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HISTOPATHOLOGICAL AND HAEMATOLOGICAL EFFECTS OF AQUEOUS EXTRACT CNIDOSCOLUS ACONTIFOLIUS ON THE LIVER AND KIDNEY IN ADULT WISTAR RAT

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ABSTRACT

The effects of chronic administration of aqueous leaf extract of cnidoscolus aconitifolius on some heamatological and histopathological parameters of the kidney and liver of wistar rats. Twenty four (24) adult wistar rats were acclimatized, weighed and sorted into four groups (A-D) of 6 animals each with corresponding body weights in the same group. Aqueous leaf extracts of cnidoscolus aconitifolius was administered orally to rats in groups B-D at 200mg/kg, 400mg/kg and 600mg/kg body weight respectively for 28 days aside the normal feed and water while group A which served as the control was given only feed and water for the same duration. At the end of administration, animals were sacrificed and blood was collected from the inferior venecava into vials containing EDTA for the various tests while the kidney and liver samples were quickly fixed in 10% formol saline. Tissue sections of the samples were processed and stained using hematoxylin and eosin stains respectively. Results showed increase in WBC (leukocytosis), glomerular degeneration as seen in proliferative glomerulopathy as well as hepatic damage evident in periportal fibrosis at the highest dose (600mg/kg). Consumption of cnidocolus acontifolius should be with caution

as it elicited a dose dependent damaging effects on the organ studied.

Keywords: *Histopathology, Haematology, Liver, Kidney and cnidocolus acontifolius*

INTRODUCTION

The need to keep body and soul together in a healthy condition for long period of time has been the major concern of many people globally today (Nelson, 1982). This has driven many people into exploring different health care methods to combat the numerous endermic micro organisms that are pathogenic in nature (Owolabi et al., 2007)

An increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several remedies (UNESCO, 1998). High cost of exploring modern medicine has made vast number of people to stick to traditional medicine which employs the use of plants and herbs in curing one disease or the other without ascertaining the active ingredients in them vis-à-vis the right dosage (Senjobi et al., 2011).

Researchers have confirmed the use traditional medicinal plants as best source of disease curing drugs (El- Said et al., 1971). This can be attributed to its availability and cheap source (Owolabi et al., 2007). Over 80% of the world's population depends on herbs with about 60% of the rural populace in Nigeria depending on them to cater for their health care needs (Ghani et al.,1989). Researches also attribute the high use of plants especially in Africa to shortage of hospitals, health care centres, trained personnel

and the prohibitive cost of modern day medicine (Senjobi et al., 2011).

One of the plant genera widely used traditionally is cnidoscolus aconitifolius which belongs to the group of arbrescent shrubs measuring 3.6cm in height. The purpose of this study is to investigate some hematological parameters and histopathological effects of chronic administration of crude aqueous extracts of cnidoscolus acontifolius on the liver and kidneys of adult male and female albino wistar rats

MATERIALS AND METHOD

Experimental Animals

Inbred male and female wistar albino rats weighing 140-215g were purchased and maintained in the Animal Holding Unit in the Faculty of Basic Medical Sciences, Delta State University, Abraka.

Plant Material

Fresh leaves of cnidoscolus aconitifolius were collected from a garden in site ii of Delta State University, Abraka and taken to the department of Botany for identification.

Study Design

The 24 adult rats were shared into 4 groups (A-D) according to body weight with 6 animals per group. Groups B, C and D were administered 200mg/kg, 400mg/kg and 600mg/kg body weight of cnidoscolus aconitifolius extract while A served as control.

Animal Care and Management

Animals were fed with grower's mash and water twice a day (morning and evening). The animals were maintained in a wooden cage which was cleaned daily and maintained with wood shavings. All treatment and handling of animals conformed to the guidelines of the National Institute of Health (NIH, publication 85-23, 1985) for laboratory animal care and use.

