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HISTOMORPHOLOGICAL AND HAEMATOLOGICAL EFFECT OF AQUEOUS EXTRACT OF CASSIA OCCIDENTALIS LEAF ON OF THE HEART OF ADULT WISTAR RATS

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ABSTRACT

This research accessed the effect of aqueous extract of *Cassia occidentalis leaf* on some hematological parameters and Microanatomy of the heart. Twenty (20) Adult rats with an average weight of 150g were randomly placed into 4 groups. Each group consisted of five (5) rats, group 1 served as the control group while groups 2, 3 and 4 were given 100mg, 200mg, and 300mg of cassia occidentalis extract respectively for 28 days after which all animals were sacrificed by cervical decapitation and the heart harvested were fixed promptly using 10% formol- saline after which the tissues were processed to obtained routine paraffin sections. The result revealed dose dependent increase in the levels of the PCV, Neutrophil, and lymphocyte while the WBC revealed a dose dependent reduction, the Eosinophil and Monocyte count were unremarkable. Histopathological studies of the heart showed a variant of tissue separation, congestion and other adaptive features which was concentration dependent and also showed a level of chronic inflammation in the 300mg/kg dosed rats. The study concluded that aqueous extract of *Cassia occidentalis* leaf has immunogenic properties however further works should be carried out so as to assess more beneficial or hazardous effect of the plant.

KEYWORDS: *Cassia Occidentalis, Wistar rat, Haematology and Histology.*

INTRODUCTION

BACKGROUND OF STUDY

The use of herbal products for medicinal benefits has an important role in nearly every culture on earth. Herbal medicine was practiced by the ancient people of Asia, Europe and the Americans (Wargovich et al.,2001) and have formed the medicinal basis of healthcare throughout the world since the earliest days of humanity and still widely used with considerable importance in international trade (Ahmad et al.,2006). Herbal medicine still remains the mainstay of about 75-80% of the world population (Kamboj, 2000).In most African countries for instance; up to 90% of the population relies exclusively on plant as a source of medicine (Hostettman, et al., 2000). This is primarily because of the general belief that herbal drugs are without side effect, besides being cheap and locally available. The reliance of plants for healing properties predates human history and forms the origin of much modern medicine. Many synthetic drugs originated from plant sources. Ancient Egyptians for example, chewed willow bark to relieve fever and headaches. Thousands of years later, scientist discovered that the bark contains salicylic acid a drug that causes the skin to peel and destroys bacteria and fungi.

A century ago, most of the effective drugs were plant-based. Examples include; Aspirin (which is a chemical copy of the analgesic chemical in the bark of willow tree), guanine (from the bark of various cinchona tree species which are used in the treatment of malaria), digoxin (from fox glove) and morphine (from the opium poppy) (Vicker and Zollman; 1999). Aloe Vera has traditionally been used for the healing of bones and wounds (Manthaisong et al., 2007). Artichoke (Cyanara cardunculus), may reduce production of cholesterol levels according to invitro studies (Gebhardt, 1998) and a chemical study. Blackberry (Rubus Fruticosus) leaf has drawn the attention of cosmetology community because it interferes with metalloproteinase's that can contribute to skin wrinkling (Blackberry leaf extract, 2007). Ginger (Zingfber officinale) administered in 250mg capsule for four days, effectively decreased nausea and vomiting of pregnancy in human clinical trial (Ozgoli et al, 2009). Few (Chrysanthemum parthenium) is sometimes used to treat migraine headache. *Ocimum gratissimum* in the coastal area of Nigeria, is used in the treatment of epilepsy, (Osifo, 1992), high fever (Oliver 1980) and diarrhea (Oliver 1980 and Sofowara, 1993). Oil from the leaves of *Ocimumgratissimum* have also been found to

possess antiseptic, anti-bacterial and antifungal activities (Ezekwesili et al, 2004). It's been observed so far the great role plants have played in the production of drugs; hence *Cassiaoccidentalis* which is a medicinal plant (Romero-Frias, 1999) has been chosen to study its effects on the haematology of the blood and histology of the heart.

In recent times mortality associated with heart diseases with heart diseases has been on the increase. *Cassia occidentalis*, which has been used for the cure of various disease and ailments, has not been understudied for its efficacy on diseases relating to heart and haemopoiesis. Thus the study focuses on the effects of cassia occidentalis on some haematological indices and histology of the heart.

