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The Effects of the Aqueous, Ethanol and Hexane Extracts of *Cnidoscolus aconitifolius* Leaf on the Hematological Indices and Liver Histological Status of Streptozotocin-induced Diabetic Rats

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Abstract

The effect of the aqueous, ethanol and hexane extracts of *Cnidoscolus aconitifolius (Ca)* leaf was examined heamatologically and histologically on Streptozotocin-induced diabetic rats**.** 50 Wistar rats were purchased but only 40 rats were given a single dose of streptozotocin (STZ) (60mg/kg body weight). 25% of STZ dosed animals died while the survivals were distributed into groups II-V. Group II was untreated while groups III, IV and V were treated to hexane, ethanol and aqueous extract of *Ca* respectively. Rats that constituted the Control group were selected from the STZ untreated animals but were allowed liberty to rat chows and normal saline. Heamatological parameters evaluated revealed significant ($P<0.05$) increase in the Hb, PCV, and Total Red Blood Count (TRBC) levels when compared with the diabetic group. Furthermore, the Total White Blood Count (TWBC), platelets, neutrophils and lymphocytes were near normal for the *Ca* extract treated groups as the control. The liver architecture of the STZ-induced diabetic group from this present result displayed distortion of the lobular pattern of the hepatocytes, with several foci of edema and congestion. These were followed by marked periportal hepatolysis and cast within parenchyma. Furthermore, between the hepatocytes were dilation of sinusoids with increased leucocytic infiltration and fibrosis. The nuclei of most cells revealed clear signs of necrosis and the hepatocytes were swollen with conspicuous cytoplasmic vacuolations. Upon treatment with aqueous, hexan and ethanol extracts of *C. aconitifolius,* sections of the hepatocytes exhibited moderate congestion and improved hepatic features with the aqueous extract exhibiting more potency than the hexane and ethanol extracts. The anti-oxidative tendency of each solvent extract depicts reduced MDA and increased GSH contrary to the diabetic-induced group alone. Hence from this study, the anti-diabetic rationale of *C. aconitifolius* was further buttressed revealing aqueous solvent extract of *C. aconitifolius* as a more potent anti-diabetic agent.
Keywords: *Cnidoscolus aconitifolius (Ca)*, heamatology, liver histopathology, streptozotocin

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1. Introduction

Diabetes mellitus (DM) is an escalating menace and demur experienced in the health precinct. It is gradually become a household disease with no age limitation. Complications emanating from DM entail arrays of dysfunctions portrayed by mal-regulation of biomolecules like carbohydrate (Defronzo *et al.*, 2015). A distinct or pronounced feature of DM is hyperglycemia associated with insulin secretion, insulin resistance or combination of both (Defronzo *et al.*, 2015). Hypoglycemic production elicits oxidative stress via glucose autoxidation, polyol pathway alteration and non-enzymatic glycation (Bonnefont-Rousselot *et al*., 2004).

DM individuals are predisposed to developing retinopathy, nephropathy and neuropathy (microvascular complications) and cardiovascular comorbidities (macrovascular complications) as a result of hyperglycaemia and metabolic syndrome (Defronzo *et al.*, 2015). However, the associated insulin resistance and impeded insulin secretion determines the kind of anti-diabetic agents required to maintain normoglycaemia (Ezebuiro *et al*., 2020). Over time, novel medications have being designed and developed, but the highest priority is based on the production of agents that can improve insulin sensitivity, pause the advancement of pancreatic β-cell failure and reverse, prevents or blocks the macrovascular and microvascular complications associated with DM (Chawla *et al*., 2016).

Insulin replacement therapy, diet regulations, exercise implementations, oral hypoglycemic drugs intake (such as sulfonylureas and biguanides) are major medicinal measures for treating and managing DM (Thomas and Stringer, 2019; Uti *et al.*, 2021). In spite of these treatment methods which are quite effective and have generated positive results, the search for treatment options continues to intensify as a result of persisting limitations of

existing ones (Padhi *et al.*, 2020).

Many native leafy herbs and plants with distinct potentials as traditional sources of food and medicine abound in third world nation with Nigeria highly diversified in folkloric herbs. These herbs and their extracts which have being undermined, under exploited, under maximized and underutilized have been excavated to possess substantial amounts of bioactive ingredients which sustained their local applications for centuries. In addition, these bioactive agents have equally sufficed as food for local consumption, food additives as spices and medicinal exigencies in disease management and treatment (Ojieh *et al.*, 2020; Mordi *et al.,* 2016).

With renewed and increased interest in trado-medicine coupled with emerging side effects with the application of orthodox medications, attention is being channeled in promoting and redesigning herbal products with the intent of harnessing both their nutritive and medicinal efficacy for societal welfare. Foliage biodiversity is noticeable with sub-Sahara countries because of enriched herbal reservoirs and medicinal plant resources. Amidst these natural endowments is the plant genera taxonomically known as *Cnidoscolus aconitifolius* (Euphorbiaceae). *C. aconitifolius* informally alludes as Chaya (Donkoh, *et al*., 1990). Several ethnic groups or locality in Nigeria has called *Cnidoscolus aconitifolius* different names such as "*efo Iyana Ipaja*"; but its traditional nomenclature and usefulness is based on its therapeutic potential, aesthetic properties, nutritional values and perhaps method of extraction (Mordi 2012; Mordi and Akanji, 2012). With the high negligence and underutilization of native herbs such as *Cnidoscolus aconitifolius,* insight into the mycobiota of *Cnidoscolus aconitifolius* phyllosphere is a springboard to yield proliferation and pathogenic species combat thereby enhancing the availability of the plant species to the growing populace (Ogbuji and Ataga, 2022)

