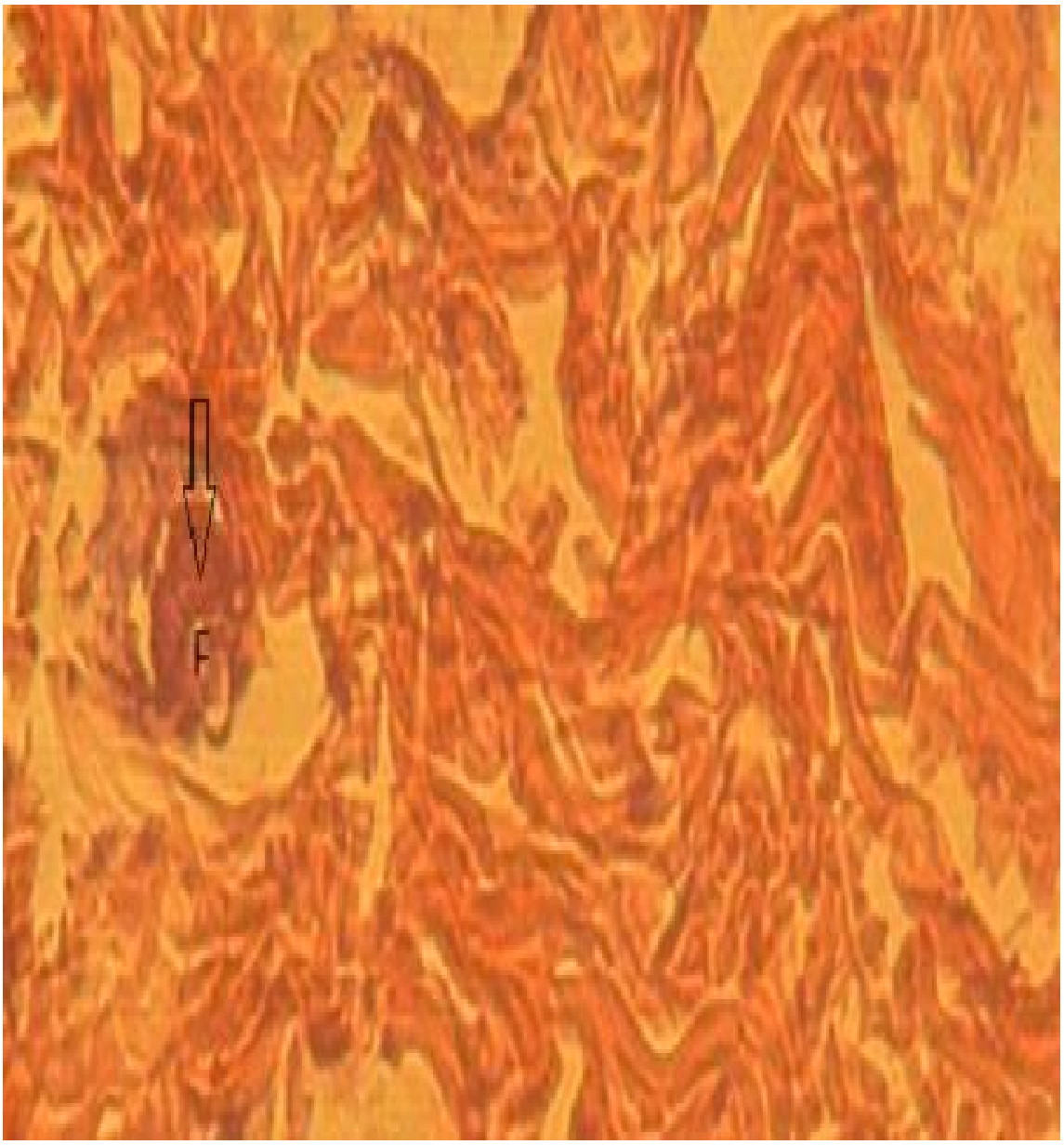

Plate 66: Section of untreated skin control group E_2 showing normal histopathological pattern of the skin, revealing the hair follicle (F). Stain uptake: Mayerôs H&E X400 magnification.

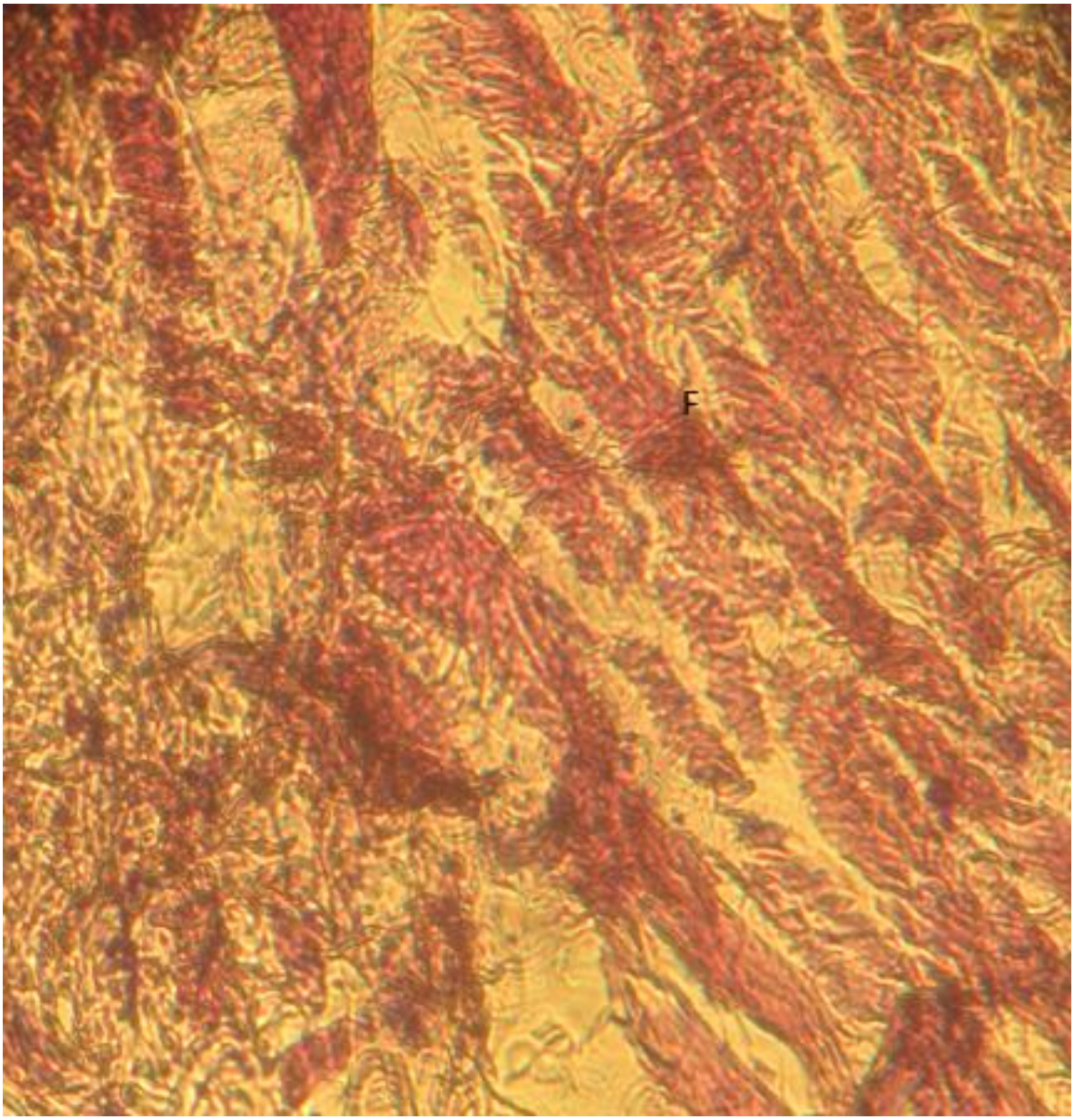

Plate 67: Section of untreated skin (control) group E_2 showing normal histopathological pattern of the skin, revealing numerous hair follicles (F). PAS X400 magnification.

Plate 68: Section of treated ratôs skin with high dose C_2 (600mg/kg b.w. subcutaneous administration of Acalypha wilkesiana crude leaf extract) showing normal histopathological pattern of the skin, with numerous hair follicles (f) and hair shaft (AS). Stain uptake: Mayerôs H&E X400 magnification.