Preparation of aqueous plant extract

The fresh leaves after being identified were rinsed and air dried. They were then pounded using a mortar and a soxhlet extractor was used to get the extract. The aqueous extract was then after filtered using whatman's filter paper and concentrated in an oven. About 78g of the extract was obtained and 8g was dissolved 100ml of distilled water to get a stock concentration of 80mg/kg body weight which was administered to animals on each day of the experiment (Adekomi et al.,2007).

Treatment of Animals with Extract

After two weeks of acclimatization, animals in group A (control) continued with feed and distilled water while animals in groups B, C and D in additional to feed received 200mg/kg, 400mg/kg and 600mg/kg body weight of the extract through an orogastric tube and a canula for a period of 28 days. (Adekomi et al.,2007)

Preparation of Animal Samples

Rats were sacrifice after an overnight fast. Liver and kidney samples were quickly harvested, rinsed and weighed and sections of the liver and kidney were fixed in 10% formol saline.

Blood collection

Blood was collected from the inferior venacava of heart of the animals into vials containing Ethylene Diamine Tetra Acetic Acid (EDTA) which prevents coagulation by complexing ca^{2}. The vials were immediately capped and the content rocked gently for about a minute by repeated inversion.

RESULTS

Table 1: Showing total body weight(g) of extract

Groups	Week 1	Week 2	Week 3	Week 4
Group A(control)	152.50±13.69	160.83±12.42	179.50±24.74	197.50±36.16
Group B (200g/kg) C.A. extract	164.17±10.68	165.83±8.61	181.67±17.79	191.67±25.03
Group C (400g/kg) C.A. extract	187.50±16.05	190.83±13.57	198.33±23.17	196.67±29.61
Group D (600g/kg) C.A. extract	202.50±12.94	203.33±14.02	214.00±20.93	219.00±32.45