MATERIALS AND METHOD

ANIMAL DIET/HOUSING

Twenty adult wistar rats were used for the study. The wistar rats were obtained and housed in the animal center of the College Of Health Sciences, Delta State, Abraka. Each group was kept in a separate cage which was cleaned daily and disinfected at interval. The animals were acclimatized for two weeks, they were kept under controlled condition of light (12 hours light, I dark cycle).

The animals were fed daily with Gower mash, manufactured by Topfeeds, Premier feed mills company limited and water ad libitum.

COLLECTION OF CASSIA OCCIDENTALIS

Cassia occidentalis leaves were collected from a plantation in Benin city and identified at the hebarium in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin. The plants were air dried for days and were taken to an oven to finally dry and totally remove the moisture content, after which the leaves were grounded into a powdery consistency.

PREPARATION OF PLANT EXTRACTS

The powdered extract was soaked in distilled water for 48 hours at room temperature. The mixture was filtered into a conical flask with watman filter paper. The filtrate was dried at temperature of 30^0C for 10 hours to prepare a gel-like extract. The extract was prepared in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin city, Edo State Nigeria.

METHODOLOGY AND GENERATION OF SAMPLES

Twenty adult wistar rats with average with average weight of 150grams were used for this study. They were divided into four groups made up of five (5) rats each. There were three test group and one control group. The test groups were administered orally with 100mg, 200mg and 300mg of aqueous extract of cassia occcidentalis for group 1(low dose),group 2 (medium dose) and group 3 (high dose) respectively for twenty eight (28) days after which they were sacrificed by cervical decapitation and the organs (heart) were harvested from each group. Organs harvested were fixed using 10% formol- saline promptly.

COLLECTION OF BLOOD SAMPLE

Blood was obtained from rats by means of cardiac puncture and 5ml syringe was used to collect blood and transferred to anti-coagulant ETDA bottles in order to prevent blood from clotting. White blood cell count (WBC).Packed cell volume (PCV) and blood differentials (neutrophils, lymphocytes, eosinophils and monocytes) were evaluated by standard methods.

PROCEDURE OF ASSAYS

WHITE BLOOD CELL (WBC)

Turk's solution of 1.0% glacial acetic acid was used as the diluents. The 1:20 dilution was then charged on an improved Neuber chamber and counted. Values were expressed in X 10^9 mg/dl.

PACKED CELL VOLUME (PCV)

Microhaematocrit method was used. The sample was collected into a heparinized capillary tube and spun at 300rpm for 10 minutes.The resultant product consisting of packed cells, Buffy coat and plasma was read with the reader with the reader and the values expressed in percentage volume.

BLOOD DIFFERENTIALS

The test for blood differentials included the neutrophils, lymphocytes, eosinophils and monocytes. The method used was the Leishman stain

PREPARATION OF TISSUES FOR MICROSCOPIC EXAMINATION

The processes of preparation of the tissue for histological analysis are separated into different stages.

- Fixation
- Tissue processing
- Staining procedure
- Photomicrography

FIXATION

This is the process of using chemicals (fixatives) to prevent tissue deterioration thereby maintaining the tissue chemistry and architecture as life like as possible after death. Fixation is also carried out to prepare the tissue for further histological procedures. The organs (heart) harvested were carefully immersed fixed using 10% formol saline for 48 hours, using plastics cassettes.

TISSUE PROCESSING

The examination of tissue using a microscope usually requires a slice of tissue thin enough to transmit light, preparation of such thin slices is called section-cutting or microtomy. In most cases, the tissues must undergo preparatory treatment before being sectioned, entailing impregnation of the specimen with an embedding medium to provide support and a suitable constituency for microtomy. This preparatory treatment is known as ‘’

tissue processing'' (Drury et al., 1967). And it follows the procedure described below.

➢ **DEHYDRATION**

This is the process of removing water from tissue. Graded solutions of alcohol are used with concentrations ranging from 70% to 100% (absolute alcohol). If dehydration is completed, the clearing agent and wax will not penetrate the tissue. These will lead to poor section cutting.

➢ **CLEARING**

This is the process of removing absolute alcohol from tissue and replacing it with a solvent which is miscible with both alcohol and paraffin wax.