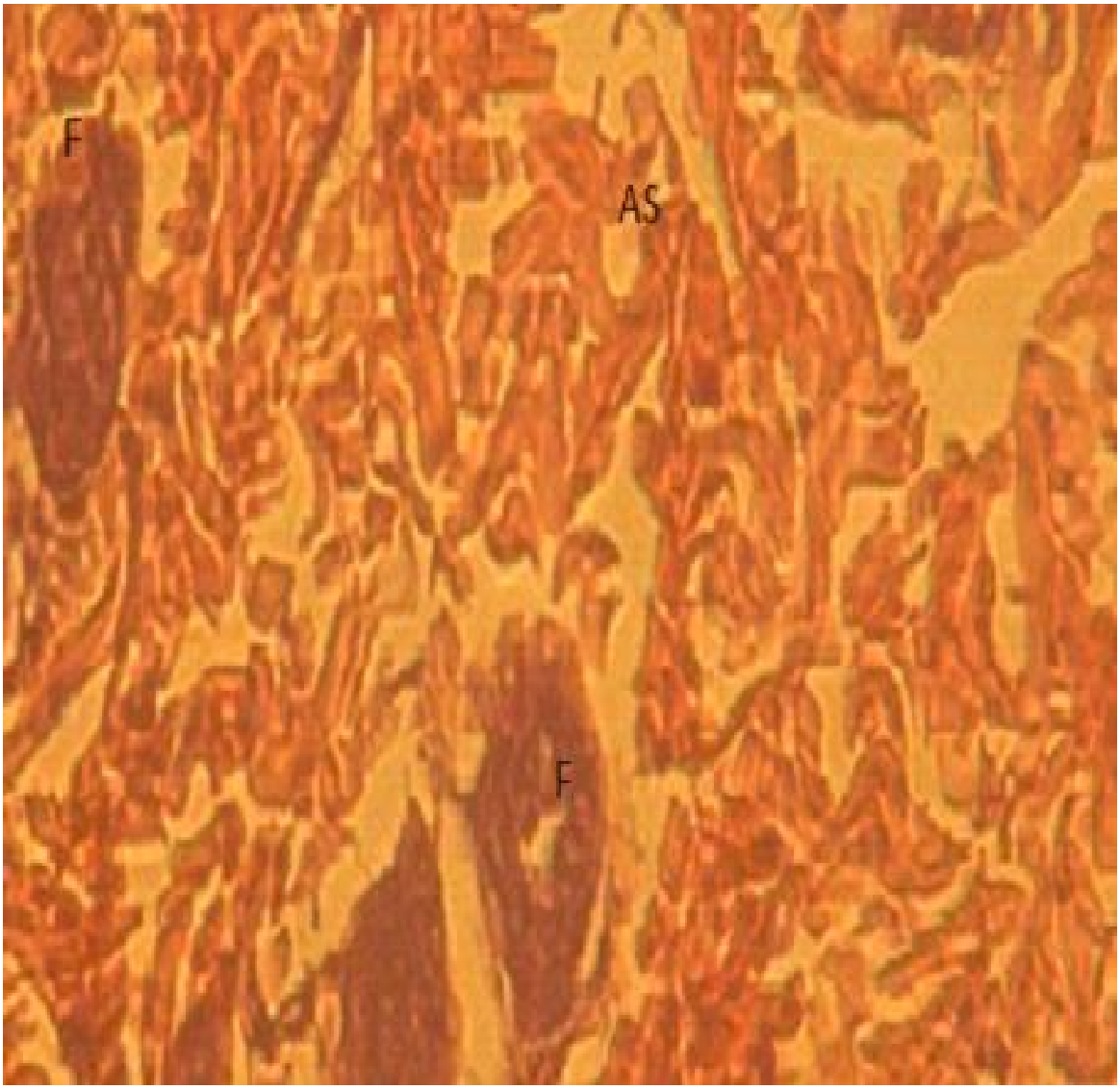

Plate 69: Section of treated ratôs skin with high dose D_2 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) showing normal histopathological pattern of the skin, with numerous hair follicles (f) and hair shaft (AS). Stain uptake: Mayerôs H&E X400 magnification.

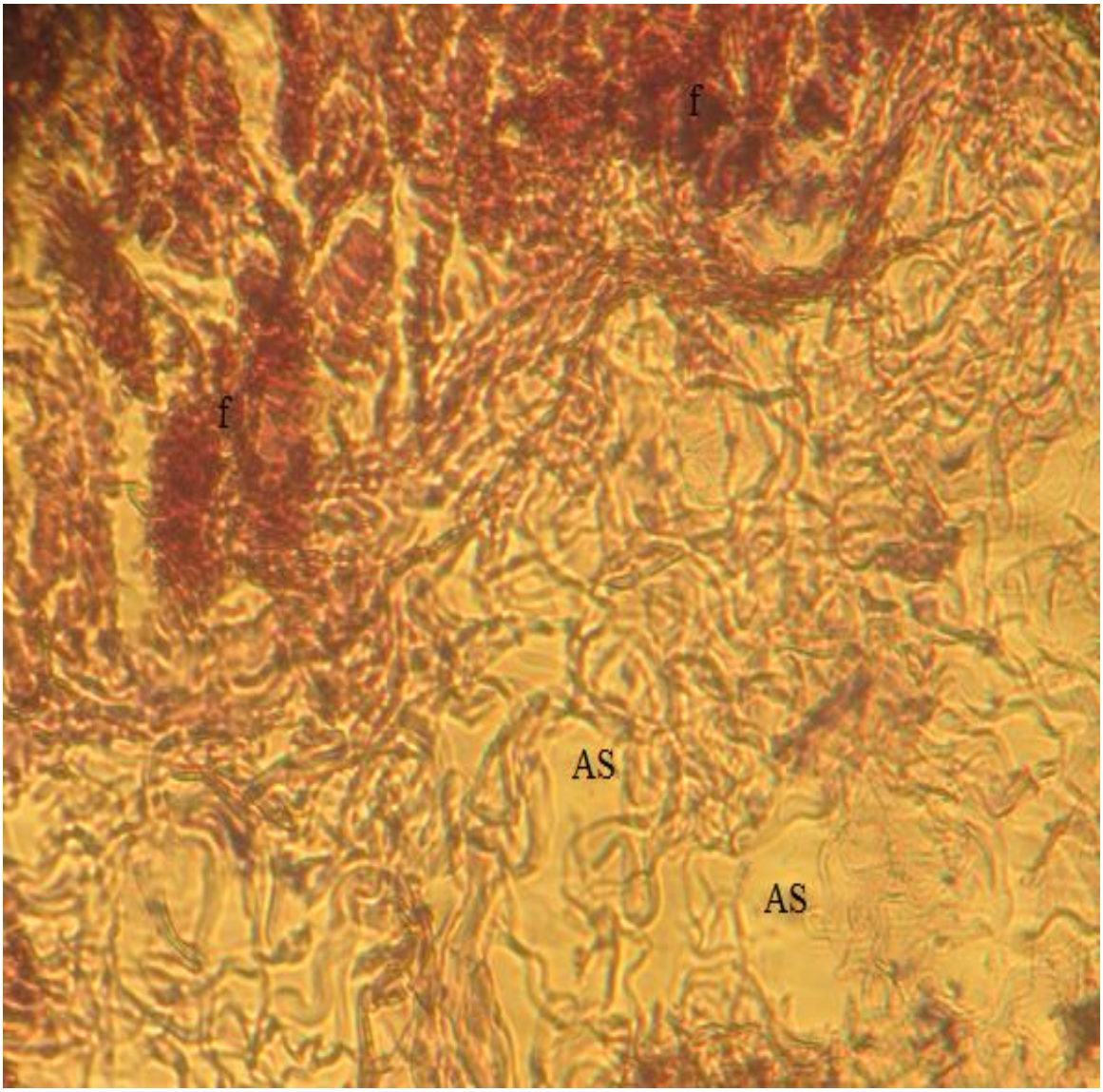

Plate 70: Section of treated ratôs skin- dermis with high dose C_2 (600mg/kg b.w. subcutaneous administration) treated rats showing normal histopathological pattern of the Skin, with normal hair follicle. Stain uptake PAS X400 magnification.