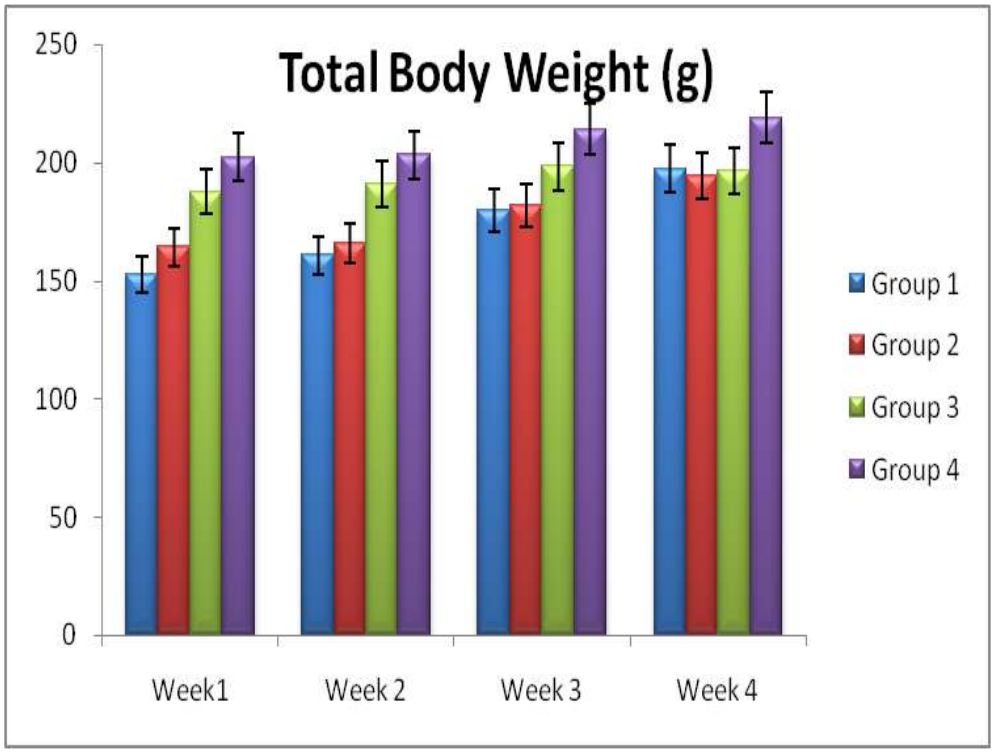

Figure 1: Showing total body weight (g) of extract

Table 2: Showing organ weight (g) group

Organs (g)	Group A	Group B	Group 3	Group 4
Kidney	0.55±0.10	0.62±0.08	0.57±0.05	0.70±0.09
Liver	6.15±0.48	6.35±0.85	6.90±1.00	8.00±1.14

HEMATOLOGICAL INDICES

Table 3 Differentials Count (%)

Differentials (%)	group A(control)	Group B	Group C	Group 4
Lymphocytes	51.50±5.05	41.67±10.56	40.176.40	40.67±
Neutrophil	39.50±13.17	39.67±6.38	45.33±8.73	47.50±6.12
Eosinophil	18.17±1.72	18.00±2.09	18.33±1.51	18.33±1.51
Basophil	0.33±0.52	0.83±0.41	0.50±0.55	0.17±0.41
Monocyte	3.83±0.98	3.60±0.82	4.33±0.52	4.17±0.75

TABLE 4: Showing Total WBC($x10^3$) and RBC($x10^6$)

Parameters	Group A (control)	Group B	Group C	Group D
WBC ($x10^3$)	7933.30±476.10	8416.70±1049.60	9050.00±1579.10	7850.00±1080.28
RBC (x106)	5.40±0.44	7.15±1.23	6.75±0.73	6.78±0.53

TABLE 5 showing PCV, HB and MCHC

Parameters (g/dl)	Group A	Group B	Group C	Group D
PCV	46.33±4.72	47.00±6.42	50.00±2.48	48.00±5.14
HB	15.45±1.56	15.68±2.12	16.65±8.73	16.07±1.67
MCHC	0.33±0.00	0.33±0.00	0.33±0.00	0.33±0.00

HISTOPATOLOGICAL RESULTS

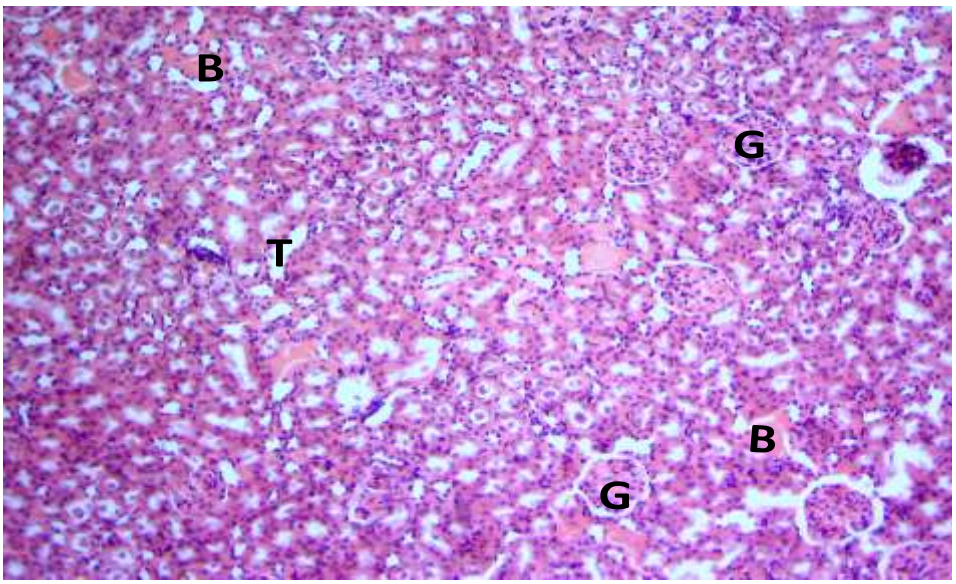

Plate I. Photomicrograph of a section of the kidney of control rats showing normal features. Glomeruli (G), tubules(T) and congested blood vessels(B). H & E X 100