Several folk claims have been attributed to the medicinal efficacy of *C. aconitifolius* for the treatment or management of numerous health complications and ailments. Its treatment value ranges from the capacity to improve vision, neutralize scorpion stings, manage insomnia, and eliminate gout (Jensen, 1997, Atuahene *et al*., 1999). Other studies have demonstrated the application of *C. aconitifolius* in alcoholism, diabetes mellitus, obesity, kidney stones, hemorrhoids (Diaz-Bolio, 1975; Kuti and Torres, 1996, Mordi, 2012) and anti-inflammation (Padilla-Camberos *et al.,* 2021).

Recently, the phenolic and amino acid composition of Ethiopian Chaya (*Cnidoscolus chayamans*a) was documented (Temesgen *et al.,* 2022). High phenolic and amino acid composition of this plant improves nutritional efficacy thereby alleviating diet-related illnesses. Subsequently, the efficacies of herbal plants are connected to the method of extraction or the kind of extract adopted (AL-Rawi, 2007; Ojeh *et al*., 2020). Ironically, there is a broad perception that herbal produce are harmless and devoid of side effects. This perception is extremely exaggerated and over simplified but not scientifically motivated or accurate. Studies have reported the existence of myriads of constituents in herbal plants of which quite a number may exude and elicit toxic side effects with adverse consequences (Shaw *et al*., 1997; Kaplowitz, 1997; Calix, 2000). With respect to diabetes, about 400 medicinal herbs have being reported and documented, albeit only a few are scientifically recognized or has scaled through clinical trials. Hence the World Health Organization Expert Committee (WHOEC) has endorsed further medicinal examinations, investigation and reassessment of new facts and claims emanating from the application of trado-herbal practices in diabetes treatment and management (WHO, 1991). Since related study had examined the effect of the aqueous extract of *Cnidoscolus aconitifolius* on Streptozotocin-induced diabetic in Wistar rats with emphasis on lipid profile (Mordi, 2012), this study intend to compare the efficacy of other solvent extracts and their possibility in treating diabetes. Folklore medication is founded on the ability of the extracting solvent adopted in the extraction of active components domiciled in the plant. Hence this present study is premised on investigating the effect of the hexane, ethanol and aqueous extracts of *C. aconitifolius* on Streptozotocin-induced diabetic in Wistar rats by assessing their heamatological potentials and histological changes in liver tissues.

2. Materials and Methods

2.1 Plant Material and Preparation of Plant Extract:

Cnidoscolus aconitifolius fresh green leaves were harvested from an abandoned farm field along Agbor-Warri road, Delta State, Nigeria. Botanical authentication and identification was conducted at the herbarium section of the Department of Botany, Delsu Abraka with a voucher number allocated and samples domesticated at the herbarium. Leaves were sun dried for about two weeks, macerated and ground into powdery form obtaining a quantity of 399g. With the aid of a soxhlet extractor, the powdery yield was extracted with a suitable solvent and a dark brownish waxy solid was obtained. The marc was then extracted with ethanol and concentrated using rotator evaporator. After the evaporation of the ethanol, a dark greenish viscous substance of 47.33 g was collected. Preceding n-hexane and ethanol extraction, the aqueous extract was evaporated to dryness and a dark brownish viscous of 41.96 g was obtained (Mordi *et al.,* 2013). Based on the treatment method, the hexane

extract was dissolved in Dimethylsulfoxide (DMSO) (AL-Rawi, 2007) and subsequently suspended in distilled H_2O while the ethanolic and aqueous extracts were directly dissolved in distilled H_2O (Mordi *et al.,* 2013).

2.2 Ethical Endorsement

With respect to animal handling, care, control and maintenance; ethical endorsement was given based on the adherence and compliance to the approved guidelines as described by Ward and Elsea (1997). Furthermore, the experimental procedures detailed for this research, were ratified by the Faculty of Basic Medical Sciences ethical committee (FBMSEC), before commencement of laboratory studies.

2.3 Experimental Animal

Fifty (50) adult Wistar rats (between 180g – 220g) were purchased from the Animal Unit of the College of Medicine, A.A.U, Ekpoma, Edo State. The animals' were first domesticated in improvised wooden cages of 25cm high by 50cm long and allowed two weeks acclimatization to laboratory conditions prior treatment. Portable H_2O and animal Chow (manufactured by Edo Flour Mill, Ewu Edo State) was made readily available to experimental rats.

2.4 Diabetes Induction

Animal induction was carried out as elucidated and described by Mordi, (2012). After 2weeks post acclimatization, 40 male Wistar rats from the initially purchased rats, were injected (ip) with streptozotocin (60 mg/kg b.wt) (Sigma chemical) after an 18 hours fast as described by Bonner-Weir *et al*. (1981). The STZ was freshly dissolved in citrate buffer (0.01M, pH 4.5) (Ozsoy-Sacan, 2000).The injection volume was prepared to contain 1.0mL/kg (Murali *et al*., 2002). Within 48hrs 25% of diabetic rats died with about 30 rats left after post streptozotocin injection, hereby maintaining each diabetic group to 7 male only. Blood glucose level was determined and rats portraying a concentration ≥ 230mg/dL were classified as diabetic (Cetto *et al*., 2000).

