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Research Article

Hypoglycaemic and Anti-inflammatory Effects of Stem Bark Extracts of *Enantia chlorantha* in Streptozotocin (STZ)-induced Diabetic Rats

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ABSTRACT

This study investigated the hypoglycemic and anti-inflammatory properties of *Enantia chlorantha* (EC) stem bark extract in streptozotocin (STZ)-induced diabetic rats. EC, a medicinal plant integral to traditional Nigerian medicine, was extracted using hydroethanol (HE) and ethyl acetate (EA) solvents. Forty-two male Wistar rats fed a high-fat diet, were randomized into six groups (n=7) plus a normal control group. The groups included diabetic and metformin controls, and test groups receiving *E. chlorantha* extracts. Treatments were administered as follows: untreated diabetic control, metformin (35 mg/kg), and *E. chlorantha* extracts (200 and 400 mg/kg for both HE and EA). After 28 days, a significant dose-dependent reduction ($p < 0.05$) in blood glucose and pro-inflammatory markers (IL1B, TNF-α, NFKB) was observed in the treated groups compared to the diabetic control. These findings suggest that *E. chlorantha* exhibits the potential to modulate blood glucose levels and inflammatory responses, supporting its therapeutic use in diabetes management. The study highlights the bioactive potential of EC and provides a basis for further research into its mechanisms and applications.

Keywords: *Enantia chlorantha,* Diabetes, Hypoglycemia, Pro-inflammatory markers, Streptozotocin

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INTRODUCTION

Medicinal plants hold immense potential in treating various ailments due to their therapeutically important phytochemicals (Falodun, 2010). Diabetes is a severe metabolic disorder with several available medications to alleviate its symptoms. However, these drugs are often expensive and associated with several complications (Arunsi *et al.*, 2020). In contrast, herbal medicines are gaining prominence due to their cost-effectiveness and improved therapeutic effects with fewer side effects (Amadi, 2018). *Enantia chlorantha* belongs to the family *Annonaceae* and is locally known as Awogba, Awopa, Osu pupa (Yoruba), Osomolu (Ikale), Dokitaigbo, Erumeru (South Eastern Nigeria), Kakerim (Boki in Cross River State, Nigeria), Erenba-vbogo Bini (Benin). It is widely distributed along the coasts of West and Central Africa. It is also very common in the forest regions of Nigeria (Adesokan *et al*., 2008). This plant has been used extensively in traditional medicine practice in the treatment of several ailments of non-related pathophysiology (Sotade *et al*., 2022).

Enantia chlorantha is particularly sought after by the rural communities in Nigeria for the treatment of many ailments. The stem bark is mostly preferred (even though the roots and the leaves may also be used), and decoctions, tinctures or infusions may be prepared (Ishola *et al* 2014). In Nigeria, *E. chlorantha* preparations can be made in the form of a drink, called 'agbo', or in the form of a powder, referred to as 'agunmu' (Ishola *et al.,* 2014)

Enantia chlorantha contains many bioactive compounds, which justify its numerous medicinal

virtues. Phytochemical screening has revealed the presence of saponins, flavonoids, alkaloids, phenols, reducing sugars, and cardiac glycosides. Among these, alkaloids are present in the highest quantity (146.22%), while flavonoids are the least (7.63%) (Sarbadhikary and George, 2022 ;Sotade *et al*.,2024).

In experimental settings, diabetes is commonly induced using Streptozotocin (STZ), a broad-spectrum antibiotic derived from the bacterium Streptomyces achromogenes (Olivier *et al*., 2015). STZ is a DNA alkylating agent often used as an antibacterial and anticancer agent, although it is not preferred for cancer treatment due to its genotoxic effects leading to drug resistance (rights are reserved by Busineni and Goud, 2015). In diabetes research, STZ damages pancreatic β-cells, resulting in hyperinsulinemia and hyperglycemia (Konda *et al*.,2020). It selectively accumulates in pancreatic β-cells via a GLUT 2 glucose transporter in the plasma membrane, making it widely used to induce experimental type 1 diabetes in rodent models (Berger and Zdzieblo, 2020).

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycaemia due to insulin resistance and/or insulin deficiency caused by the failure of pancreatic β-cells. DM is classified into four clinical categories: type 1 diabetes (autoimmune destruction of β-cells), type 2 diabetes (insulin resistance and progressive insulin secretory defect), gestational diabetes mellitus (GDM), and other specific types of diabetes due to genetic defects, drug- or chemical-induced alterations, and diseases of the exocrine pancreas ("Diagnosis and classification of diabetes mellitus," 2009). One potential implication of the many studies suggesting a relation between inflammation and diabetes is that inflammatory markers may be used to refine diabetes risk prediction and thus better target individuals for lifestyle interventions This study evaluated the anti-diabetic effects of *E. chlorantha* stem bark extracts in STZ-induced diabetic rats, focusing on its impact on blood glucose levels and pro-inflammatory markers.