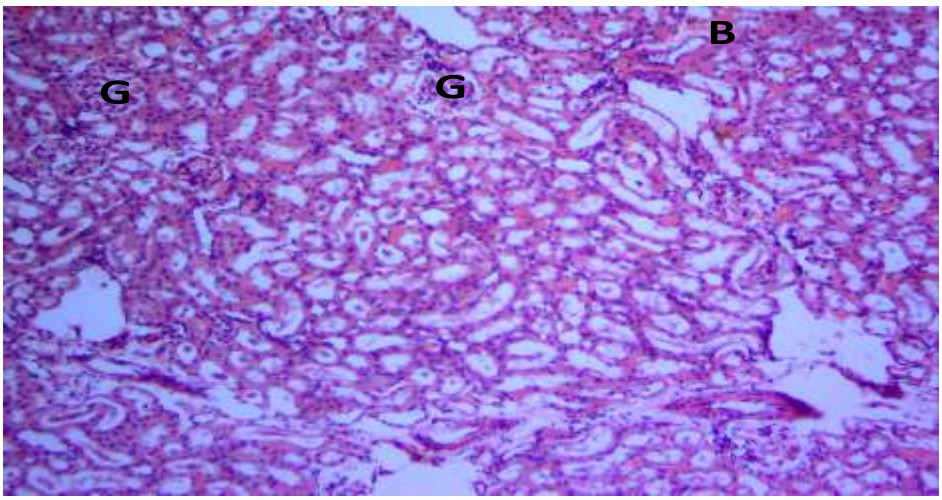

Plate 2. Photomicrograph of a section of the kidney of rats administered 200mg/kg body weight of C.A. leaf extract showing mild tubular degeneration and presence of inflammatory and marked congestion. H & E X 100

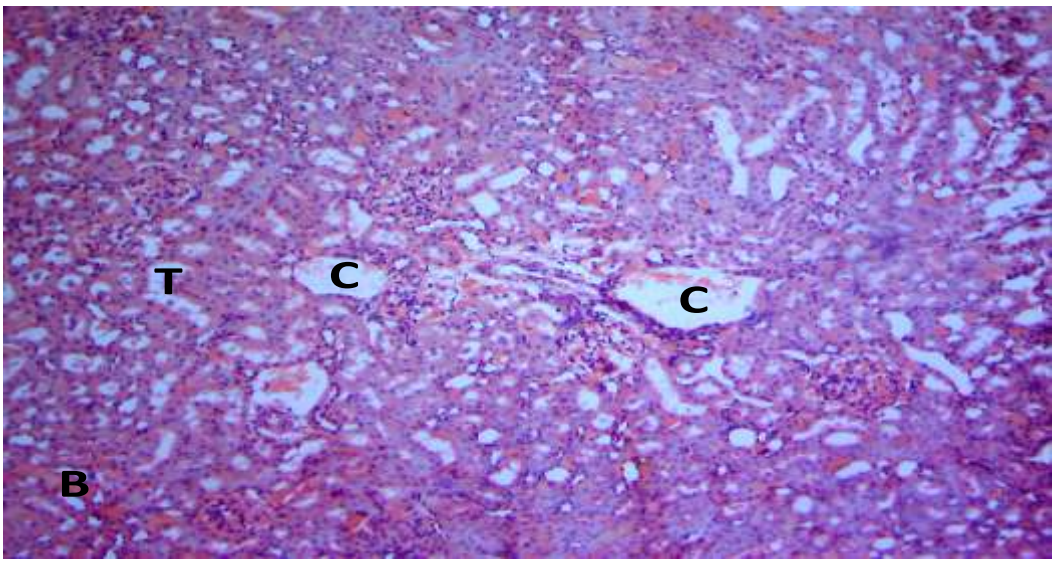

Plate 3. Photomicrographs of a section of the kidneys of rats administered 400mg/kg body weight of C.A. extract showing tubules filled with cast(C),interstitial edema and congested blood vessels (B). H & E X 100.