2.5 Experimental Design

Animal distribution, treatment and randomization were done thus: Group I (Control) received normal saline solution (0.9% NaCl w/v, 5 mL/kg). Group II (Diabetic) rats after being induced with STZ 60 mg/kg body weight got no extract treatment. Group III, IV and V which were already induced with diabetes were administered Hexane *Ca* extract, Ethanol extract and *Ca* aqueous extract respectively. Each group had 7 rats per group (n=7). *Cnidoscolus aconitifolius* extracts were freshly prepared, hexane extract (50 mg / kg b.wt), ethanolic and aqueous extracts (100 mg / kgb. wt). Administration of the various extracts was done by oral intubation and daily observation for signs of morbidity, mortality and body weights were measured and documented from the start to finish of the treatment duration.

2.6 Blood and liver Collection

At the close of the experimental period of 8 weeks, animals were subjected to 12 hours of fasting and further anaesthetized with ether. Whole blood was easily collected into tubes containing anticoagulant (EDTA) prior heamtological estimations. The liver were dissected group-wise and small pieces were fixed in 10% formal saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraplast at 56 - 60ºC and from each paraffin block cut at 5µm on rotary microtome. Staining of the paraffin sections was carried out from each paraffin block (H&E) for histological studies and examined under light Leitz microscope.

2.7 Hepatic Antioxidant Chemistry

1kg of the removed liver was blotted and homogenized at 4 volume ice-cold homogenizing buffer (pH 7.4). Antioxidant assay conducted are Superoxide dismutase (SOD) as described by Fridovich (1989), Hepatic reduced gluthathione (GSH) was estimated by colorimetric method using Ellman's reagent as described by Sedlak and Lindsay (1968). Catalase determination was quantified and estimated as described by Aebi (1984) and Malondialdehyde (MDA) was spectrophotometrically estimated as described by Varshney and Kale (1990).

2.8 Statistical Evaluation

Expression of results were depict as Mean ± SD for six animals in each group. Subsequently, one-way ANOVA

alongside Dunnets Post Test on the difference between the control and *Cnidoscolus aconitifolius* extract administered groups were conducted by using GraphPad Prism Version 4.00 for Windows, USA. Value of <0.05 was considered as significant.

3. Result and Discussion

Osmotic diuresis and consequently polyuria (excessive urine production) are usually generated as an aftermath of high renal concentration manifested in insulin dependent diabetes (AL-Rawi, 2007). Secondary to insulin deficiency are hepatic dysfunctions, lipolysis and enormous cellular protein catabolism (Mayne, 1994). These cumulative metabolic alterations may have contributed to the myriads of biochemical and histological complications evaluated in this study of which weight loss in the STZ treated groups was implicated. Weight loss as observed in Table 1 of this study, was obviously detected in the diabetic group when juxtaposed with the control and treatment groups. The visible weight loss might also have emanated from the escalated oxidative stress connected with diabetic complications. Studies relating to oxidative disposition of the diabetic animals revealed that STZ-induced apoptosis in diabetic rats (Latha *et al*., 2004); meanwhile hyperglycemic induced oxidative stress impaired insulin signaling thereby accelerating insulin resistance. Evidently from this result, oxidative stress markers such as MDA (lipid peroxidation index) and GSH were significantly ($p<0.05$) different from the non-diabetic (control) and solvent treatment groups with aqueous extraction been the most effective (Table 2). The increased MDA and reduced GSH activities respectively are indicative of oxidative stress in the pathogenesis of diabetes. GSH depletion can culminate into ROS promotion thereby affecting cellular functionality and structural integrity (De Leve *et al.,* 1996). Scientifically, free radical reaction is an intricate component in the mechanism of lipid peroxidation. Scavengers or radical quenchers may both terminate perioxidation reaction and enhance the quality and stability of cells (Bran-Williams *et al*., 1995; Chen and Ho, 1997).

The near body weight stability observed in the diabetic-extract co-administration (Table 1) may have occurred as a result of suppressed lipid peroxidation, cellular water conservation, inhibition of lipolysis and decline in protein catabolism. Studies have demonstrated and established the anti-oxidative ability of *Cnidoscolus aconitifolius* (Perez-Gonzalez *et al*., 2017; Mordi *et al*., 2013; Johnston *et al*., 2016). A more recent study has demonstrated the phenolic acid and amino acid composition of *Cnidoscolus aconitifolius* which however substantiates its antioxidant principles (Temesgen *et al.,* 2022). Although the mechanism on how *Cnidoscolus aconitifolius* performs its anti-oxidative ability is still been deciphered and investigated, it can be inferred that the *Cnidoscolus aconitifolius* solvent extract decrease bio-activation of diabetes to reactive species. This research corroborates these facts by reducing lipid peroxidation with corresponding increase in the endogenous enzymatic antioxidant activities such as SOD and Catalase (Table 2).