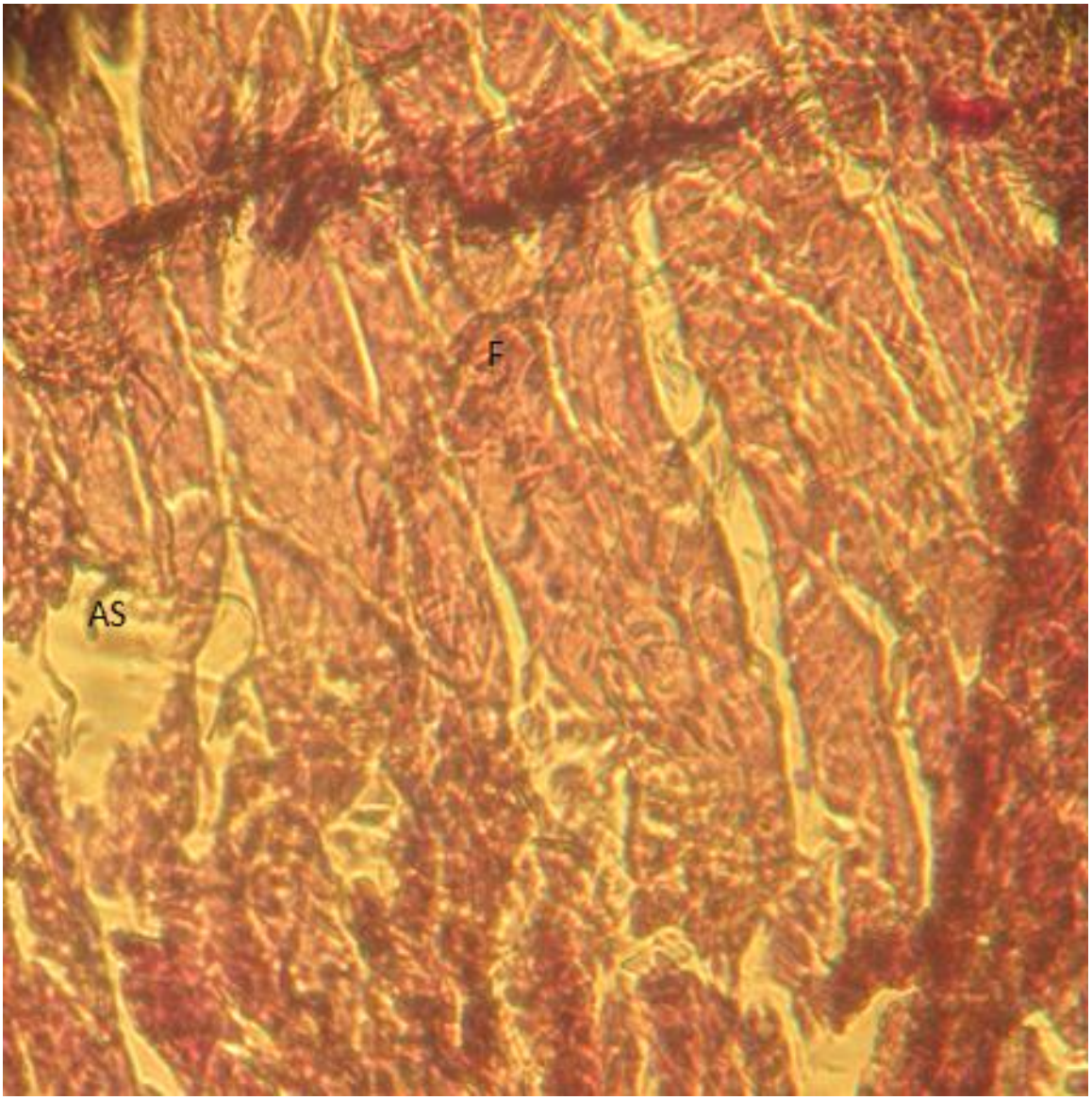

Plate 71: Section of treated ratôs skin- dermis with high dose D_2 (800mg/kg b.w. subcutaneous administration) treated rats, showing normal histopathological pattern of the Skin, revealing hair follicles and hair shaft. Stain uptake: PAS X400 magnification.

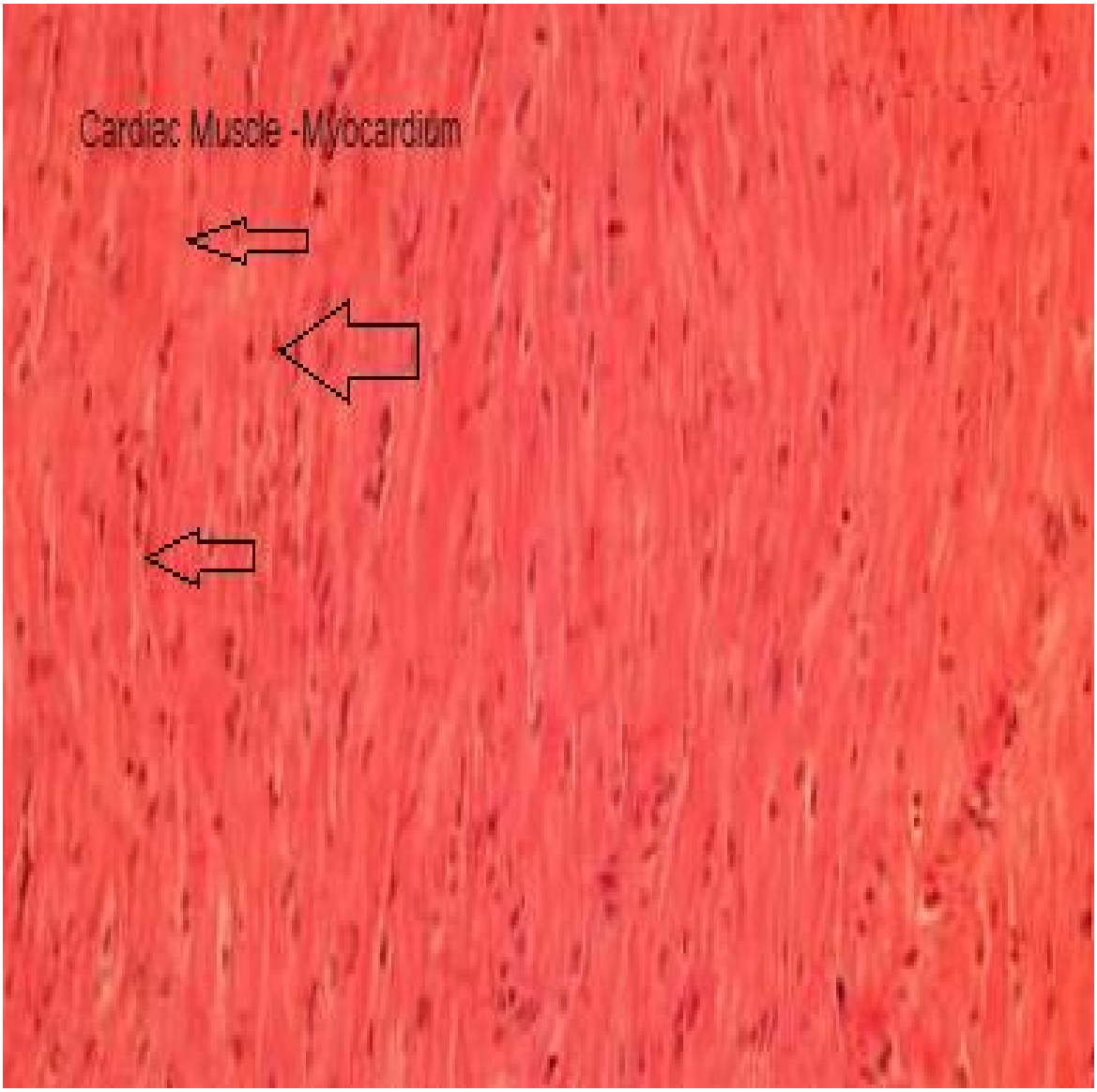

Plate 72: A section of the untreated ratôs cardiac muscle (control) taken from the myocardium showing histopathological pattern of the cardiac muscle. Stain uptake: Mayerôs H&E X400 magnification.

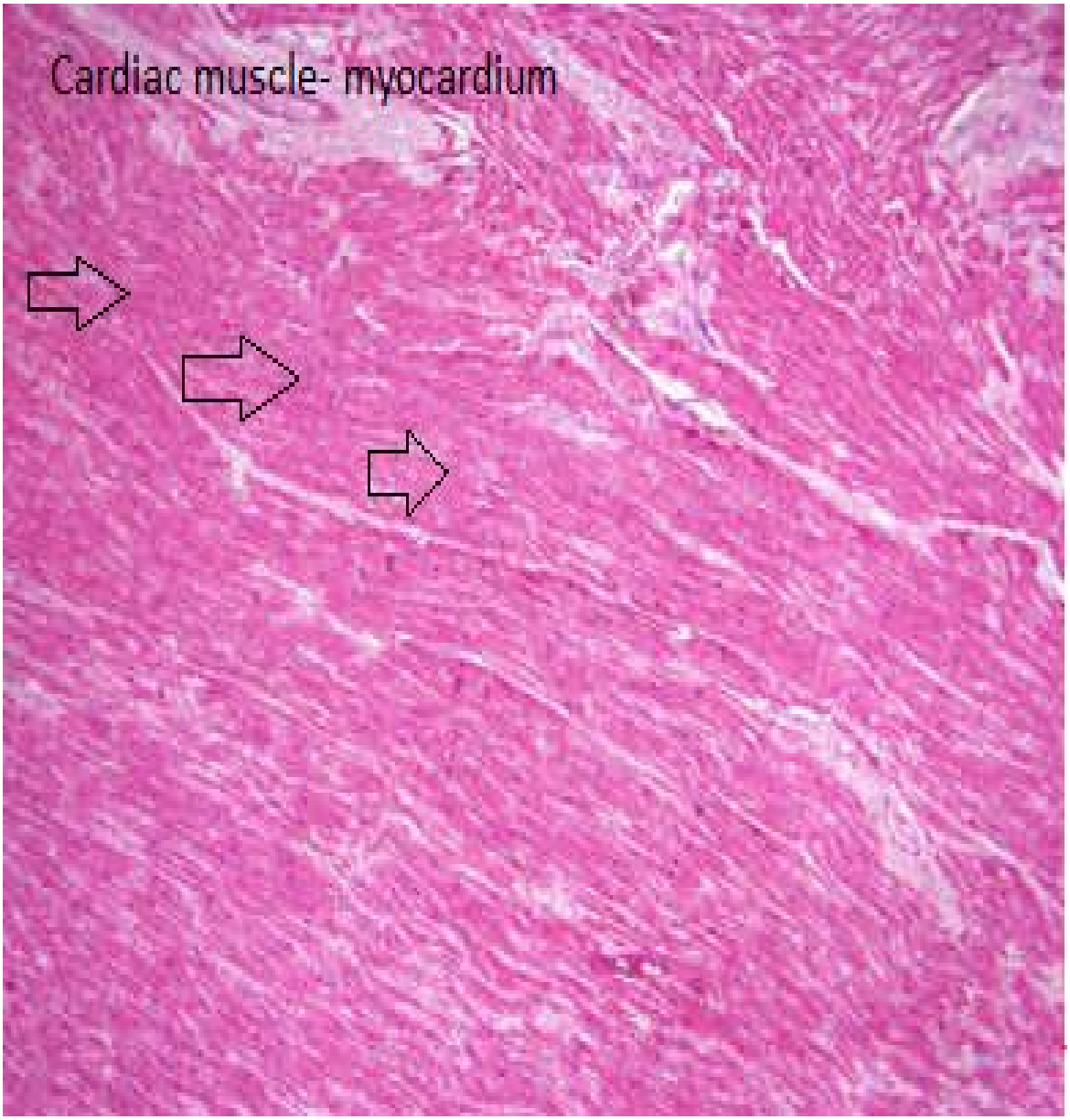

Plate 73: A section of the untreated ratôs cardiac muscle (control) taken from the myocardium showing normal histopathological pattern of the cardiac muscle (Striated). Stain uptake: PAS X400 magnification.