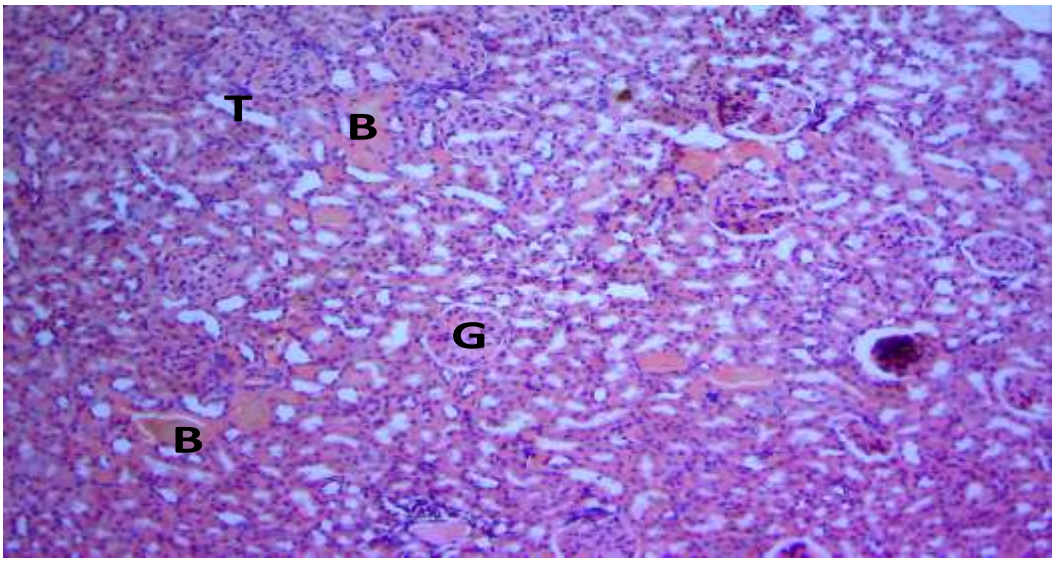

Plate 4. Photomicrograph of a section of the kidney of rats administered 600mg/kg body weight of C.A. extract showing glomeruli(G), marked congestion of the Interstitium (B). H & E X 100

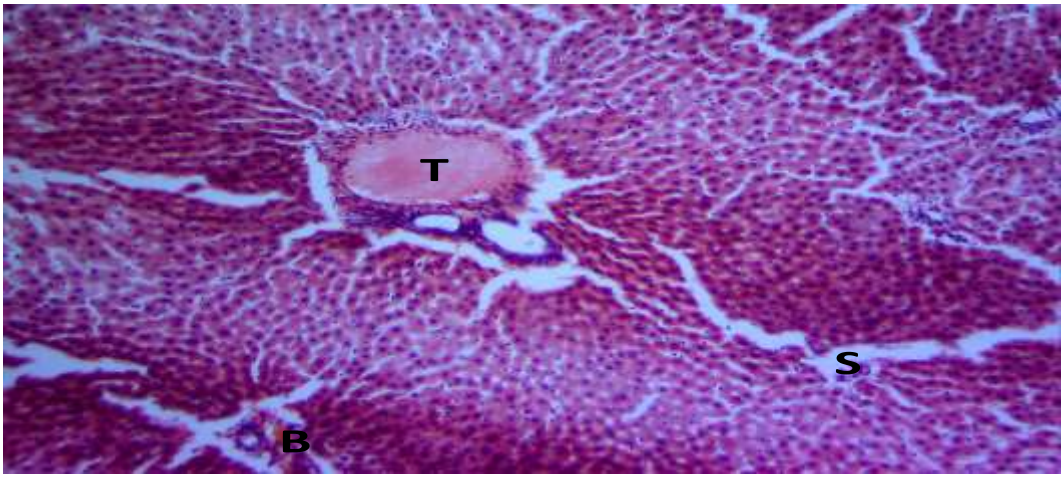

Plate 5. Photomicrograph of controlled sections showing portal triad (T), sinusoids(S), hepatocytes and few congested blood vessels (B) the Interstitium is free from inflammatory cells and congestion. H & E X 100