Associated diseases such as obesity and type 2 diabetes, represents a broad spectrum of diseases which ranges between necrosis to cirrhosis and steatosis (NAFLD) to steatosis with inflammation (Caldwell *et al.,* 2006). Similar study portrayed macrovesicular steatosis, hepatocellular injury, parenchymal, portal inflammation, pericellular and sinusoidal fibrosis (Caldwell *et al.,* 2006). This work reveals that STZ induced periportal inflammatory cells, moderate fibrosis and marked congestion of the intervening veins in the diabetic group (Slide 1). Histological examination of the STZ-induced diabetic group form this present results also, displayed distortion of the lobular pattern of the hepatocytes, with several foci of edema and congestion (Slide 2). These were followed by marked periportal hepatolysis and cast within parenchyma (Slide 3). Furthermore, between the hepatocytes, was dilation of sinusoids with increased leucocytic infiltration (Slide 4). The nuclei of most cells revealed clear signs of necrosis and the hepatocytes were swollen with conspicuous cytoplasmic vacuolations (Slide 4).

Contrary to STZ-diabetic group, result reported remarkable improvement of histological biochemical alterations noticed in STZ-diabetic rats after treating rats with, water, hexane and ethanol extracts of *Cnidoscolus aconitifolius*. However, there was periportal infiltration of inflammatory cells with mild fibrosis in extract treated groups, however, hepatocytes were moderately congested and hepatic features were preserved and kept (Slide). Padilla-Camberos *et al*., (2021) expounded the anti-inflammatory properties of *Cnidoscolus aconitifolius* of which its bioactive components have been implicated.

Numerous documented studies have explicitly demonstrated the effects of *Cnidoscolus aconitifolius* on haematological parameters as it relates to diabetes. Alloxan-treated rats with *Cnidoscolus aconitifolius* significantly increased PCV, RBC, Hb, WBC, and MCV with corresponding increase in the platelet values (Azeez *et al*., 2010). Similar result was replicated by Ezebuiro *et al.* (2020) at a high dose of 400mg/kg of

Cnidoscolus aconitifolius.
Currently as depicted from this results of *Cnidoscolus aconitifolius* on haematological parameters in STZ-induced diabetic rats showed a significant (P<0.05) increase in the Hb, PCV, and TRBC levels when compared with the diabetic group. STZ-induced diabetes depletes TRBC, causes anemia microcytic hypochromic from haematuria which was corrected with the introduction of *Cnidoscolus aconitifolius* treatment to promote cell health. The TWBC, platelets, neutrophils and lymphocytes were significantly (P<0.05) higher in STZ-induced diabetic group compared to the control group. They were significantly (P<0.05) lowered in extract treatment groups (DCAHE, DCAEE and DCAAE) when compared with STZ-induced diabetic group. The possible explanation to the abnormal increase in TWBC in STZ-induced diabetic group may be ascribed to resultant hyperplasia of the pancreases. With the introduction of the *Cnidoscolus aconitifolius* extracts a reduce level was observed which was statistically significant for all solvent extract. This is indicative that the immune system components were not compromised as consequential of diabetic complications suggesting that an immune boosting potential may have emanated from the extract administration through its phytochemicals content.

5. Conclusion

No doubt previous research studies has evaluated the anti-diabetic potentials of *Cnidoscolus aconitifolius*, however, this study does not only concretize this scientific assertions but also comparatively examines the best possible solvent extract to achieve a more effective treatment and management process with less deleterious effect. Comparatively, this present results indicated that the potency of the aqueous extract is higher than the hexane and the ethanolic extracts of *Cnidoscolus aconitifolius* in ameliorating the histological disturbances in the diabetic liver tissue. This may be possible because bioactive ingredients are effectively expressed from plant materials based on their solubility in the extracting solvent. Based on the phytochemical screening of the dried aqueous extract of *Cnidoscolus aconitifolius* documented by Mordi and Akanji (2012), the high content of phlobatannin and saponin must have acted synergistically as antioxidants cushioning the diabetic consequences as revealed by the histological slides. Hence, the scientific rationale in the use of *Cnidoscolus aconitifolius* as antidiabetic agent was confirmed in this study; however further targeted investigations to unveil the chemical structure of the bioactive molecule, their mechanisms of action and molecular disposition.

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Table 1: Body weight of control, diabetic and *Cnidoscolus aconitifolius* (CA) extracts treated rats at the start and the end of the experiment

TREATMENT TIME	Control	Diabetic	Diabetic + Ca Hexane extract	Diabetic + Ca Ethanol extract	Diabetic + Ca Aqueous extract
At the start of the Experiment	199.3± 6.2	212.4± 4.9	209.7± 6.7	212.1± 8.0	211.6± 7.4
At the end of the Experiment	295.6±17.1	179.0±18.3*	218.5±21.1**	215.1±19.7**	218.7±15.1**
* % Change	48.3	- 18.7	4.03	1.41	3.36

Data represents Mean ± SD of 7 rats. (*) connotes significant difference at P > 0.05 as compared to control. (**) indicates significant difference at P< 0.05 as compared to diabetic group.