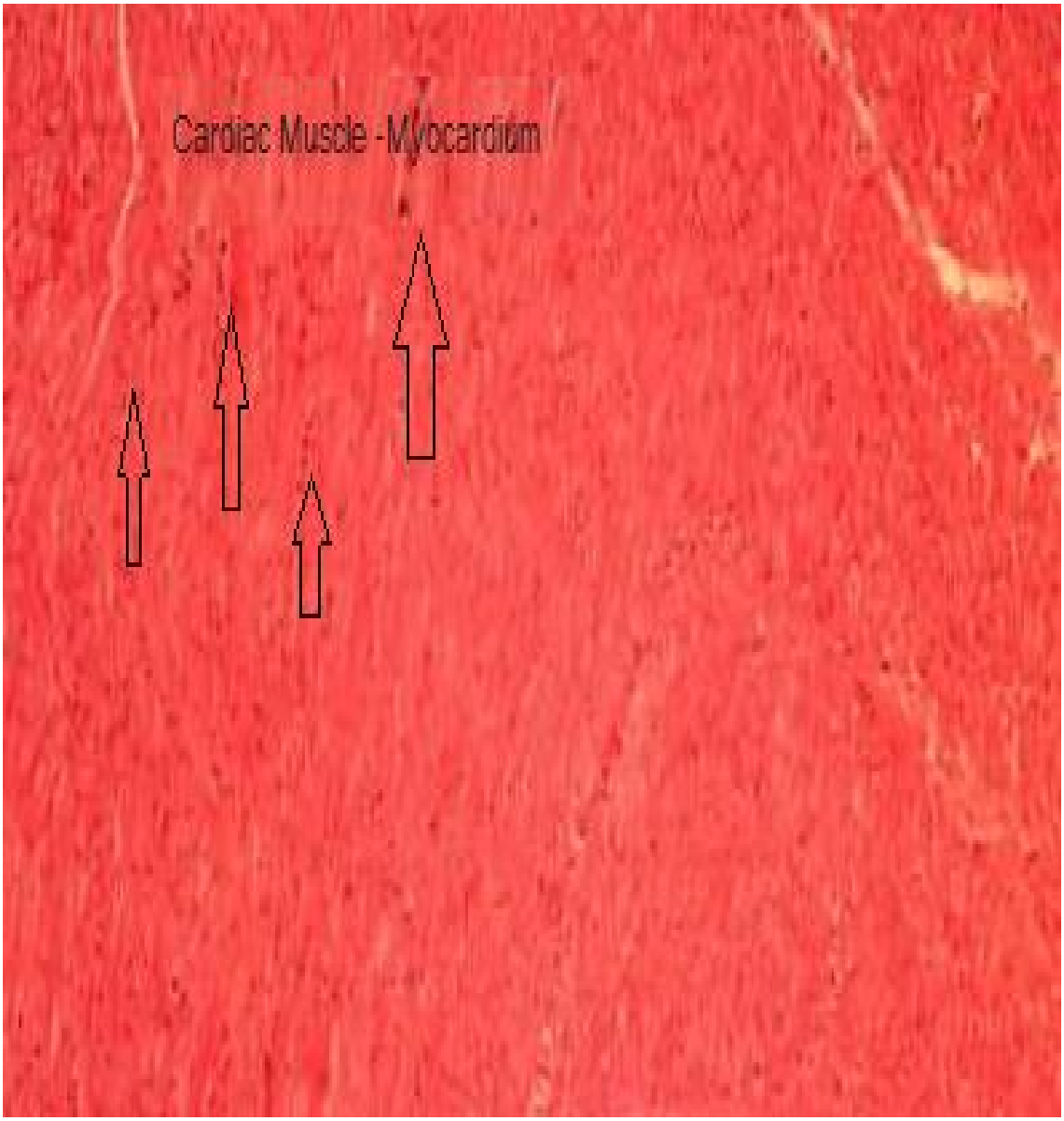

Plate 74: A section of the treated ratôs cardiac muscle with high dose C_2 (600mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) taken from the myocardium, showing normal histopathological pattern of the cardiac muscle. Stain uptake: Mayerôs H&E X400 magnification.

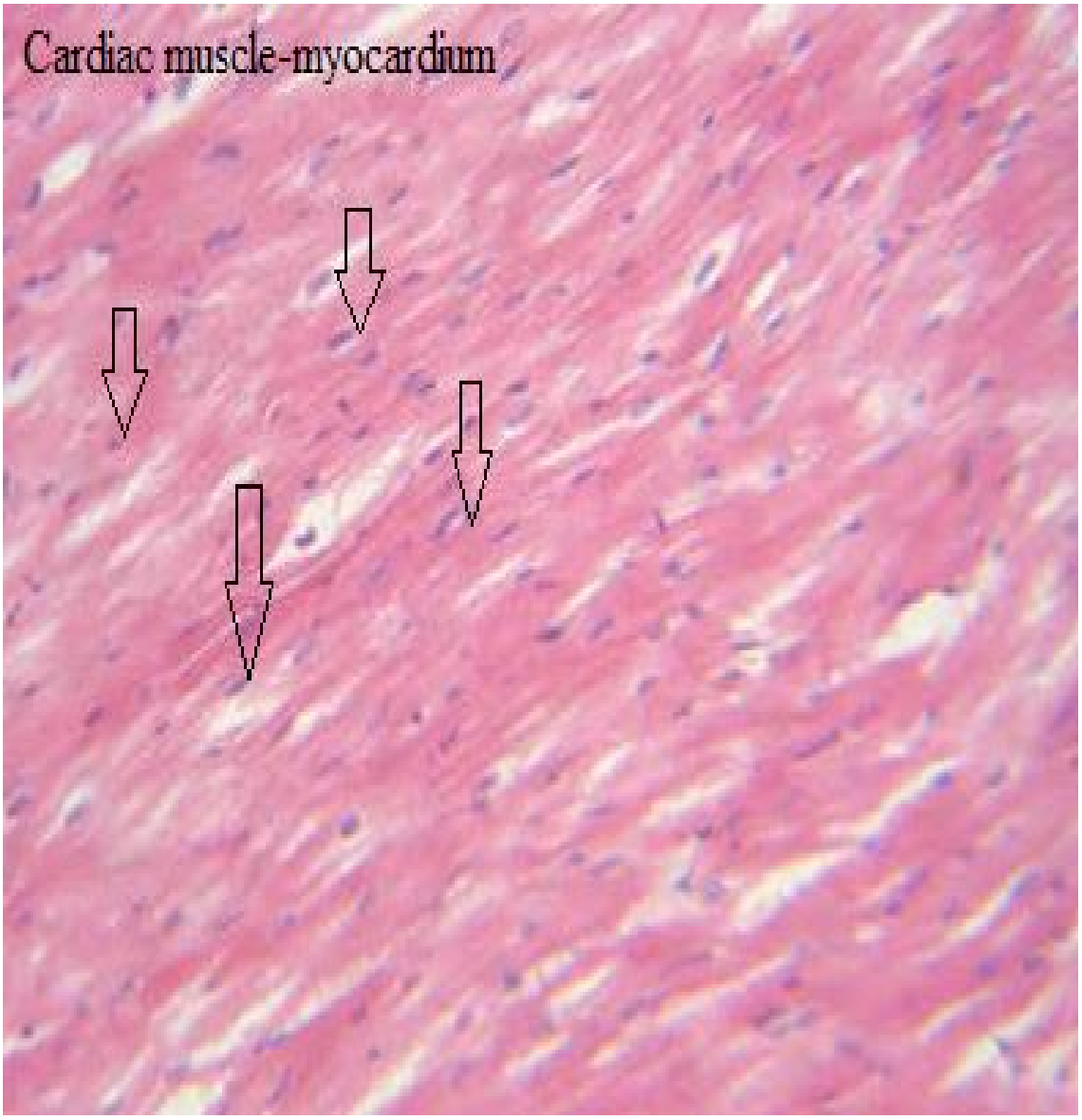

Plate 75: A section of the treated ratôs cardiac muscle with high dose C_2 (600mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) taken from the myocardium, showing normal histopathological pattern of the cardiac muscle. Stain uptake: PAS X400 magnification.