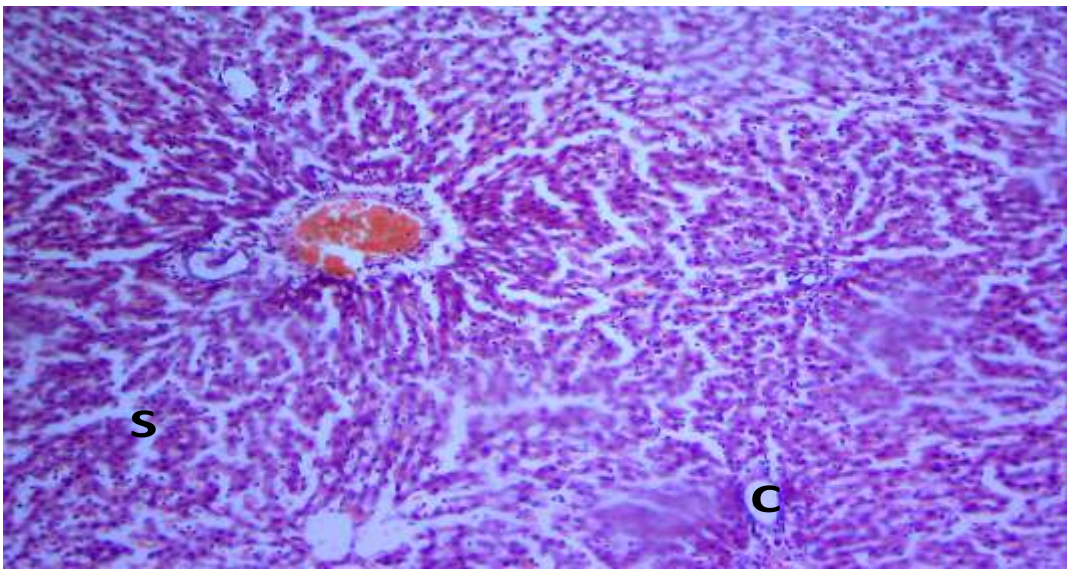

Plate 6. Photomicrograph of a section of the liver of rats administered 200mg/kg body weight of C.A. extract showing features with no marked change; sinusoid(S), central vein(C). H & E X 100