➢ **INFILTRATION**

This stage is also called impregnation. It is a process of replacing a clearing agent agent or an antemedium with molten paraffin wax.

➢ **EMBEDDING**

This is the process of burying or immersing a tissue in molten paraffin wax. The paraffin wax becomes a solid firm structure when it is cold. This forms a solid support medium for the tissue during microtomy.

➢ **SECTIONING AND MOUNTING**

Sections of the heart were cut using rotary microtome and floated in a hot water bath. The floated section were picked and mounted on microscopic slides for staining (Avwioro, 2002).

STAINING PROCEDURE

The procedure employed for staining in this study was haematoxylin and eosin Staining technique includes.

1. Dewaxing and hydrating
2. Staining in Erhlich's haematoxylin
3. Rinsing in water for 15 minutes
4. Differentiated in HCL in 70% alcohol for 1 minute
5. Rinse in water
6. Blued in tap water for 2 minutes
7. Counter stained with 1% eosin for 1 minute
8. Rinsed in water
9. Dehydrated, cleared and mounted (Avwioro, 2002).

PHOTOMICROGRAPHY

The stained tissue images were captured using a digital microscopic eyepiece ''SCOPETEX''DCM 500, 5.0 mega pixel connected to a computer.

STATISTICS

The results were expressed as Mean ±SD, the mean of the experimental and the control groups was performed and compared using single factor analysis of variance. (ANOVA) P values less than 0.05 were considered significant.

RESULTS

SECTION A: CHANGES IN WEIGHT.

Table 1: Effect of *cassia occidentalis on the* heart weight of wistar Rats.

PARAMETER	100 mg/ml	200mg/ml	300mg/ml	CONTROL
ORGAN WEIGHT (g)	0.94±0.2	0.62±0.08	0.62±0.13	0.48±0.13

All Values are presented as Mean±SD

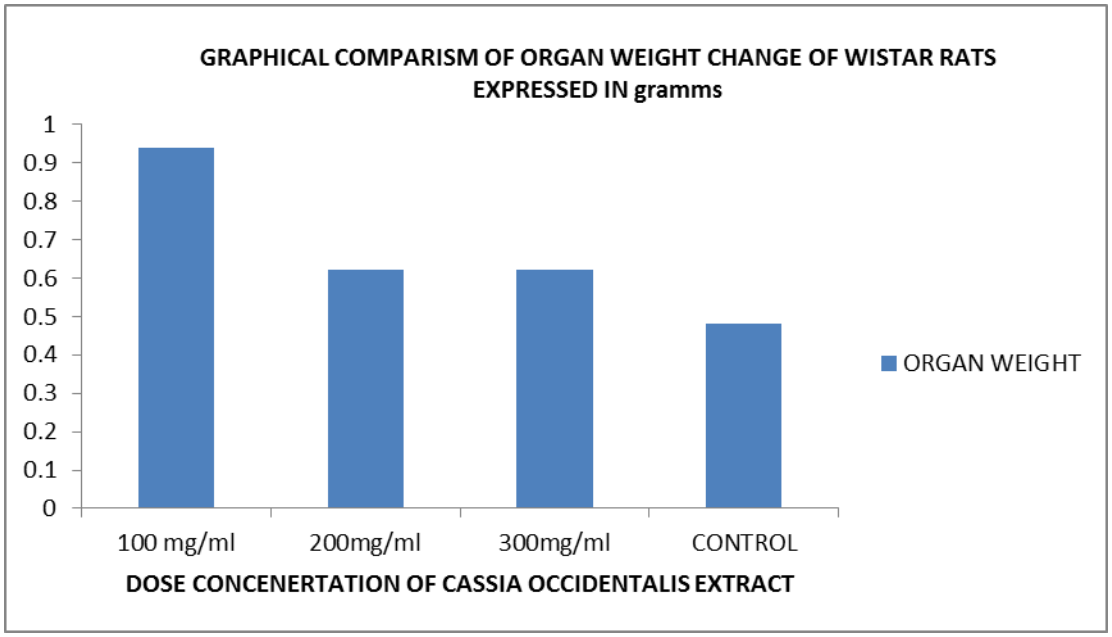

Fig. 1: Graphcal Comparism Of Variation In organ Weight.

Table 2: Effect of *cassia occidentalis on the* body weight of wistar Rats.