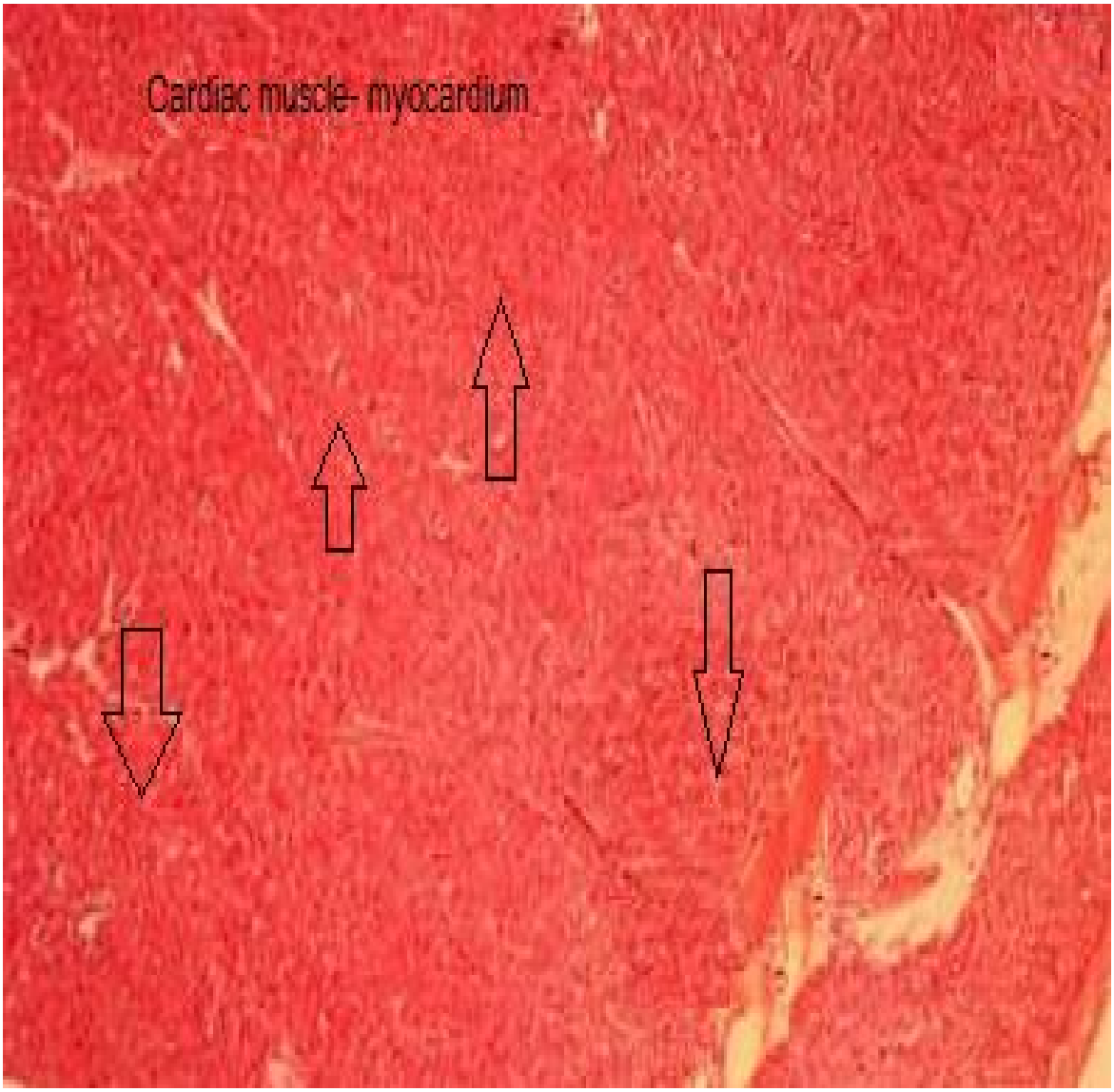

Plate 76: A section of the treated ratôs cardiac muscle with high dose D_2 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) taken from the myocardium, showing normal histopathological pattern of the cardiac muscle. Stain uptake: Mayerôs H&E X400 magnification.

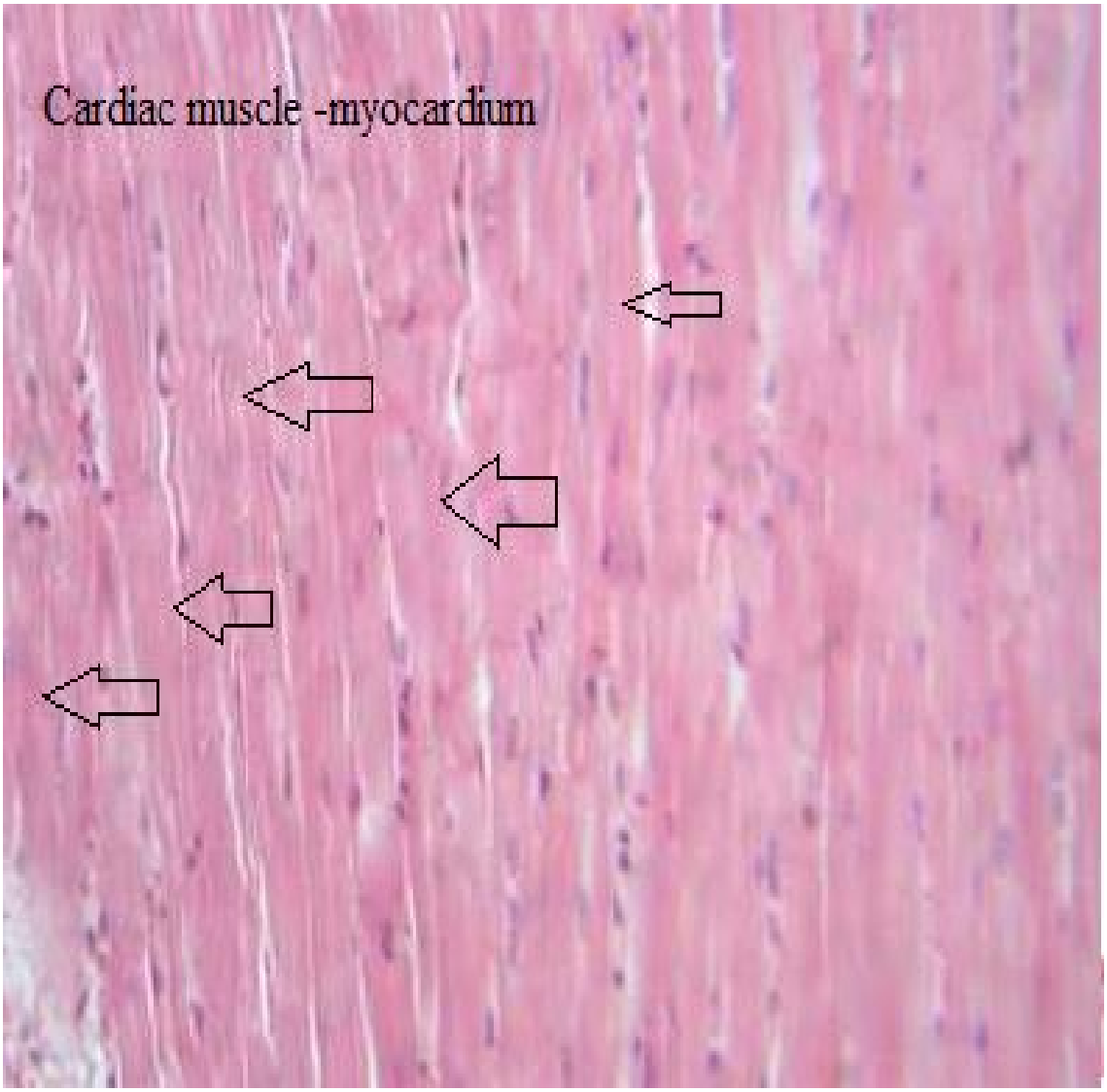

Plate 77: A section of the treated ratôs cardiac muscle with high dose D_2 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) taken from the myocardium, showing normal histopathological pattern of the cardiac muscle. Stain uptake: PAS X400 magnification.