MATERIALS AND METHODS

Collection and Identification of Plant

The stem bark of *E. chlorantha* was harvested from an open forest in Irrua, Esan-Central Local Government Area, Edo State, Benin City, Nigeria. The sample was washed, air-dried, and pulverized at the Department of Pharmacognosy Laboratory, University of Benin. Samples of the stem bark were taken to the Department of Plant Biology and Biotechnology, University of Benin, Nigeria for identification and UBH-J386 was assigned as a voucher number. The stem barks were cleaned to remove adhering dirt, and air-dried for six weeks. Thereafter, the dried stem bark was pulverized by an electric blender and weighed.

Preparation of Plant Extracts

The pulverized stem back of EC were soaked separately in hydroethanol (80% ethanol and 20% distilled water) and ethyl acetate for three days. The mixtures were stirred and allowed to stand. After three days, the marc was separated from the crude extract using filter paper. The crude extract was stored in clean jars. The marc were soaked again in the same hydroethanol and ethyl acetate solutions separately for another three days to ensure exhaustive extraction. The resultant extracts, HE and EA were then freeze-dried.

Animals

Male Wistar rats (16 weeks old) were purchased and kept in clean cages. They were allowed to acclimatize for two weeks with free access to grower mash and water. After acclimatization, the rats were divided into seven groups (n=6 per group) and marked using picric acid for identification. All experimental procedures involving animal care adhered to the standard protocol outlined in the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals" manual.

Diet Formulation

High-fat diet (HFD) and normal diet (ND) were prepared. The HFD included corn starch, fish meal, soybean oil, butter, sucrose, cellulose, vitamin mix, and mineral mix, while the ND excluded butter. Ingredients were mixed, pelleted, and dried before feeding the rats.

Table 1. **Composition of the constituents in Normal Diet**

Raw Materials	Source	Amount (g)
Corn starch	Carbohydrate	2,562.50
Cellulose	Fiber	875.00
Sucrose	Simple sugar	625.00
Fish meal	Protein	312.50
Soybean oil	Fat and oil	250.00
Mineral mix	Minerals	62.50
Vitamin mix	Vitamins	218.75

Table 2. Composition of the constituents in High Fat Diet

Raw Materials	Source	Amount (g)
Corn starch	Carbohydrate	5,125.0
Cellulose	Fiber	625.00
Sucrose	Simple sugar	1,250.00
Fish meal	Protein	1,750.00
Soybean oil	Fats and oil	500.00
Mineral mix	Mineral	437.50
Vitamin mix	Vitamin	125.00
Butter	Fats and oil	2,687.50

Experimental Design

The study consisted of seven groups:

Group 1: Normal rats (control) fed Normal Diet (ND) and water.

Group 2: Diabetic rats (untreated control) fed High Fat Diet (HFD) and water.

Group 3: Diabetic rats treated with 500 mg/kg bwt metformin.

Group 4: Diabetic rats treated with 200 mg/kg bwt *E. chlorantha* hydrothanol extract (HE).

Group 5: Diabetic rats treated with 400 mg/kg bwt *E. chlorantha* HE.

Group 6: Diabetic rats treated with 200 mg/kg bwt *E. chlorantha* ethyl acetate extract (EA).

Group 7: Diabetic rats treated with 400 mg/kg bwt *E. chlorantha* EA.

Administration of Extracts

The HE and EA acetate extracts were administered daily using an orogastric tube. Caution was taken not to inflict injuries to the experimental animals.

Animal Sacrifice and Tissue Collection

Following 28 days of treatment, the animals were fasted overnight and euthanized the following morning. To minimize pain and distress, euthanasia was performed according to established protocols for rodent Carbon Dioxide inhalation (Hickman, 2022). Upon euthanasia, a laparotomy was performed to gain access to the abdominal cavity. Blood samples were collected via cardiac puncture using 5 ml syringe and stored in EDTA and lithium heparin containers. The livers were then carefully excised. Tissues were immediately rinsed in ice-cold saline (0.9% NaCl) to remove excess blood. Following rinsing, the organs were patted dry on pre-weighed Whatman filter paper before measurement of organ weights using a calibrated analytical balance

Induction of Diabetes Mellitus with Streptozotocin

Streptozotocin (STZ) was administered intraperitoneally to induce diabetes. Fasting blood glucose levels were checked after three days. Rats with fasting blood glucose >200 mg/dL were considered diabetic.

Fasting Blood Glucose Measurement

Fasting blood glucose levels were measured using a glucometer. Blood samples were collected from the tail tip after cleaning with methylated spirit. This was performed every three days post-STZ administration.

Biochemical Assays

Total RNA was isolated from tissues using TRI reagent. RNA quality and quantity were assessed, and cDNA was synthesized using M-MuLV Reverse Transcriptase. PCR amplification was performed using specific primers, and products were resolved on 1.5% agarose gels.