DAYS/GROUPING	100 mg/ml	200mg/ml	300mg/ml	CONTROL
Day 7	173±16.43**	196±20.43**	157±11.5**	162.6±7.6**
Day 14	162.4±10.06**	194±28.6**	154±8.2**	164.4±5.63**
Day 21	170±7.9**	198±25.15**	158±7.5**	166±7.4**
Day 28	178±12.54**	206±24.84**	165±12.24**	164.8±6.1**

All Values are presented as Mean±SD Values followed by ** are considered significant P< 0.05

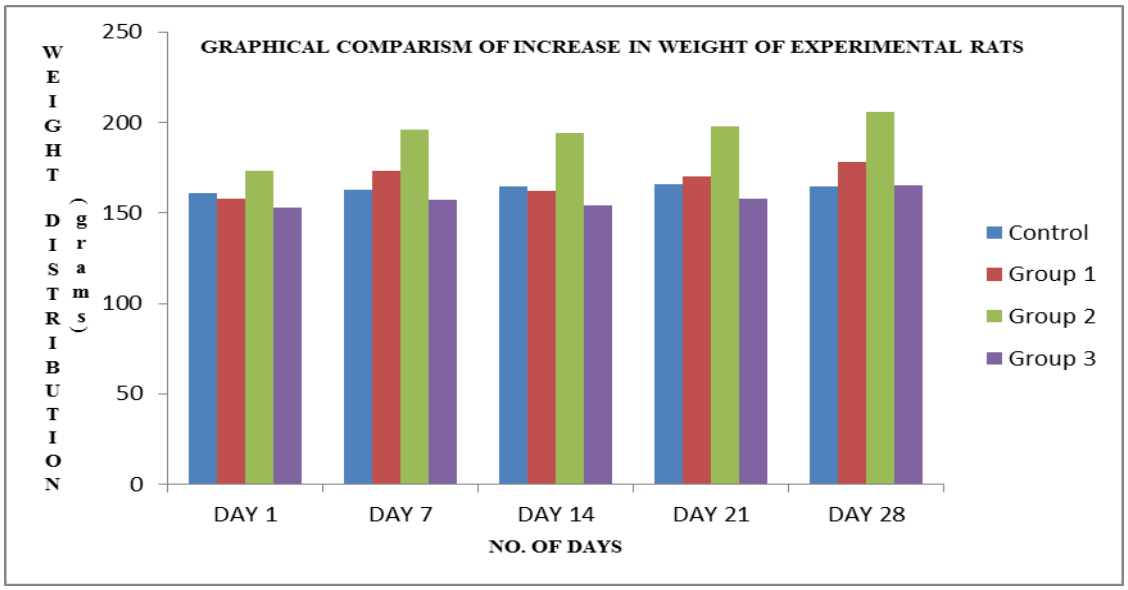

Fig. 2: A graphical comparism of weight variation of Rats dose *Cassia Occidentalis.*

SECTION B: HAEMATOLOGICAL PARAMETERS

Table 3: Effect of *Cassia Occidentalis* on Haematological Parameters.

PARAMETERS	Control	100mg/kg	200mg/kg	300mg/kg
PCV	41.8±5.89	46.8±6.38	46.6±1.5	50.6±6.5
WBC	6450±4432.8	5240±2265.61	4400±2265.6	1160±151.7
NEUTROPHILS	47.6±7.7**	31.8±4.71**	41.4±11.1**	66.4±8.44**
LYMPHOCYTES	50.2±7.9**	63.6±3.8**	55.4±11.1**	31.4±8.3**
EOSINOPHILS	1.6±0.55	1.4±0.55	1.6±0.55	1.2±0.45
MONOCYTES	1±0	2±1	1.6±0.89	1±0

Table 3. shows the result of the effect of *Cassia occidentalis* on haematological parameters. Values are presented as Mean±SD values followed by ** shows a level of significant difference at P<0.05.