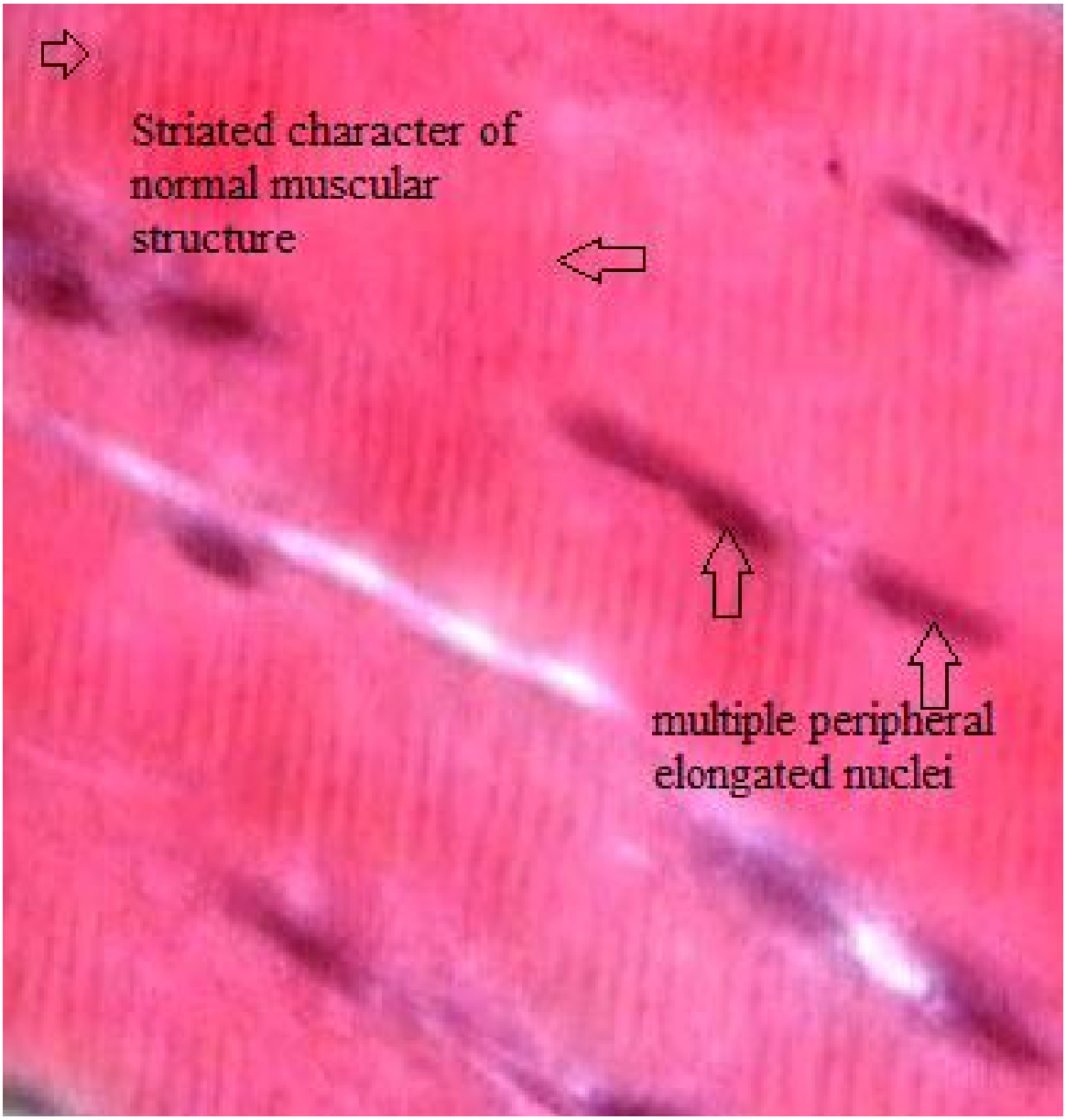

Plate 78: A section of the untreated ratôs skeletal muscle (Control) taken from the left thigh, showing normal histopathological pattern of the skeletal muscle. Stain uptake: Coleôs H&E X400 magnification.

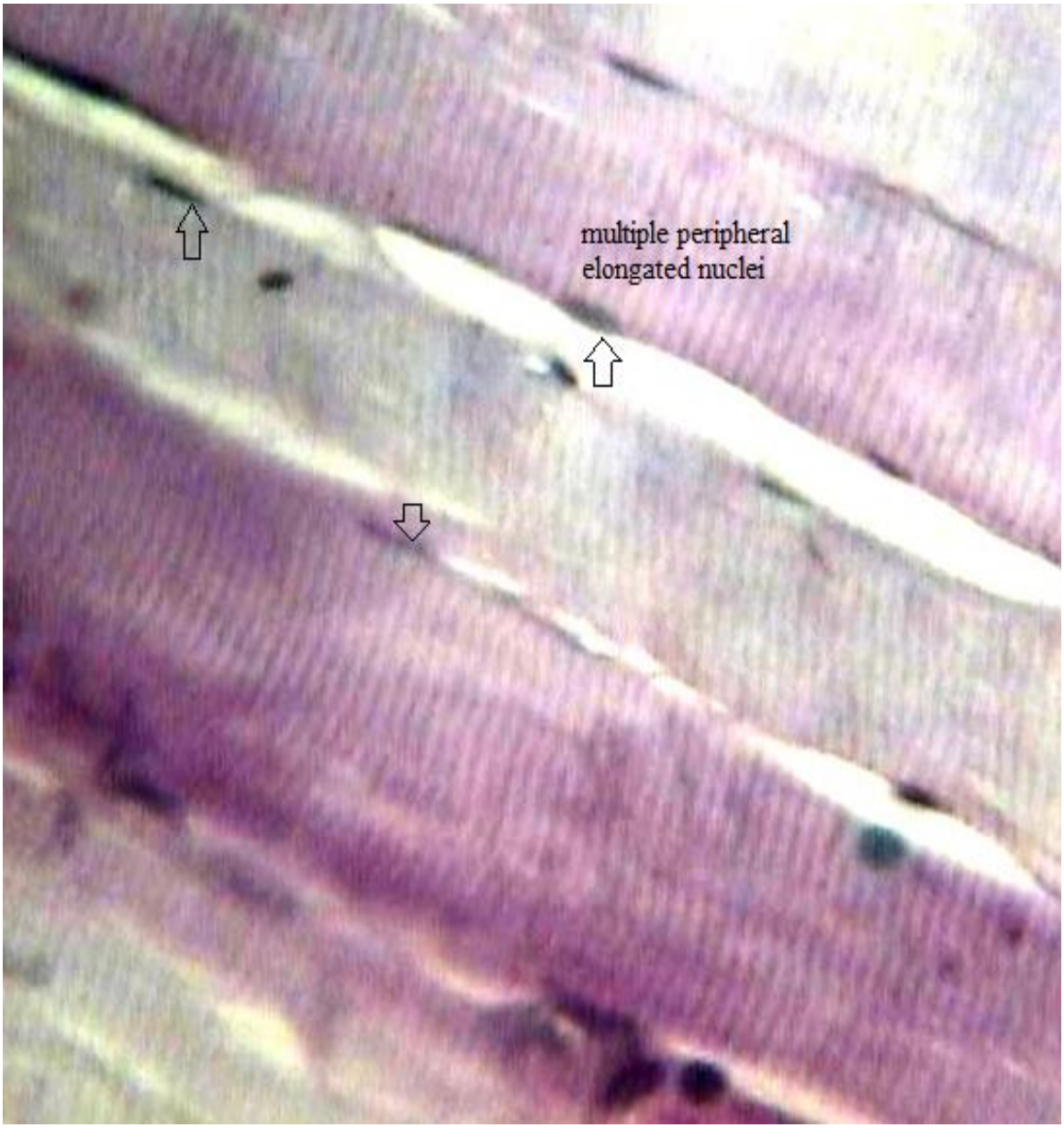

Plate 79: A section of the untreated ratôs skeletal muscle (Control) taken from the left thigh showing normal histopathological pattern of the skeletal muscle. Stain uptake: PAS H&E X400 magnification.