TABLE 2: Antioxidant activity of control, diabetic and *Cnidoscolus aconitifolius* (CA) extracts on Streptozotocin-induced Diabetic Rats

Group	CAT µ/mg Protein	GSH µmol/mg protein	SOD µ /mg protein	MDA µmol/g protein
Control	101.14 ± 0.11^{a}	43.32 ± 0.11^{a}	45.94 ± 2.50^{a}	3.25 ± 0.45^{a}
Diabetic	127.55± 2.76^{c}	30.93 ± 1.25^{c}	66.94 ± 1.75^{c}	9.25 ± 0.17^{b}
DCHE	113.16 ± 2.20^{c}	34.60 ± 0.42^{c}	48.65 ± 1.81^{b}	6.55 ± 0.23^{c}
DCEE	116.30 ± 0.63^{c}	43.10 ± 0.51^{a}	50.73 ± 1.80^{b}	5.50 ± 0.11^{b}
DCAE	112.51 ±2.04^{c}	45.34 ± 0.17^{a}	52.50 ± 1.84^{b}	5.25 ± 0.72^{c}

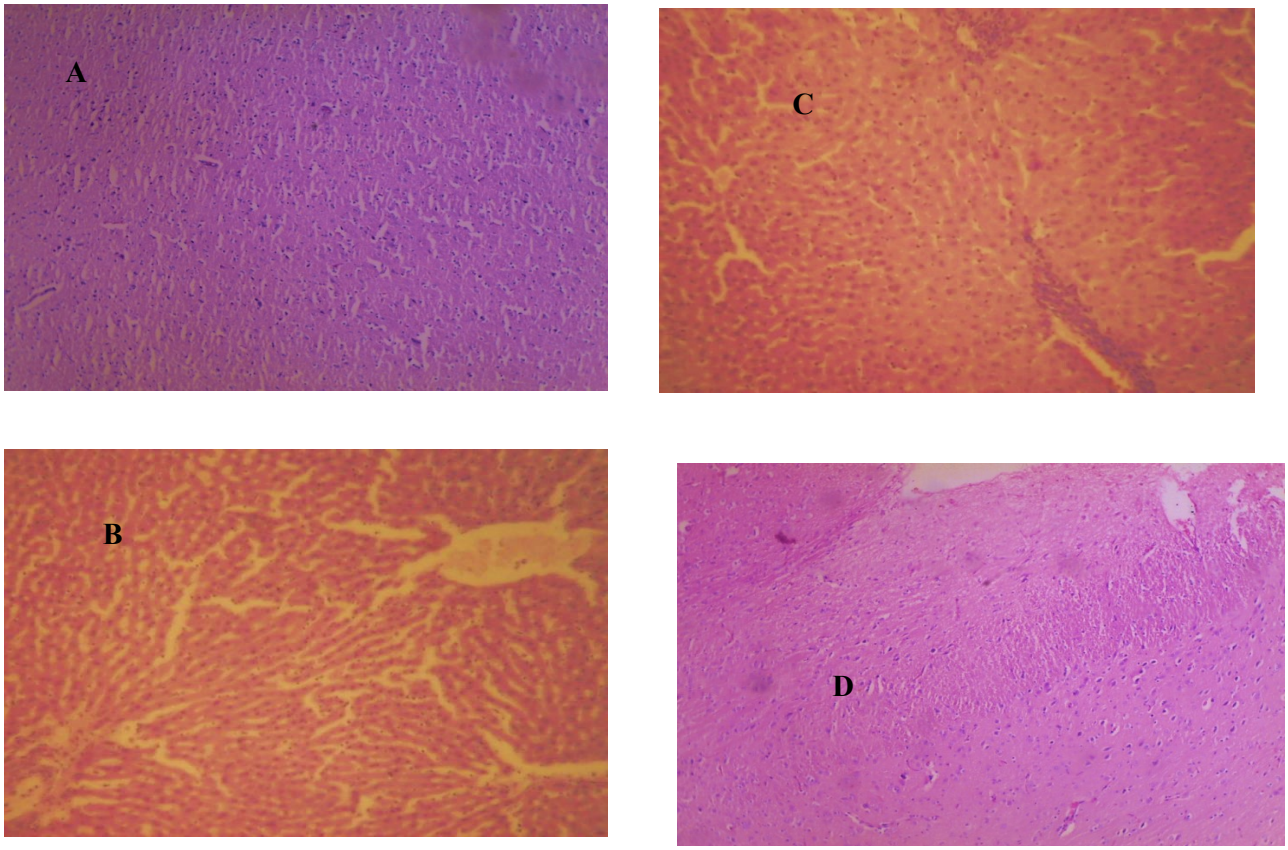
Data is presented as Mean± SD. DCHE (Diabetic+ *CA* Hexane extract), DCEE (Diabetic + *CA* Ethanol extract), DCAE (Diabetic + *CA* Aqueous extract). CAT Catalase, GSH: Glutathione, SOD – Superoxide dismutase, MDA: Malondialdehyde.

TABLE 3: Effect of *Cnidoscolus aconitifolius* (CA) on Haematological Parameters Streptozotocin-induced Diabetic Rats