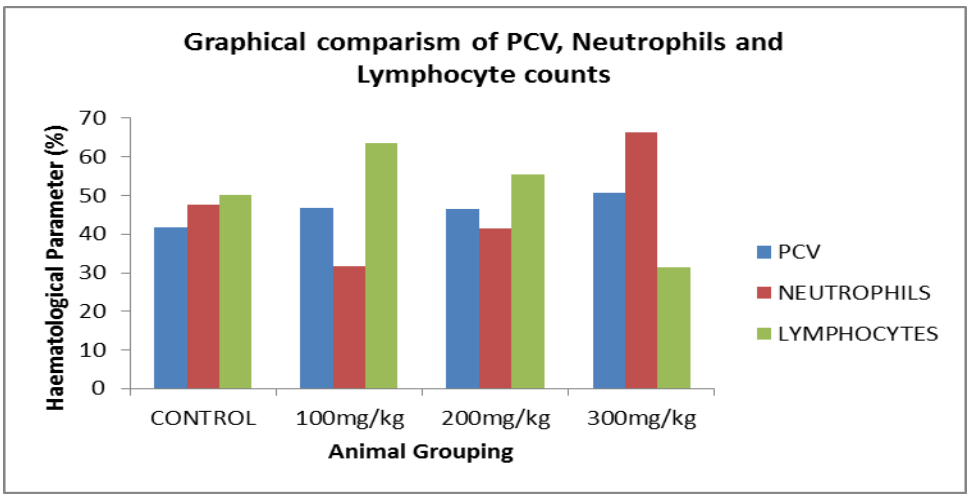

Fig.3: A graphical comparism of variation in PCV, Neutrophils and Lymphocyte counts of Rats dosed *Cassia Occidentalis.*

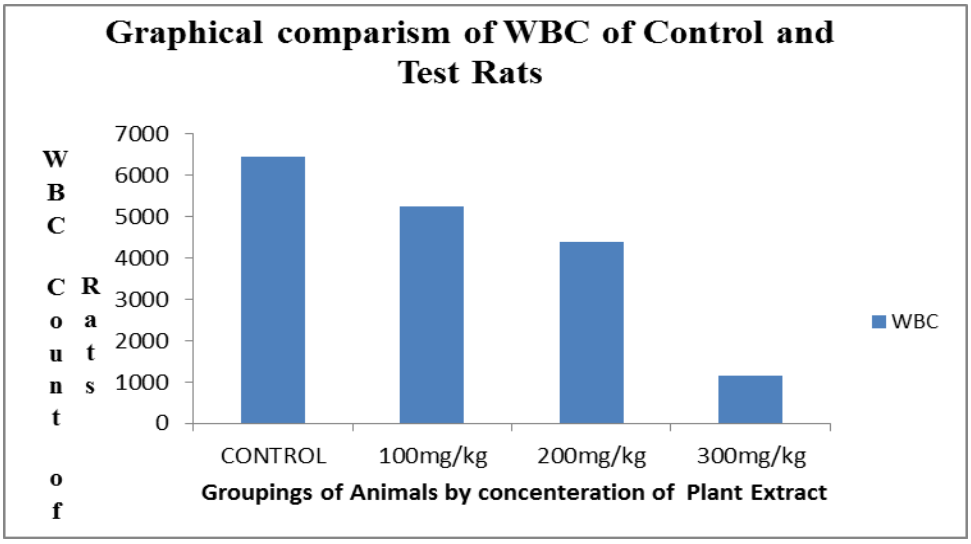

Fig. 4: A graphical comparism of variation in WBC of Rats dose *Cassia Occidentalis.*