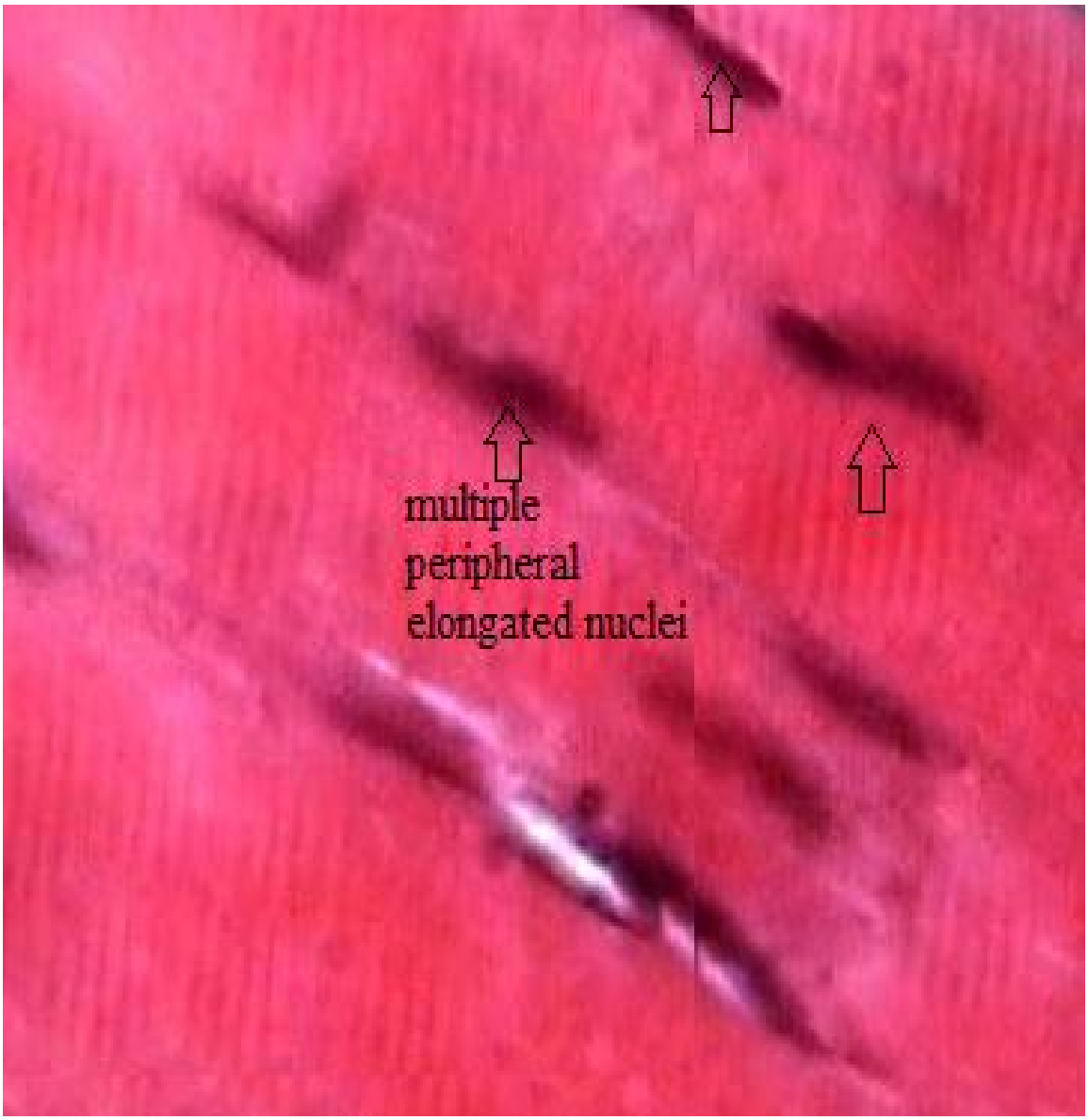

Plate 80: A section of the treated ratôs skeletal muscle with high dose D_1 (600mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) taken from the left thigh, showing normal histopathological pattern of the skeletal muscle. Stain uptake: Coleôs H&E X400 magnification.

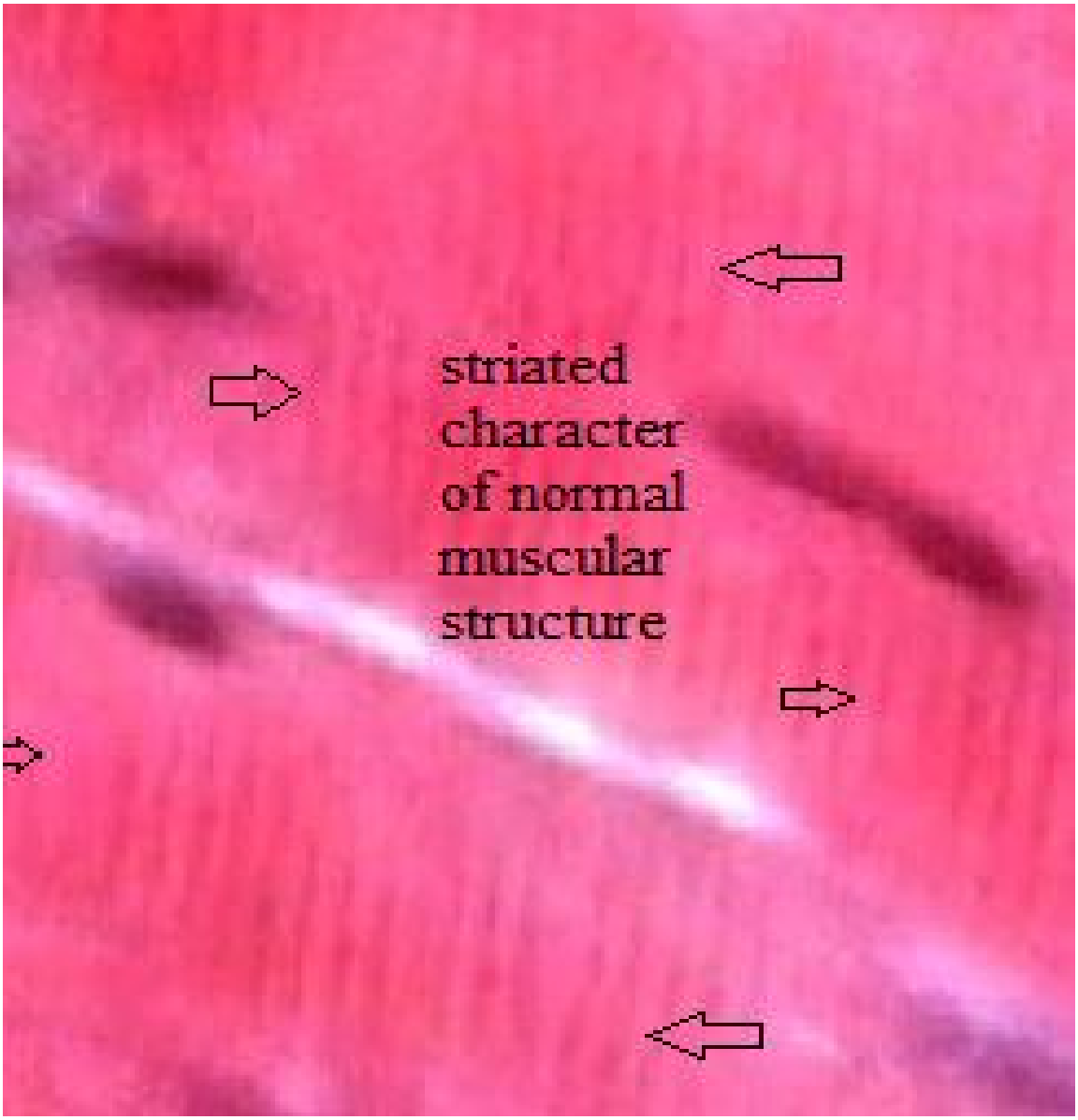

Plate 81: A section of the treated ratôs skeletal muscle with high dose D_2 (800mg/kg b.w. subcutaneous administration of *Acalypha wilkesiana* crude leaf extract) taken from the right thigh, showing normal histopathological pattern of the cardiac muscle. Stain uptake: Coleôs H&E X400 magnification.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

The rate of abuse of medicinal plants (herbal preparations) are quite alarming in our society today (Onocha and Olusanya, 2010). At any primary onset of fever and some petty illness, patients place themselves on the popular yoruba decoction called ñagboò or gbogbo li sheò for relief (Owoyele *et. al.* 2011). These acts may result in cellular adverse effects if adequate dosage is not taken into consideration. This study was set out to examine and characterize possible adverse effects of such herbal preparation from the plant *Acalypha wilkesiana* extracts as reference point on the organs at the cellular level.

The effect of aqueous extract (10%w/v) of the leaf of *Acalypha wilkesiana* (a popular medicinal plant used in Nigeria for the treatment of fever in infants) was studied in the liver and kidney of albino rats. The reports from Alli *et al.,* (2011); showed that the administration of the aqueous extract resulted in a significant reduction in the enzymes activities ($p<0.05$) in the liver that was complimented by an increased activity of these enzymes in the serum. The result obtained further revealed that prolonged usage or overdose of the aqueous extract of *Acalypha wilkesiana* could exhibit a dose dependent toxicity. In contrast, histological indices in this study are of normal representation. However, opinions of the former were drawn from the assessment of liver enzymes and may be dose dependent when compared to the latter. In addition to the foregoing, proper identification of the plant is also necessary, especially with the different cultivars available in Nigeria. Hence, if researchers are not careful, one cultivar could be

misrepresented for another. This single act may have contributed in part to the variation in reports by Alli *et al.,* (2011) as compared to the present study.

To further justify the claim by Ikewuchi *et al.*, (2010; 2011a); the leaf and aqueous extract of *A. wilkesiana* were reported as being very rich in tannins and tannic acid which confirms the views of Madziga *et al.,* (2010); Gotep *et al.,* (2009) and in the present study. Tannins was reported as positive in the plant sample by the formation of greenish-black colouration. Likewise, saponin was inferred to be present if frothing or foams were observed in a plant sample. Saponin, like tannins was found to be present in the plant used for this study. Therefore, the present result correlates with Borokini and Omotayo, (2012); Owoyele *et al.,* (2011); Ikewuchi *et al.* (2010, 2011a); Mittal *et al.,* (2010); Pithayanukul *et al.,* (2009); Akinyemi *et al.* (2006); Oladunmoye, (2006); Alade and Irobi, (1993); in that the presence of tannins and saponins in *A. wilkesiana* leaf extract was earlier reported. Though, Madziga *et al.,* (2010); Gotep *et al.,* (2009); reported the absence of Saponin in *A. wilkesiana* leaf extract but in agreement with the same report which showed that tannins was present. It is of importance to know that tannins is well known for its anti-microbial properties; therefore such actions suggests that they may be useful in the treatment of venereal diseases / sexually transmitted infections (STIs) (Haruna *et al.,* 2013).