Total RNA isolation

Total RNA was isolated from whole tissues following a method described by Omotuyi *et al.* (2018). Briefly, tissues were homogenized in cold (4 °C) TRI reagent (Zymo Research, USA, Cat: R2050-1-50, Lot: ZRC186885). Total RNA was partitioned in chloroform (BDH Analytical Chemicals, Poole, England Cat: 10076-6B) following centrifugation at 15,000 rpm/15 min (Abbott Laboratories, Model: 3531, Lake Bluff, Illinois, United States). RNA from the clear supernatant was precipitated using equal volume of isopropanol (Burgoyne Urbidges and Co, India, Cat: 67-63-0). RNA pellet was rinsed twice in 70% ethanol (70 ml absolute ethanol (BDH Analytical Chemicals, Poole, England Cat: 10107-7Y) in 30 ml of nuclease-free water (InqabaBiotec, West Africa, Lot no: 0596C320, code: E476-500ML)). The pellets were air-dried for 5 min and dissolved in RNA buffer (1 mM sodium citrate, pH 6.4).

cDNA conversion

Prior to cDNA conversion, total RNA quantity (concentration (µg/ml) = 40 * A_{260}) and quality (≥ 1.8) was assessed using the ratio of A_{260}/A_{280} (A=absorbance) read using spectrophotometer (Jen-way UV-VIS spectrophotometer model 6305, UK). DNA contamination was removed from RNA was removed following DNAse I treatment (NEB, Cat: M0303S) as specified by the manufacturer. 2 µl solution containing 100 ng DNA-free RNA was converted to cDNA using M-MuLV Reverse

transcriptase Kit (NEB, Cat: M0253S) in 20 µlfinal volume (2 µl, N^9 random primer mix; 2 µl, 10X M-MuLV buffer; 1 µl, M-MuLV RT (200 U/µl); 2 µl, 10 mM dNTP; 0.2 µl, RNase Inhibitor (40 U/µl) and 10.8 µl nuclease-free water). The reaction proceeded at room temperature O/N. Inactivation of M-MuLV Reverse transcriptase was performed at 65°C/20 min.

PCR amplification and agarose gel electrophoresis.

PCR amplification for the determination of genes whose primers (Primer3 software) [3] are listed below (table 1.0) were done using the following protocol: PCR amplification was performed in a total of 25 µl volume reaction mixture containing 2 µl cDNA (10 ng), 2 µl primer (100 pmol) 12.5 µl Ready Mix Taq PCR master mix (One Taq Quick-Load 2x, master mix, NEB, Cat: M0486S) and 8.5 µl nuclease-free water. Initial denaturation at 95 °C for 5 minutes was followed by 20 cycles of amplification (denaturation at 95 °C for 30 seconds, annealing (see TM values for each primer pair on table 1.0) for 30 seconds and extension at 72 °C for 60 seconds) and ending with final extension at 72 °C for 10 minutes. In all experiments, negative controls were included where reaction mixture has no cDNA. The amplicons were resolved on 1.5% agarose gel (Cleaver Scientific Limited: Lot: 14170811) in Tris (RGT reagent, china, Lot: 20170605)-Borate (JHD chemicals, China, Lot 20141117)-EDTA buffer (pH 8.4).

Ampliconimage processing

In-gel amplicon bands images captured on camera were processed on Keynote platform as previously reported (Omaththage *et al.,* 2015) and quantified using image-J software. All graphs were plotted as mean +/- SEM using graph-pad prism.

Primers:

(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AAGGACCCTACACAGAGGCT	Plus	20	1194	1213	59.88	55.00	4.00
3.00								
Reverse primer	AAGGTGGGCTCAGCTTCTTC	Minus	20	1311	1292	59.96	55.00	8.00
0.00								
Product length	118							
Exon junction	1298/1299 (reverse primer) on template NM_033230.3							
Products on intended targets								
> NM_033230.3 Rattus norvegicus AKT serine/threonine kinase 1 (Akt1), mRNA								
>NM_031144.3 Rattus norvegicus actin, beta (Actb), mRNA								
Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCACAGCAGCCTCAGATACT	Plus	20	1320	1339	59.17	55.00	3.00
3.00								
Reverse primer	CTGGCTACTTGGGGCTGTTC	Minus	20	1420	1401	60.68	60.00	2.00
0.00								
Product length	101							
Exon junction	1334/1335 (forward primer) on template NM_032080.1							
Products on intended targets								
>NM_032080.1 Rattus norvegicus glycogen synthase kinase 3 beta (Gsk3b), mRNA								
(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GGACGTCACAGCAGAATGAAGA	Plus	22	4296	4317	60.61	50.00	6.00
0.00								
Reverse primer	TGAGGTCCTGGTTGTGAATCG	Minus	21	4399	4379	60.00	52.38	5.00
2.00								
Product length	104							
Exon junction	4309/4310 (forward primer) on template NM_012969.2							
Products on intended targets								
>NM_012969.2 Rattus norvegicus insulin receptor substrate 1 (Irs1), mRNA								

(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCACACCACTTCCGAAGGC		Plus	19	40	58	61.62	63.16	7.00	3.00
Reverse primer	GGGTTCCCCATCTTCAGAGC		Minus	20	185	166	60.11	60.00	4.00	2.00
Product length	146									
Exon junction	173/174 (reverse primer) on template NM_012751.1									
Products on intended targets										
>NM_012751.1 Rattus norvegicus solute carrier family 2 member 4 (Slc2a4), mRNA										