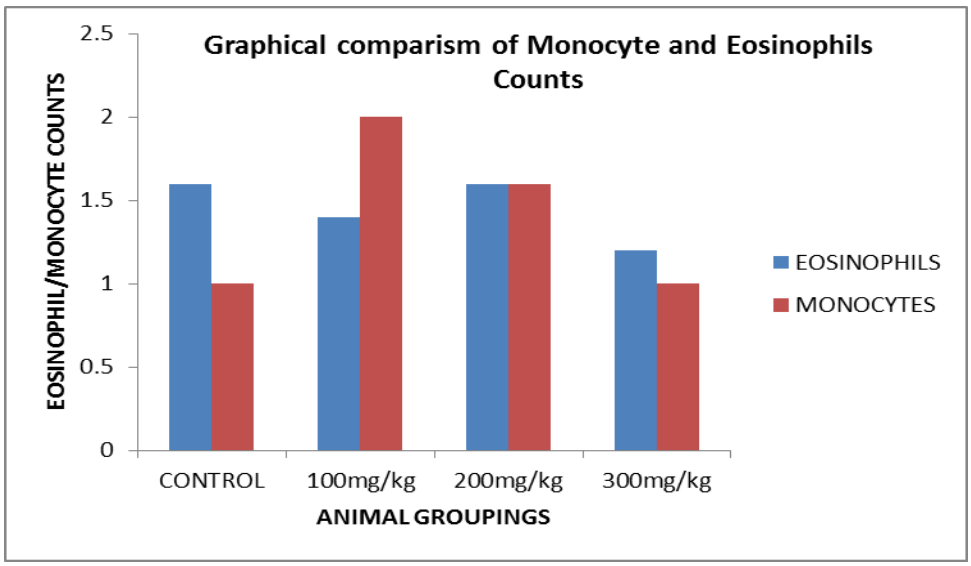

Fig. 5: A graphical comparism of variation in monocyte and Eosinophil count of rats dosed *Cassia Occidentalis.*

HISTOPATHOLOGY OF THE HEART

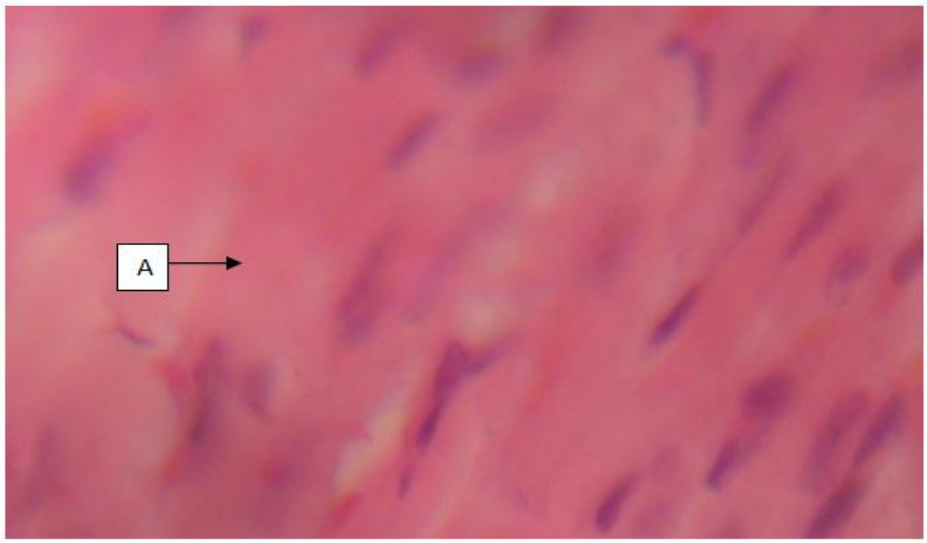

Fig 4.6: Control: Normal Rat Heart Myocardium showing bundles of myocytes in syncytium A (H&E x 40)

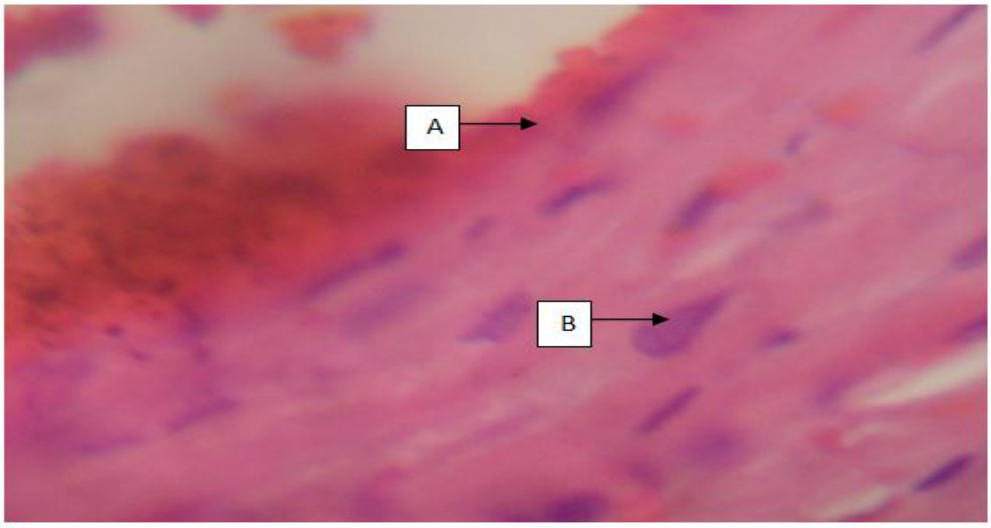

Fig 4.7: Rat Heart treated with 100mg/kg *Cassia Occidentalis* for 29 days showing mild vascular congestion and dilatation A and mild infiltrates of chronic inflammatory cells B (H&E x 40)