Ikewuchi *et al,* (2011) revealed that tannins contained in aqueous extract of the leaf of *A. wilkesiana* may be responsible for the hepato-protective activity which was observed and it reflected in the present study where all liver sections appeared normal. The hepato-protective activity of tannic acid is well documented Mittal *et al.,* (2010); Pithayanukul *et al.,* (2009) with regards to Vitamin C, one of the major constituents of the leaf of *A. wilkesiana* which may have contributed to, or be responsible for the hepato-protection of the liver (Ikewuchi and Ikewuchi, 2009; Ikewuchi *et al,* 2011). Studies have shown that hepatic microsomal drug metabolism are improved with vitamin C supplementation, probably due to its augmentation of cytochrome

P450 (Ikewuchi *et al,* 2011). Therefore, the present study strongly agrees with the views that aqueous extract of the leaf of *Acalypha wilkesiana* showed ability to protect normal functional status of a poisoned liver (Ikewuchi *et al.,* 2011). Though the rats used in this study were not poisoned, the mechanism by which the extract produces its hepato-protective activity are not certain, but it is possible that ϐ-sitosterol, a constituent of the extract of the leaf of *Acalypha wilkesiana* is at least partly responsible for the protective activity against carbon tetrachloride hepatotoxicity (Ikewuchi *et al.,* 2011a).

Furthermore, the formation by which orange precipitate are formed from the extract of *A. wilkesiana* stirred with hydrochloric acid and the filtrate treated with few drops of Picric acid solution is strongly indicative of an alkaloid as observed in this study. Notwithstanding, Borokini and Omotayo, (2012); Owoyele *et al.,* (2011); Akinyemi *et al.,* (2006); Oladunmoye, (2006); earlier reported that *A. wilkesiana* contains alkaloids and are justified in the present study. However, this study does not agree with the findings by Madziga *et al.,* (2010); Gotep *et al.,* (2009) by which the absence of alkaloids in *A. wilkesiana* was reported. Alkaloids are the most revered of all the phytochemicals present and are said to be pharmacologically active while their actions are felt in the autonomic nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant diseases and malaria (Trease and Evans, 1989). In addition, a cross reference of the pharmacological actions with the ethnomedicinal profile of the plant indicated that alkaloids may be responsible in part for the antimalarial actions of *A. wilkesiana* that was reported with therapeutic effects for malaria and fever (Borokini and Omotayo, 2012; Oladunmoye, 2006). This may also reflect a high quantity of alkaloids in the plant samples because of the pharmacological actions of alkaloids in the gastrointestinal tract, it is suggested that it may be responsible for the ethnomedicinal uses of some of the plant *(A. wilkesiana)* in treating stomach ache, vomiting, intestinal worms, constipation, dysentery and diarrhea (Borokini and Omotayo, 2012).

The presence of cardiac glycosides, flavonoids and anthraquinones in this study agrees with many authors in the past (Borokini and Omotayo, 2012; Anokwuru *et al.,* 2011; Owoyele *et. al.,* 2011; Akinyemi *et al.,* 2006 and Alade and Irobi, 1993). Meanwhile, Madziga *et al.,* 2010; Gotep *et al.,* (2009) reported the presence of steroid and the absence of anthraquinones that do not agree with the report from this study but in agreement with Borokini and Omotayo, (2012); Oladunmoye, (2006); by which the absence of steroid was reported. This report strongly agrees with the previous study by Akinyemi *et al.,* (2006); haven reported that *A. wilkesiana* contained tannins, saponins, alkaloids, flavonoids and anthraquinones. Although, Alade and Irobi, (1993) reported that *A. wilkesiana* was said to contain saponin, tannin, anthraquinones and cardiac glycoside in the sample investigated. Nonetheless, the variations in phytochemical composition of the plant could be attributed to the different locations where the plant was collected: Benin City in Edo state, Nigeria for the present study.

Gotep *et al.,* (2009), suggested a relationship between chemical composition of plants and geographical location. Moreso, Rao and Rout (2003), reported differences in phytochemical composition of essential oils of *Jasminum sambac*. (L.) collected from different parts of India. The composition of bee propolis has also been found to depend on geographical source (Evans, 2005). It contains flavonoids and phenolic esters in temperate regions but these compounds were absent in propolis obtained from tropical regions, although both exert antibacterial properties (Gotep *et al.,* 2009). Seasonal changes in the antimicrobial constituents of *A. wilkesiana* have also been reported. The report attributed the antimicrobial property of the extract to its tannins: geraniin, corilagin and gallic acid (Adesina *et al*., 2000). Therefore, the contrast and variation so recorded may not be far from lack of proper identification of the exact cultivar or species worked upon by various authors.

The phytochemicals present were juxtaposed with the ethno-medicinal significance and from these; several suggestions were deduced on the secondary metabolites responsible for the pharmacological actions of the plant (Borokini and Omotayo, 2012). In agreement with Owoyele *et al.,* (2011); Akinde and Odeyemi (1987) where the use of *Acalypha species* was reported for the treatment of malaria, dermatological and gastro-intestinal disorders and for dermatological purpose, subcutaneous application of A*calypha* leaf extract in this study has no histological / or deleterious effect on the skin. Meanwhile, the use of A*calypha* leaf extract for gastro-intestinal disorders and oral consumption of the decoction showed no adverse effect on the organs of the test animals, it strongly support the views of some researchers as being free and safe for human consumption. Nevertheless, oral and subcutaneous administration of *Acalypha wilkesiana* leaf has no histological or deleterious effects on the cardiac muscles as seen in this study. This study thus support the use of the leaf in the management of hypertension Ikewuchi *et al.,* (2009) especially, as the sections of the cardiac muscles from the high dose treated animals appeared normal with the untreated groups.

5.2 Conclusion

The results indicated an existence of a wide diversity in the distribution of the secondary metabolites present in the plant. However, it could be said that the ethno medicinal significance of *A. wilkesiana* plant extract used for this study corresponded to the pharmacological actions of the secondary metabolites they contain. Therefore, this study suggest that daily oral intake of an aqueous extract of the leaf of *Acalypha wilkesiana* (infusion or boiled decoction) may be fit for human consumption without possible deleterious / histopathological effects exerted on vital organs. It may be used for the purpose of weight reduction and could be applicable in obsessed human subjects.

5.3 Recommendation

The following recommendations are hereby made:

1. More research should be carried out on the toxicity of the plant in order to know the safety and establish a safe dosage regimen since the infusion or decoction of the leaf is often taken orally.

2. It is pertinent that further studies be carried out on the qualitative analysis of the plant. From that point the specific metabolites of the plant can be screened and separated to undergo proper pharmacological processes and become a potent drug.

3. Further studies should attempt to look into proper identification of the plant as well as the purification and characterization of the specific phytochemical agents present.

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GLOSSARY OF ABBREVIATIONS

C. = Cage

b.w. = Body weight

NR. = No. of rats

AW. = Average weight

Nml = number of milliliter given

AD. = Administration.

cm: centimeter

g: gram

g/l: gram per litter

MIC: Minimum inhibitory concentration

MBC: Minimum Bactericidal Concentration

mg: milligram

kg: kilogram

ml: milliliter

mm: millimeter

Rpm: rotation per minute

RH: relative humidity

SAR: systemic acquired resistance

Spp.: species

MRSA: methicillin-resistant staphylococcus aureus

L: litre

hr: hour

L.D: lethal dose

G.I.T.: Gastrointestinal tract

DPX: Distrene plasticizer xylene

CO: Cardiac output

PR: Peripheral resistance

BP: Blood pressure

ALP: Alkaline Phosphatase

ALT: Alanine Transaminase

AST: Aspartate Transaminase

MHA: Mueller Hinton Agar

PDA: Potato Dextrose Agar

LM: Light microscope

EM: Electron microscope

CT: Connective tissue

CCK: Cholecystokinin

GIP: Gastric inhibitory peptide

APPENDIX I

STATISTICAL FORMULAR

1. Mean ($\bar{x}$) = $\frac{x}{n}$ where x = total values obtained

   $\bar{x}$ = mean

   N = number of occurrences values

2. Standard Deviation (SD):

$$SD = \frac{(x - \bar{x})^2}{n - 1}$$

3. Standard Error (SE)

$$SE = \frac{SD}{n}$$

4. Test of significance (t)

$$Z = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}}$$

Where $\bar{x}_1$ & $\bar{x}_2$ = mean of 2 sets of sample.

SD_1 & SD_2 = Corresponding number of occurrences of values in the 2 sets of sample.

n_1 & n_2 = Corresponding number of occurrences of values in the 2 sets of sample.

APPENDIX II

DISSECTING INSTRUMENTS

- 1 dissecting pan
- 1 Lg. Freezer Bag
- 4 pieces of string (25 in each)
- 2 forceps (fine & blunt)
- 1 scalpel
- 2 blunt probes
- 4 sharp probes
- 12 pins
- 2 scissors (tissue & paper)
- Water dropper & cup
- Ruler
- Index card (labels)