(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AAGGACCCTACACAGAGGCT		Plus	20	1194	1213	59.88	55.00	4.00	3.00
Reverse primer	AAGGTGGGCTCAGCTTCTTC		Minus	20	1311	1292	59.96	55.00	8.00	0.00
Product length	118									
Exon junction	1298/1299 (reverse primer) on template NM_033230.3									
Products on intended targets										
> NM_033230.3 Rattus norvegicus AKT serine/threonine kinase 1 (Akt1), mRNA										

5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTCCGGGAGAGGATGTGAGA		Plus	20	354	373	60.11	60.00	7.00	1.00
Reverse primer	CATTCCCGGGCACACCTC	Minus	18	462	445	60.44	66.67	6.00	0.00	
Product length	109									
Exon junction	448/449 (reverse primer) on template NM_017071.2									
Products on intended targets										
>NM_017071.2 Rattus norvegicus insulin receptor (Insr), mRNA										

Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGCTGGTGATCAGAAGGCTG		Plus	20	73	92	60.04	55.00	6.00	2.00
Reverse primer	TGTGTCAACCATGGTAATTTCAGT		Minus	24	213	190	58.86	37.50	8.00	2.00
Product length	141									
Exon junction	193/194 (reverse primer) on template NM_001145366.1									
Products on intended targets										
>NM_001145366.1 Rattus norvegicus peroxisome proliferator-activated receptor gamma (Pparg), transcript variant 2, mRNA										

Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGGCCGTACTATGGTGGTCC		Plus	20	435	454	61.33	60.00	5.00	3.00
Reverse primer	GGCAATCCCAATCACACCAC		Minus	20	552	533	59.47	55.00	3.00	0.00

(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Product length 118								
Exon junction 450/451 (forward primer) on template NM_017050.1								
Products on intended targets								
>NM_017050.1 Rattus norvegicus superoxide dismutase 1 (Sod1), mRNA								
Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGCTCCCAACTACTACCCCA		Plus	20	1332	1351	59.88	55.00	3.00	0.00
Reverse primer	AGAATGTCCGCACCTGAGTG		Minus	20	1471	1452	60.04	55.00	4.00	3.00
Product length 140								
Exon junction 1458/1459 (reverse primer) on template NM_012520.2								
Products on intended targets								
>NM_012520.2 Rattus norvegicus catalase (Cat), mRNA								
Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CAGCTTTCGACAGTGAGGAGA		Plus	21	91	111	59.73	52.38	6.00	3.00
Reverse primer	TGTCGAGATGCTGCTGTGAG		Minus	20	228	209	60.11	55.00	4.00	1.00
Product length 138								
Exon junction 103/104 (forward primer) on template NM_031512.2								
Products on intended targets								
>NM_031512.2 Rattus norvegicus interleukin 1 beta (Il1b), mRNA								
Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGGGCTCCCTCTCATCAGT		Plus	20	341	360	60.03	55.00	4.00	2.00
Reverse primer	GCTTGGTGGTTTGCTACGAC		Minus	20	446	427	59.76	55.00	3.00	2.00
Product length 106								
Exon junction 433/434 (reverse primer) on template NM_012675.3								
Products on intended targets								
>NM_012675.3 Rattus norvegicus tumor necrosis factor (Tnf), mRNA								
Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGTAGATGACCATGAGTCGC		Plus	20	454	473	57.13	50.00	6.00	2.00
Reverse primer	TGTCCTGCTGTATGCTGCTT		Minus	20	593	574	59.67	50.00	4.00	3.00
Product length 140								
Exon junction 469/470 (forward primer) on template NM_031789.2								
Products on intended targets								
>NM_031789.2 Rattus norvegicus nuclear factor, erythroid 2-like 2 (Nfe2l2/NRF2), mRNA								
Exon junction 387/388 (forward primer) on template NM_001276711.1								
Products on intended targets								
>NM_001276711.1 Rattus norvegicus nuclear factor kappa B subunit 1 (Nfkb1), mRNA								

(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GACCATCAGCAAGCAGGTCA	Plus	20	28	47	60.32	55.00	4.00
1.00								
Reverse primer	CACCAGGTGAGGACCACAAA	Minus	20	165	146	59.82	55.00	7.00
1.00								
Product length	138							
Exon junction	43/44 (forward primer) on template NM_019129.3							
Products on intended targets								
>NM_019129.3 Rattus norvegicus insulin 1 (Ins1), mRNA								
Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCATCTTCTTGTGCAGTGCC	Plus	20	36	55	60.11	55.00	5.00
3.00								
Reverse primer	GAGAAGGCAGCCCTGGTAAC	Minus	20	140	121	60.39	60.00	5.00
3.00								
Product length	105							
Exon junction	None for forward primer, None for reverse primer							
Products on intended targets								
>NM_017008.4 Rattus norvegicus glyceraldehyde-3-phosphate dehydrogenase (Gapdh), mRNA								