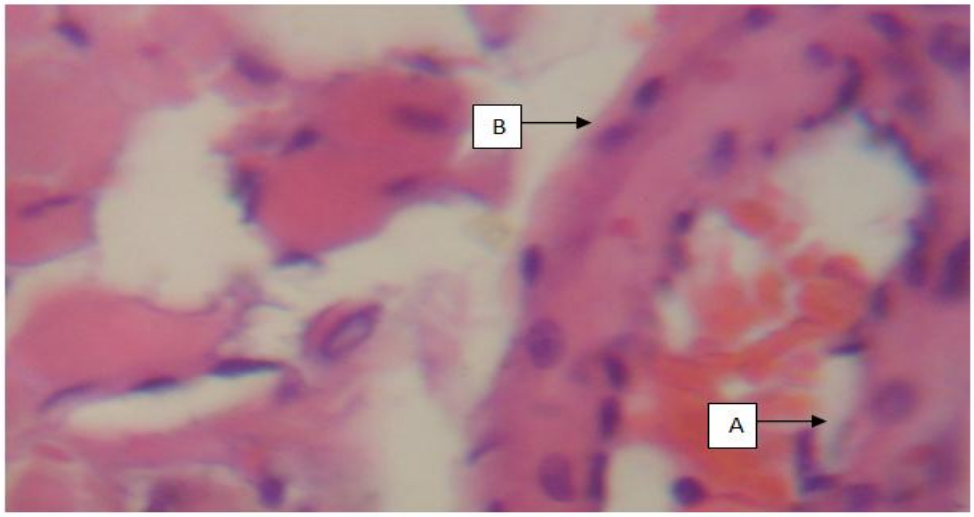

Fig 4.8: Rat Heart treated with 200mg/kg *Cassia Occidentalis* for 29 days showing mild vascular congestion and hypertrophy A and moderate tissue separation B (H&E x 40).

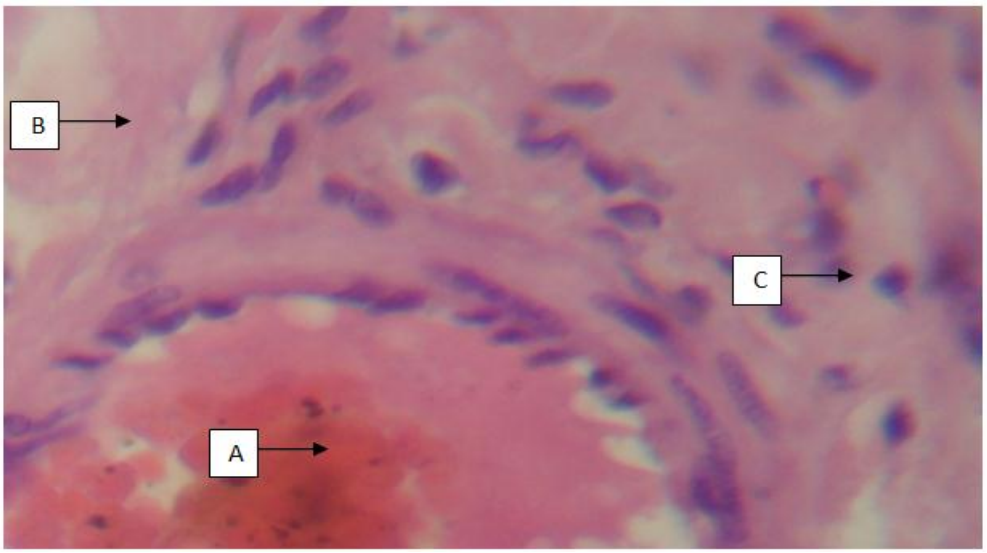

Fig 4.9: Rat Heart treated with 300mg/kg *Cassia Occidentalis* for 29 days showing mild vascular congestion and hypertrophy A, mild tissue separation B and mild infiltrates of chronic inflammatory cells C (H&Ex 40)

DISCUSSION OF FINDINGS

Several studies have been carried out on the effects of cassia occidentalis on some organs this include the works of Jafri et al., 2008, Chris-Ozoko et al 2012, Chris-Ozoko 2013 etc. This present study focused on the effects of *Cassia occidentalis* on haematological parameters and the histology of the heart.