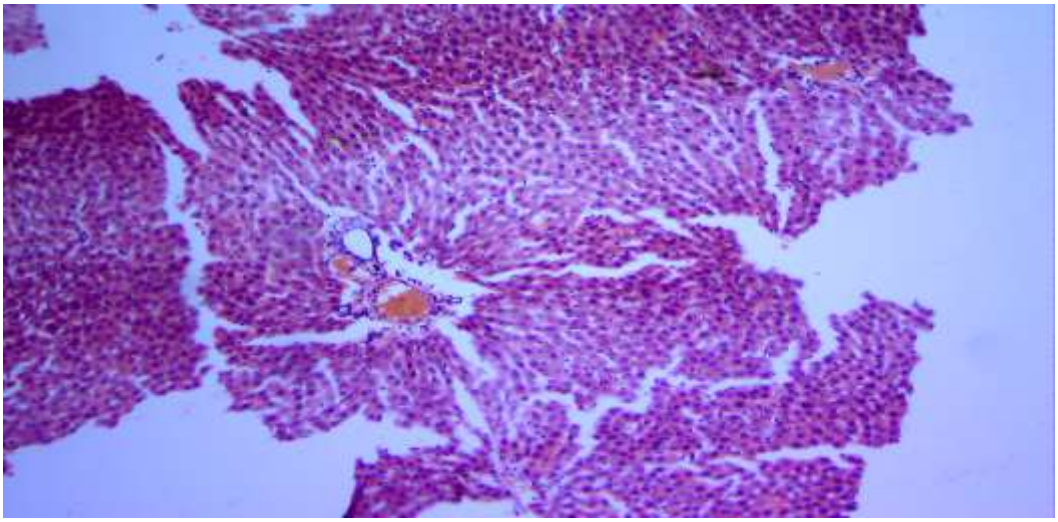

Plate 7. Photomicrograph of a section of the liver of rats administered 400mg/kg body weight of C.A. extract showing periportal inflammatory cells and congested vascular channels

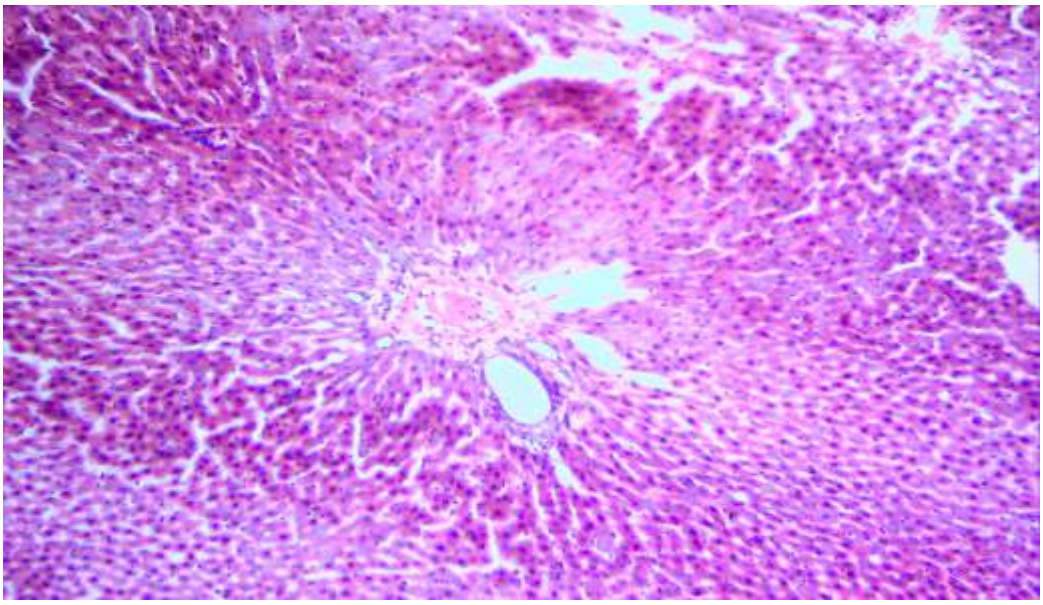

Plate 8. Photomicrograph of a section of the liver of rats administered 600mg/kg body weight of C.A. extract. Showing periportal fibrosis and inflammatory cells. H & E X 100

DISCUSSION

Herbs are widely perceived by the general public as being free from side effects; however, herbal therapies have not been effusively researched or standardized to enable clinical application.

Morphological, Histopathological Hematological and Biochemical effects after use of some medicinal herbs have been reported in the works of Ekundina et al 2014, Eboh and Ekundina, 2013 Ebeye et al 2013 and Chris-Ozoko et al 2013.
The present studies examined the effects of chronic administration of crude aqueous extracts of cnidoscolus aconitifolius on hematological parameters and the histological effects on the kidneys and liver of wistar albino rats.

Phytochemical screening revealed nine bioactive compounds detected in the aqueous extract of the plant this compounds are; Alkaloids, Saponins, Phenolics, Tannins, Flavonoids, Anthraquinones, Phlobatanins, Triterpenes and cyanogenic glycosides however there quantity were not assessed. This active ingredient were reported present in previous researches by Iwuji et al., 2011, Mordi and Akanji, 2011.