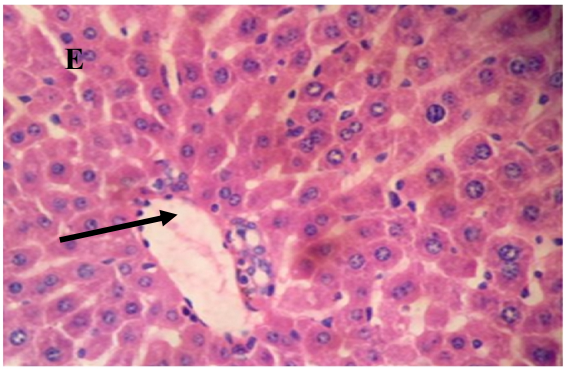
GROUP	HB g/dl	PCV (%)	TWBC x 10^{9}/l	Platelet x 10^{9}/l	TRBC x 10^{12}/l	N (%)	L (%)	E (%)	M (%)	B%
Control	14.3 ± 0.28^{b}	50.0 ± 1.85^{a}	8.6±1.25^{c}	290.0 ± 2.15^{c}	6.6 ± 0.17^{b}	28.0 ± 0.17^{c}	59.0± 2.50^{c}	ND	01	ND
Diabetic	10.3 ± 0.45^{c}	39.0 ± 1.50^{c}	13.2 ± 2.13^{b}	470.0± 3.50^{a}	4.8 ± 0.05^{c}	40.0 ± 1.50^{a}	70.0± 3.00^{a}	01	ND	ND
DCAHE	13.1 ± 0.25^{b}	41.0 ± 1.35^{b}	10.5 ± 0.50^{a}	320.0± 2.50^{b}	5.0± 0.15^{b}	37.0 ±2.50^{b}	60.0± 7.15^{b}	01	ND	01
DCAEE	14.5 ± 0.25^{b}	48.0 ± 0.85^{b}	10.1± 1.35^{b}	335.0± 1.75^{b}	6.7 ± 0.05^{b}	35.0 ± 2.10^{b}	55.0± 3.15^{b}	ND	ND	ND
DCAAE	16.8 ± 0.20^{a}	54.0 ± 2.16^{a}	8.5± 1.20^{c}	810.0± 1.00^{c}	7.8 ± 2.30^{a}	30.0 ± 1.52^{b}	40.0 ± 1.65	ND	01	ND

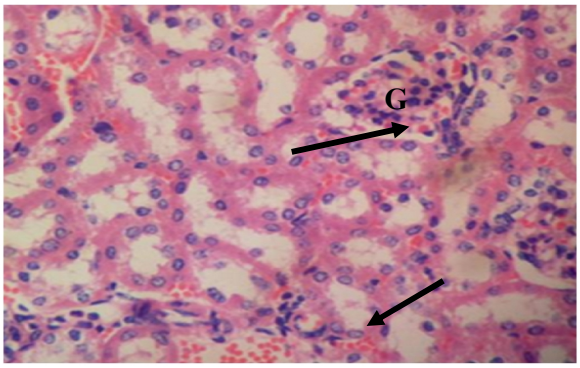
Data is presented as Mean± SD. DCHE (Diabetic+ *CA* Hexane extract), DCEE (Diabetic + *CA* Ethanol extract), DCAE (Diabetic + *CA* Aqueous extract), Hb (Haemoglobin), PCV (Packed Cell Volume), TWBC (Total white blood count), TRBC (Total Red blood count), N (Neutrophils), L (lymphocytes), E (Eosionophils) M (Monocytes), B (Basophil). ND (Not detected).

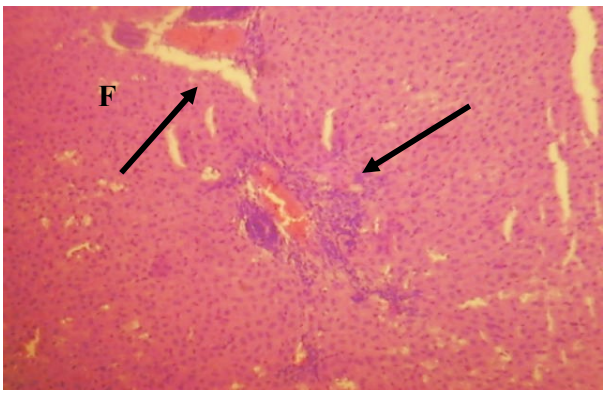
HISTOLOGY OF THE LIVER

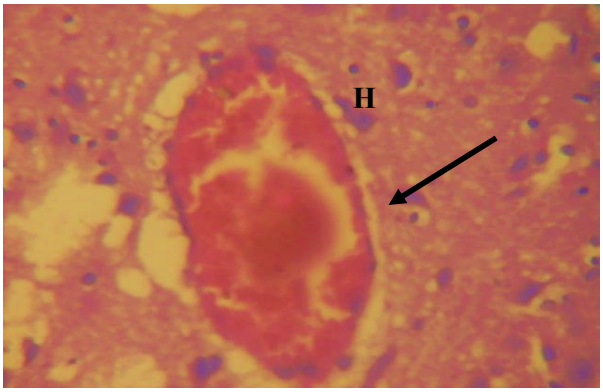


SLIDE A-D (CONTROL GROUPS): Liver tissues of control depicting regular (**A**) and intact hepatocytes with normal architecture (**B**). Edema cells absent with no vascular congestion **(C)**. Hepatic necrosis absent and central vein absent (**D**). **H&E X 100**