MORPHOMETRIC RESULTS (MEAN TOTAL BODY WEIGHT AND MEAN ORGAN WEIGHT).

Effects of aqueous extract of cassia occidentalis on the total body weight showed a varying weight difference among and between groups; however it was observed a uniform/dose dependent weight increase in animals in all groups, since this was observed in the control group it makes the effect of the extract on the total body weight unremarkable. It could however be said that oral consumption of this extract would not necessary cause a weight gain or weight loss.

The mean cardiac weight as shown in table 1 and fig 1 showed an increase in the mean Heart weight (0.94±02) which was statistically significance in group 1 administered with 100mg/ml when compared with the control group and this is followed by a decline in 200mg/ml and 300mg/ml (0.62±08) and (0.62±13) respectively. This swelling may be due inflammatory response as reported by Ferrero-Miliani et al., 2007 and also been reported by Eboh and Ekundina 2013.

The result revealed dose dependent increase in the levels of the PCV, Neutrophil, and lymphocyte while the WBC revealed a dose dependent reduction, the Eosinophil and Monocyte count were unremarkable.

HAEMATOLOGY PARAMETERS

Table 3 Revealed the result of cassia occidentalis on some haematological parameter the packed cell volume PCV, Neutrophil and lymphocyte showed a dose dependent increase among groups and as such the plant, could be said to have haemopoietic and immunogenic properties as reported in some extract Ebeye et al., (2013) however some hematological indices like white blood cell count (WBC) showed a reduction when compared to control.

The significant difference ($P< 0.05$) in the Lymphocyte and Neutrophilic count, these variations which led to an increase in the Neutrophilic count of 100mg/kg, and 200mg/kg dosed group but reduced in the 300mg/kg group. The eosinophillic count and monocyte was unremarkable.

Lymphocytes have been known to be made up of two special cells which include the T cells (thymus cells) and B cells (bursa-derived cells). These cells are the major cellular components of the adaptive immune response and are involved in cell-mediated immunity, whereas B cells are primarily responsible for humoral immunity (relating to antibodies). Neutrophils on the other hand, are normally found in the blood stream. During the beginning (acute) phase of inflammation, particularly as a result of infection, environmental exposure, and some cancers, Neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation (Kumar et al , 2004).

Implications of this observation could be that the plant *Cassia occidentalis* may b e toxic thus the need for immune response as seen in the rising level of the lymphocytes.

HISTOPATHOLOGY OF THE HEART

A further study of the heart histology as shown in figures 1-4 revealed that the heart of the normal rats in the control group showed myocardium displaying bundles of myocytes in syncytium A. However, Rat Heart treated with 100mg/kg *Cassia occidentalis* for 29 days showed mild vascular congestion and dilatation as seen in point A and mild infiltrates of chronic inflammatory cells seen in point B while rat Heart treated with 200mg/kg *Cassia occidentalis* for 29 days showed mild vascular congestion and hypertrophy as seen in point marked A and moderate tissue separation in point marked B. But Heart treated with 300mg/kg *Cassia occidentalis* for 29 days showed mild vascular congestion and hypertrophy in point marked A, mild tissue separation in point marked B and mild infiltrates of chronic inflammatory cells in point marked C.

Cassia occidentalis in this study.

It is worth mentioning that studies in cardiac anatomy and physiology have severally implicated symptoms of heart failure to involve reduction of cardiac output (fatigue, weakness) or to excess fluid retention (dyspnea, orthopnea, and “cardiac wheezing”) thus with progression hepatic congestion of the cardiac cells occurs (Davie et al., 1997), which was very evident in the various groups dosed 100-300 mg/kg body weight of *Cassia occidentalis* but this could be due to the environmental factors.

CONCLUSION

Based on the observations made from the test results, it can be concluded that aqueous extract of *Cassia occidentalis* leaf activated the immune system which could be an inflammatory response to the extract administration. Further works should be carried out so as to assess more beneficial or hazardous effect of the plant to corroborate findings from this study.

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