Histopathology

In the histopathological analysis, liver tissues were collected from all experimental groups, including control and treated diabetic rats, at the end of the study. The tissues were promptly excised and fixed in 10% neutral-buffered formalin for 24 hours at room temperature to preserve tissue architecture. Following fixation, the liver tissues were subjected to routine histological processing. The tissues were dehydrated in a series of graded ethanol solutions, cleared in xylene, and embedded in paraffin wax. Paraffin blocks were then prepared and sectioned into 5 µm thick slices using a rotary microtome. The paraffin-embedded tissue sections were mounted on glass slides and deparaffinized with xylene. The sections were rehydrated through a descending series of ethanol concentrations and finally rinsed in distilled water. For general histological evaluation, the sections were stained with haematoxylin and eosin (H&E). Slides were immersed in haematoxylin solution for 5-10 minutes to stain cell nuclei, followed by brief differentiation in 1% acid alcohol. The sections were then placed in running tap water for 10 minutes to enhance nuclear staining. Subsequently, the slides were stained with eosin solution for 1-2 minutes to provide a counterstain, highlighting the cytoplasmic and extracellular matrix components. After staining, the slides were dehydrated through ascending grades of ethanol, cleared in xylene, and mounted with a coverslip using a synthetic resin mounting medium. The stained sections were examined under a light microscope at various magnifications to evaluate histopathological changes. Photomicrographs were captured to document representative histopathological findings, with specific attention given to the assessment of hepatic architecture, cellular integrity, presence of necrosis, inflammation, and other pathological changes. Histopathological changes in liver tissues were semi-quantitatively scored by a blinded pathologist based on the severity and extent of observed lesions. The scoring system included the evaluation of hepatocyte degeneration and necrosis, inflammatory cell infiltration, sinusoidal congestion, and fatty changes (steatosis), each parameter scored on a scale from 0 to 3, where 0 indicates no change, 1 indicates mild change, 2 indicates moderate change, and 3 indicates severe change. The histopathological scores were analyzed statistically to compare differences between control and treated groups.

Data Analysis

The Data obtained were analyzed using GraphPad Prism 9. Results are expressed as mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. A p-value <0.05 was considered significant.

RESULT

Antidiabetic effects of *E. chlorantha* stem bark extract In STZ-induced diabetic rat.

The antidiabetic activity of extracts of *E. chlorantha* stem bark in Type 1 diabetes model is shown in Table 3. The rats induced with Type 1 diabetes showed significant increase in the blood glucose level, when compared with the normal control group on Day 3. However, there was a dose-dependent reduction in blood glucose levels in the diabetic rats on Days 7 and 14 when compared with the diabetic control. The hydroethanol extract showed greater amelioration at the higher dose of 400 mg/kg bwt. Also, administration of the standard drug, metformin, significantly ($p < 0.05$) lowered the increased glucose levels throughout the experimental period.

Table 3: Antidiabetic effects of *E. chlorantha* stem bark extract in STZ-induced diabetic rat.

Group/Dose	Fasting Blood Glucose Level (mg/dl)			
	Day 0	Day 3	Day 7	Day 14
Group I (Normal control)	80.00 ± 2.06^{a}	71.00 ± 2.66^{a}	97.00 ± 2.06^{a}	85.62 ± 3.09^{a}
Group II (35 mg/kg bwt STZ)	^{a}88.00 ± 1.19*	^{b}297.50 ± 1.46*	^{c}449.00 ± 7.04*	^{c}454.00 ± 7.80*
Group III (200 mg/kg bwt MF)	^{a}79.50 ± 0.84	^{b}357.00 ± 4.90**	^{c}103.50 ± 1.46**	^{d}84.5.80 ±1.46**
Group IV (200 mg/kg bwt HE)	^{a}80.00 ± 1.19	^{b}383.00 ± 3.36**	^{c}223.00 ± 2.06**	^{d}195.00 ±3.76**
Group V (400 mg/kg bwt HE)	^{a}78.00±1.18	^{b}313.50±1.46**	^{c}289.5±6.00**	^{d}177.00±3.14**
Group VI (200 mg/kg bwt EA)	^{a}80.5±1.46	^{b}317.5±0.84**	^{c}366.50±0.84**	^{d}390.5±4.03**
Group VII (400 mg/kg bwt EA)	^{a}82.50±1.85	^{b}351.50±0.84**	^{c}173.00±4.44**	^{d}81.5±2.22**

Values are expressed as mean ± SEM (n=7); values with different alphabet letters are significantly ($p < 0.05$) different from one another. *mean is significant ($P < 0.05$) when compared with the control; ** mean is significant ($P < 0.05$) when compared with diabetic control group. EA: Ethylacetate, HE: Hydroethanol, MF: Metformin, STZ: Streptozotocin,

The effects of *E. chlorantha* stem bark on Tumor Necrotic Factor (TNF-α ACTIN Expression in STZ-induced diabetic rats

The effects of *E. chlorantha* stem bark extract on TNF- **α** in STZ induced diabetic rat is presented in figure 1. The result showed that the expression of the Tumor Necrotic Factor (TNF-α) gene in group I which is the normal control is repressed in the liver. The induction of diabetes in group II increased the expression of TNF- α gene in the liver. In group III, metformin reduced the expression of TNF- α gene. In group IV, the induction of 200 mg of crude hydro ethanol extract reduced the expression of TNF- α gene. In group V, the induction of 400 mg of crude hydro ethanol extract further reduced the expression of the gene.