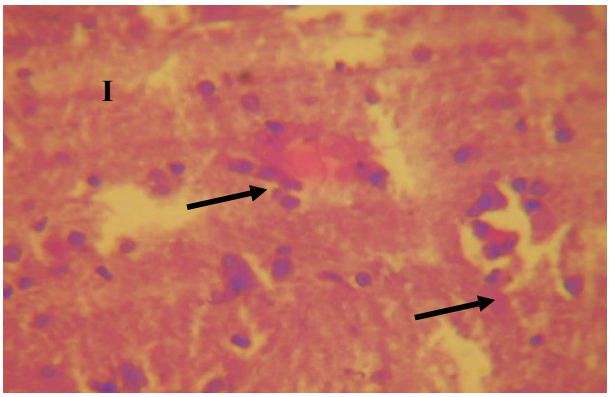


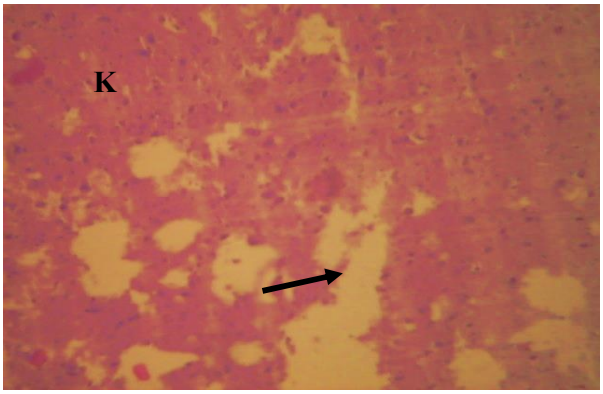


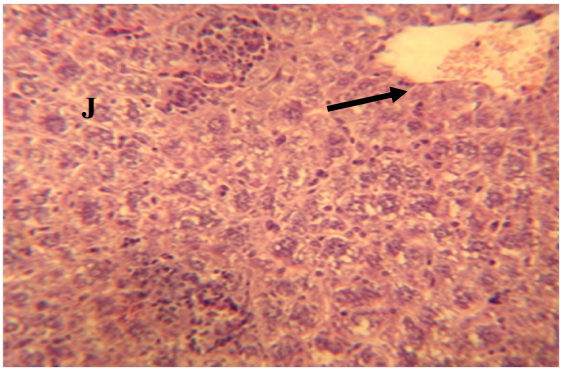


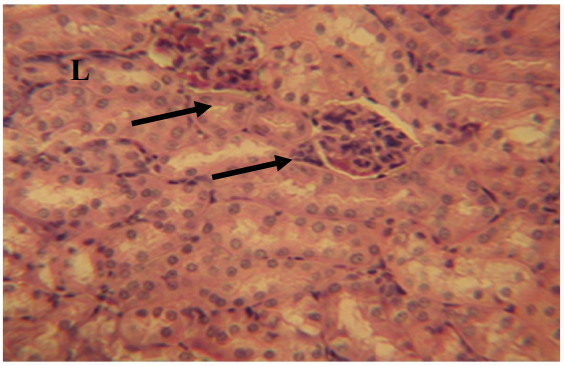


SLIDE E-H (DIABETIC GROUP ALONE - WITHOUT TREATMENT): Liver sections displaying distortion of the lobular pattern of the liver with several foci of edema and congestion (**E**). Also observed are areas with periportal inflammatory cells, extensive fibrosis (**F**) and marked congestion of the intervening veins (**G**). Liver tissue features are in keeping with marked periportalhepatolysis and cast within parenchyma **(H)**. Magnification **H&E X 100**