The presence of tannins suggests the ability of the plant to play a major role as an antidiarhoe and antihaemorrhagic agent (Asguith and Butler, 1996). Saponins have been shown to have immense significance as antihypercholesterol, hypertensive and cardiac depressant properties (Trease and Evans, 1995) while phlobatanins suggest the diuretic properties of the plant (Okuda,1991) which explains why the plant is been used as a diuretic agent. Flavonoids indicate the plant as a source of natural antioxidants which may interfere

with the functions of highly oxidative organs which have high demand for oxygen consumption (Kuti and Konuru, 2004). The plant was also found to contain cyanogenic glycosides which is a source of cyanide poison (Leslic, 2010). Alkaloids as found in the aqueous extract indicates the detoxifying and antihypertensive properties of the plant (Trease and Evans 1997). Alkaloids have also been found to reduce progesterone level (Gocze et al., 1996).

Morphologically; there was an increase ($p>0.05$) in the weights of animals administered with cnidoscolus aconitifolius extract compared to control. This increase was dose dependent although there was a slight decrease in group 3. This increase in weight confirms the nutritional value of the plant as being rich in proteins, minerals like calcium, phosphorus, magnesium and iron; vitamins A, B and C (Lelie 2010).

Hematologically there was an increase($P>0.05$) in the PCV of rats administered 200mg/kg (47±6.42), 400mg/kg (50±2.45) and 600mg/kg (48±5.14) compared to the control (46.33±4.7). Hemoglobin values also increased as well as WBC and RBC. These increases in the values of PCV, Hb, RBC and WBC corresponded with the results of Henry (2012) but varied with the results of Temitope et al (2010).

WBC differentials showed a significant difference($p<0.05$) in the eosinophil value, an increase in the values of monocytes and neutrophil and a decrease in the lymphocytes.
The increased WBC (leukocytosis) could be a possible infection caused by the extract since it was the only administration given to animals. White blood cells are cells of

the immune system which help the body to fight against infections and diseases.

Histopathologically sections of the liver of rats in groups1 (control), 2(200mg/kg) and 3(400mg/kg) revealed hepatocytes disposed in sheath. Also seen was the portal triad, containing portal vein, artery and bile ductuli within the sheaths of hepatocytes. These hepatocytes were composed of a centrally placed nucleus and eosinophilic cytoplasm. A few congested blood vessels were also seen. However, liver sections of rats administered 600mg/kg (group 4) showed features in keeping with periportal fibrosis.

Histopathological examination of sections of the kidneys of rats in group 1(control) and group 2(200mg/kg) showed several normal sized glomeruli disposed within a loose connective tissue. Intermixed with the glomeruli were several tubules whose lumen were filled with cast. These tubules were lined by a single layer of epithelia cells. Also seen were a few congested blood vessels(plates 1 and 2). There were no degenerative changes. Sections of the kidneys of rats administered 400mg/kg (group 3) showed several variable sized renal corpuscle disposed within a loose connective tissue stroma in which were several tubules some of which had hemophore cast in their lumen. A few dilated and congested blood vessels were also seen within the tissue.

Sections of the kidney of rats administered 600mg/kg (group 4) showed several variable sized renal corpuscle. These corpuscles were composed of a Bowman's capsule and highly cellular glomeruli. The surrounding tubules were also lined by a single layer of epithelium and showed moderate dilatations on

their lumen. Several diluted blood vessels were also seen. Features were those of proliferating glomerulopathy.

CONCLUSION

Results from this study shows that aqueous extracts of cnidoscolus aconitifolius administered to rats for the period of 28 days showed both positive and negative effects on the rats. It should be noted however that despite its nutritional values and medicinal efficacies, a high dose of cnidoscolus aconitifolius leaf extract is considered to possess degenerative properties as revealed in this work.

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