The effects of *E. chlorantha* stem bark on Nuclear Factor Kabba b(NK-kb) / TNF-α ACTIN Expression STZ induced diabetic rats

The effects of *E. chlorantha* stem bark extract on NF-kb- TNF-α ACTIN in STZ induced diabetic rat is presented in figure 2. The result also showed that the Nuclear Factor Kabba b (Nf-kb) gene expression is repressed in group I which is the normal control. In group II, which is the diabetic control group, the induction of diabetes increased the expression of Nf-kb gene. In group III, the induction of metformin reduced the expression of the Nf-kb gene. In group IV, the expression of Nf-kb gene was fully expressed, the induction of 200 mg of crude hydro ethanol extract increased the expression of Nf-kb gene. In group V, the induction of 400 mg of crude hydro ethanol extract reduced the expression of Nf-kb gene. In group VI, the induction of 200 mg of ethyl acetate increased the expression of Nf-kb gene. In group VII, the induction of 400 mg of ethyl acetate decreased the expression of Nf-kb gene.

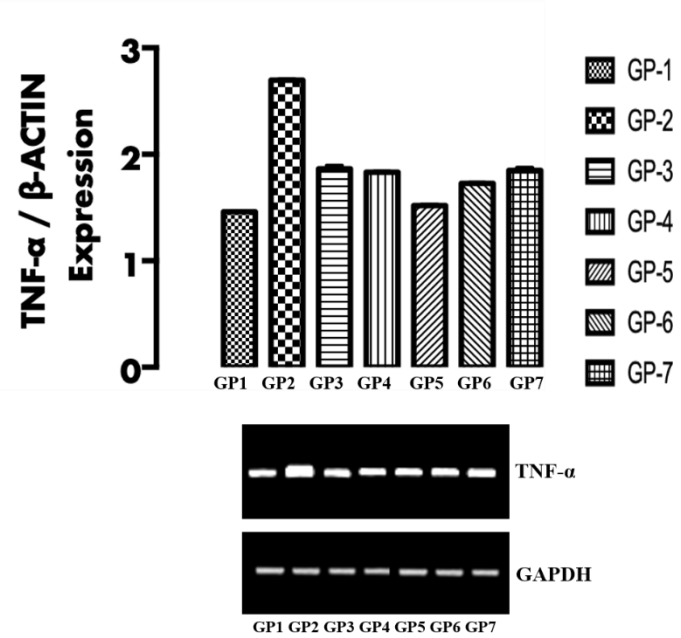

Figure 1: *E. chlorantia* stem bark modulate liver Tumor Necrotic Factor (TNF α) gene with reference to gatekeeper gene (GAPDH) in STZ induced diabetic rats. Data are represented as mean±SEM, n =4. GP-1: group 1, GP-2: group 2, GP-3: group 3, GP-4: group 4, GP-5: group 5, GP-6: group 6, GP-7: group 7.

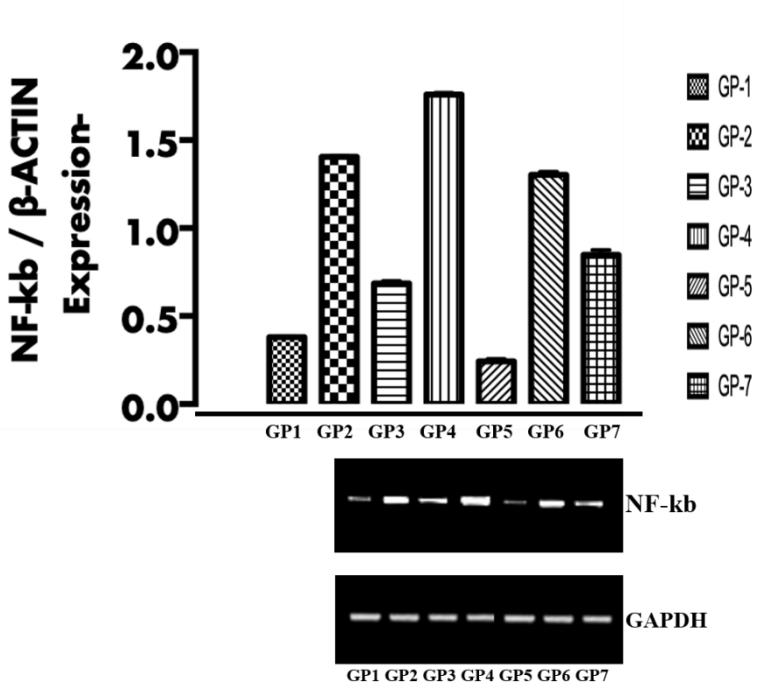

Figure 2: The effects of *E. chlorantia* stem bark on liver Nuclear Factor Kabba b (Nf-kb) gene with reference to gatekeeper gene (GAPDH) in STZ induced diabetic rats. Data are represented as mean±SEM, n =4. GP-1: group 1, GP-2: group 2, GP-3: group 3, GP-4: group 4, GP-5: group 5, GP-6: group 6, GP-7: group 7

Histopathology

Histological assessment of the liver shown in plates 1 to 7 depicted shows that normal hepatocytes containing round nuclei, portal tract and central vein in the control [Group 1].