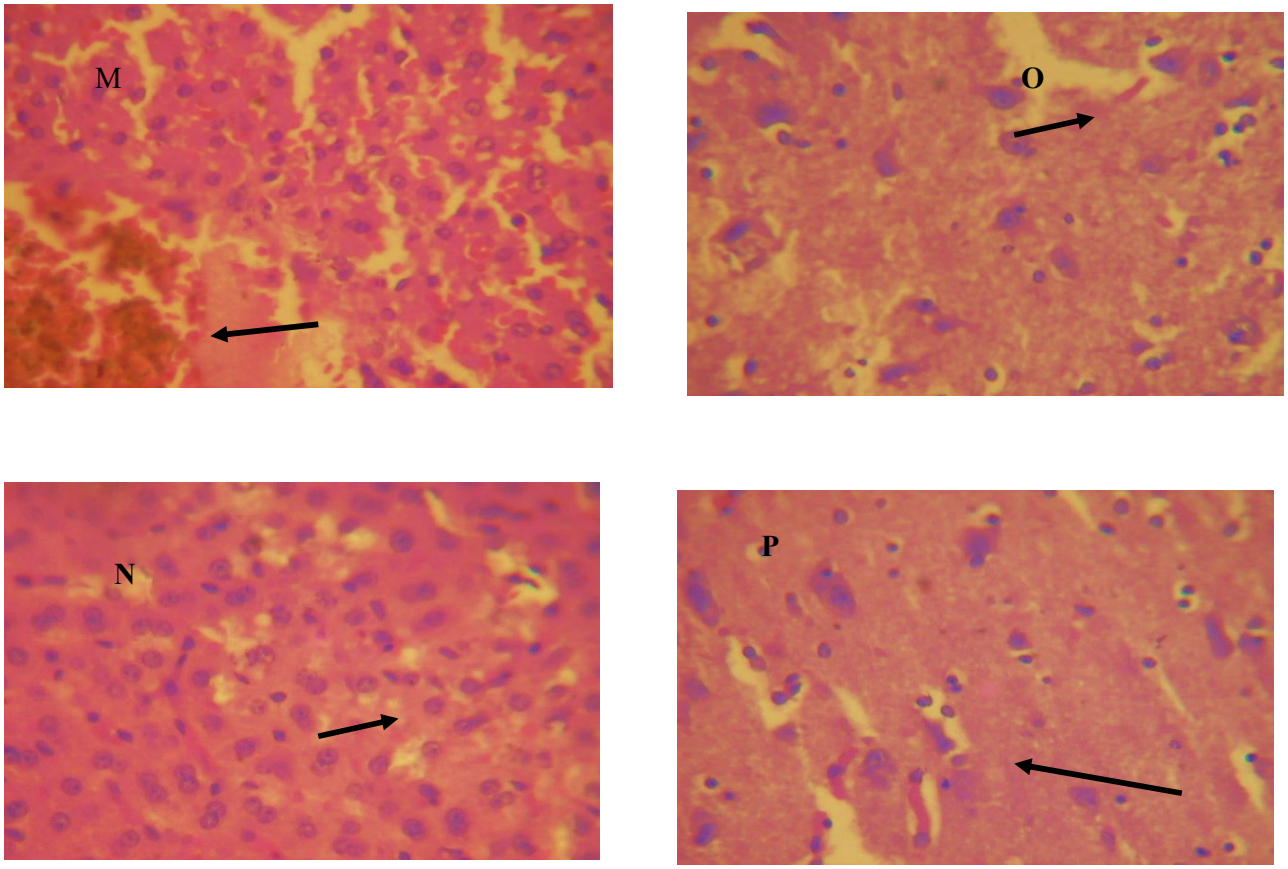




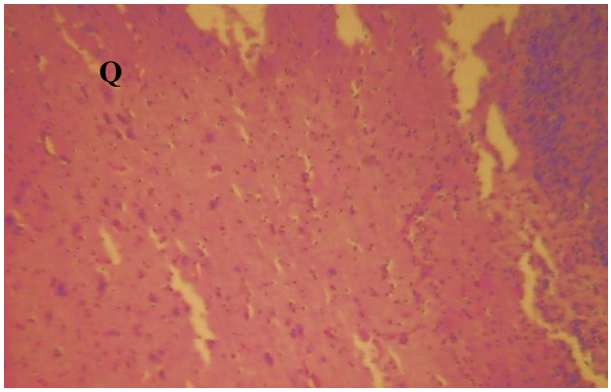


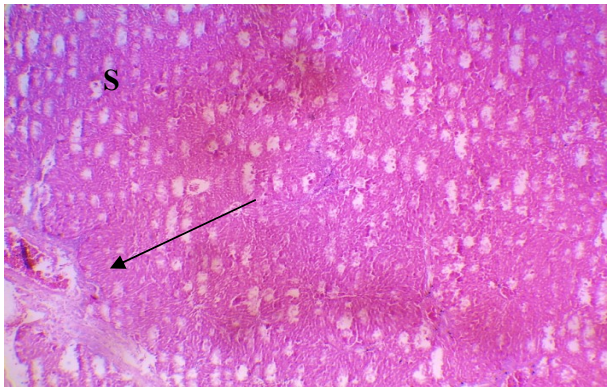


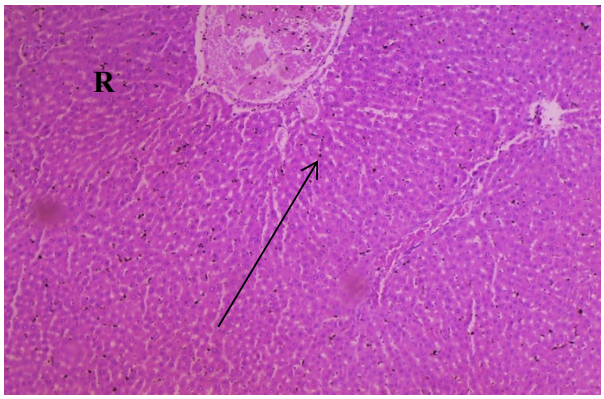
SLIDE I-L (DIABETIC+ CA HEXANE EXTRACT): Sections of the liver showing moderate distortion of the lobular architecture**(I)** with congested blood vessel channels**(J)**. There is periportal infiltration of inflammatory cells and mild fibrosis (**K**). Liver tissue features are in keeping with periportal hepatitis and moderately congestion **(L).**

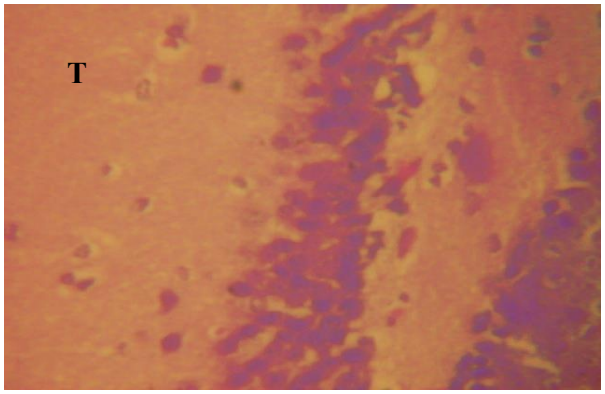


SLIDE M-P (DIABETIC+ CA ETHANOLIC EXTRACT): Sections of the liver depict marked distortion of the lobular architecture with several foci of hepatolysis**(M)**. In some foci, there are cast within parenchyma (**N)**. Liver tissue features indicative of necrosis with degenerative changes (**O)**. Hepatic vessels further showed congestion with periportal fibrosis (**P)**.**H&E X 100**









SLIDE Q-T (DIABETIC+ CA AQUEOUS EXTRACT): Sections of the liver showing distortion of the normal architecture with foci of edema and cell loss (**Q)**. In few areas are also foci of vascular congestion (**R)**. Hepatic tissue; features are in keeping with moderately congested hepatocytes (**S**).Eosinophilic deposits are few in this section with few apoptotic bodies also seen x 100.