In the *E. chlorantha* HE (200 mg/kg) group, the liver sections showed moderate improvements with

reduced necrosis and hepatocyte degeneration. Inflammatory cell infiltration was still present but reduced whilst in the *E. chlorantha* HE (400 mg/kg) group, There was significant improvement in liver histopathology. Hepatocyte structure was more preserved, and inflammatory infiltration was minimal. In the *E. chlorantha* EA (200 mg/kg) group, moderate improvement was observed with reduced necrosis and hepatocyte degeneration. However, some inflammatory cells were still present. In the *E. chlorantha* EA (400 mg/kg) group significant histopathological improvements were noted, with hepatocyte structure resembling the normal control group and minimal inflammatory cell infiltration.

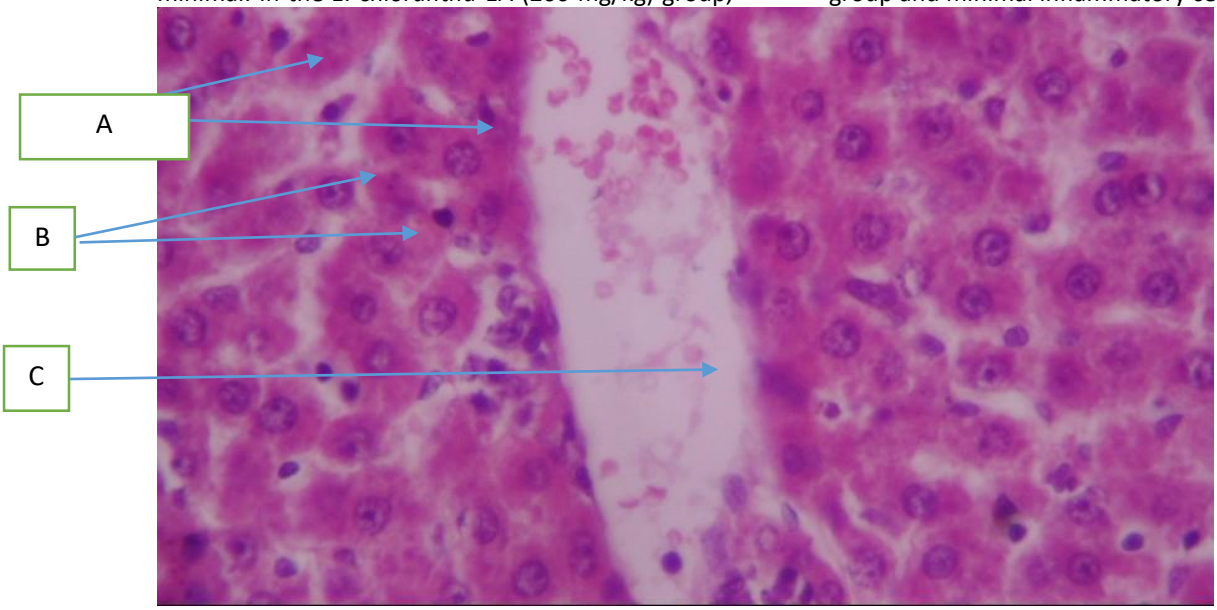

Plate 1. Control. Composed of: **A**, hepatocytes, **B**, sinusoids and **C**, central vein (H&E x 400)

The control group displayed normal liver histology with well-preserved hepatocytes, sinusoids, and a central vein. The hepatocytes appeared polygonal with a central nucleus, and the sinusoids were regularly spaced, demonstrating the typical structure of a healthy liver (H&E x 400).

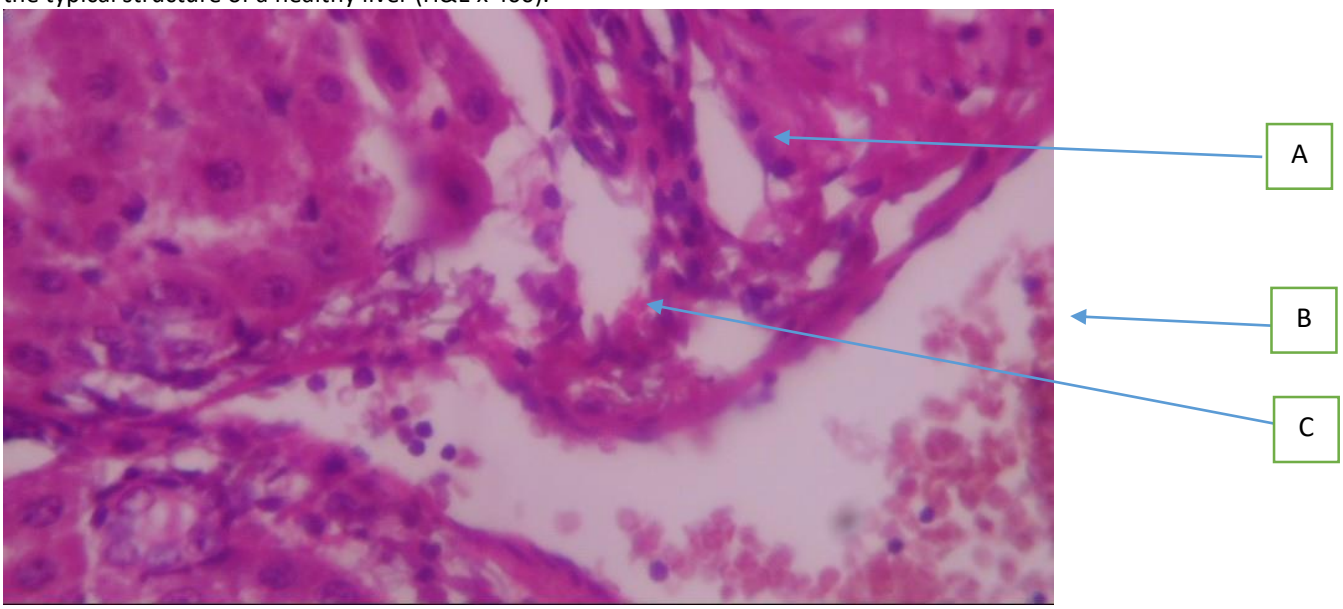

Plate 2. Rat given Streptozotocin (STZ) only showing: **A**, periportal infiltrates of inflammatory cells, **B**, portal congestion and **C**, zonal necrosis (H&E x 400)

Rats administered Streptozotocin (STZ) showed significant liver damage characterized by periportal infiltrates of inflammatory cells, portal congestion, and zonal necrosis. The inflammatory infiltrates were primarily localized around the portal triads, and necrosis was evident in specific zones of the liver lobule, indicating severe hepatocellular injury induced by STZ (H&E x 400).

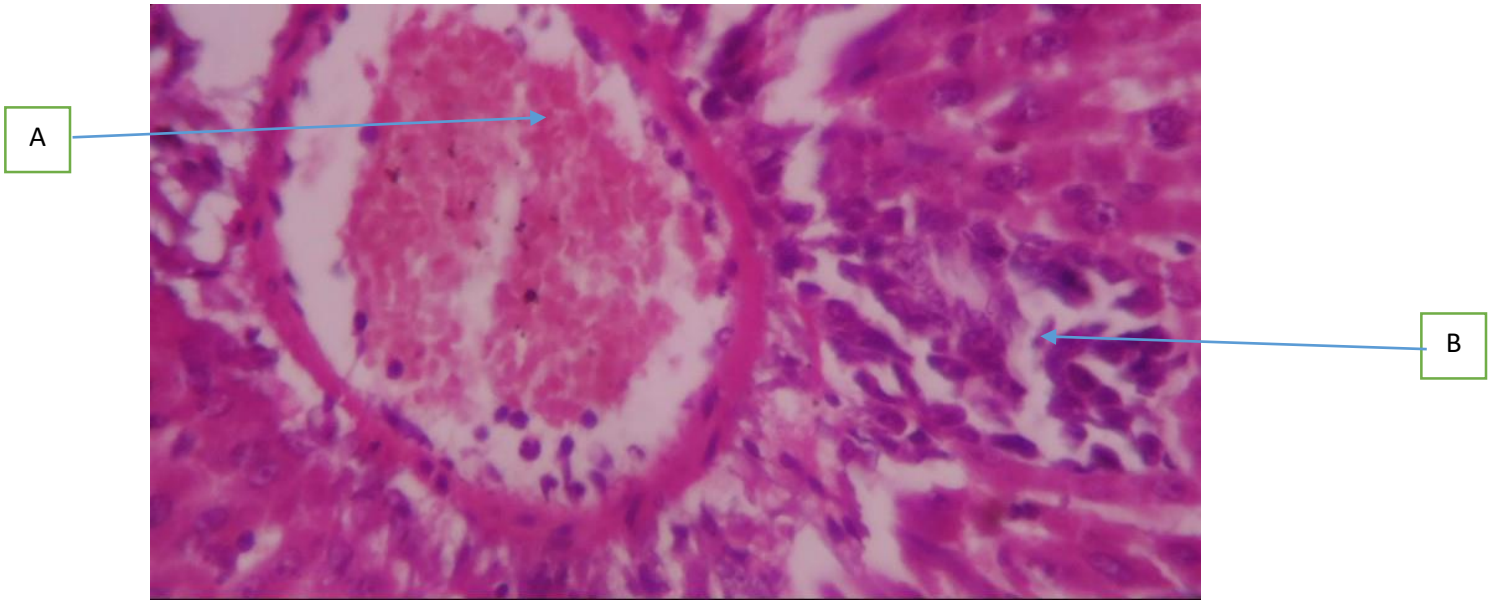

Plate 3. Rat given Streptozotocin (STZ) + Metformin showing: **A**, portal congestion and **B**, focal periportal infiltrates of inflammatory cells (H&E x 400)

Treatment with Metformin in STZ-induced diabetic rats resulted in noticeable histopathological improvements. There was a reduction in periportal inflammatory infiltrates and portal congestion, with only mild signs of zonal necrosis. This suggests that Metformin has a protective effect on liver histology in diabetic conditions (H&E x 400).

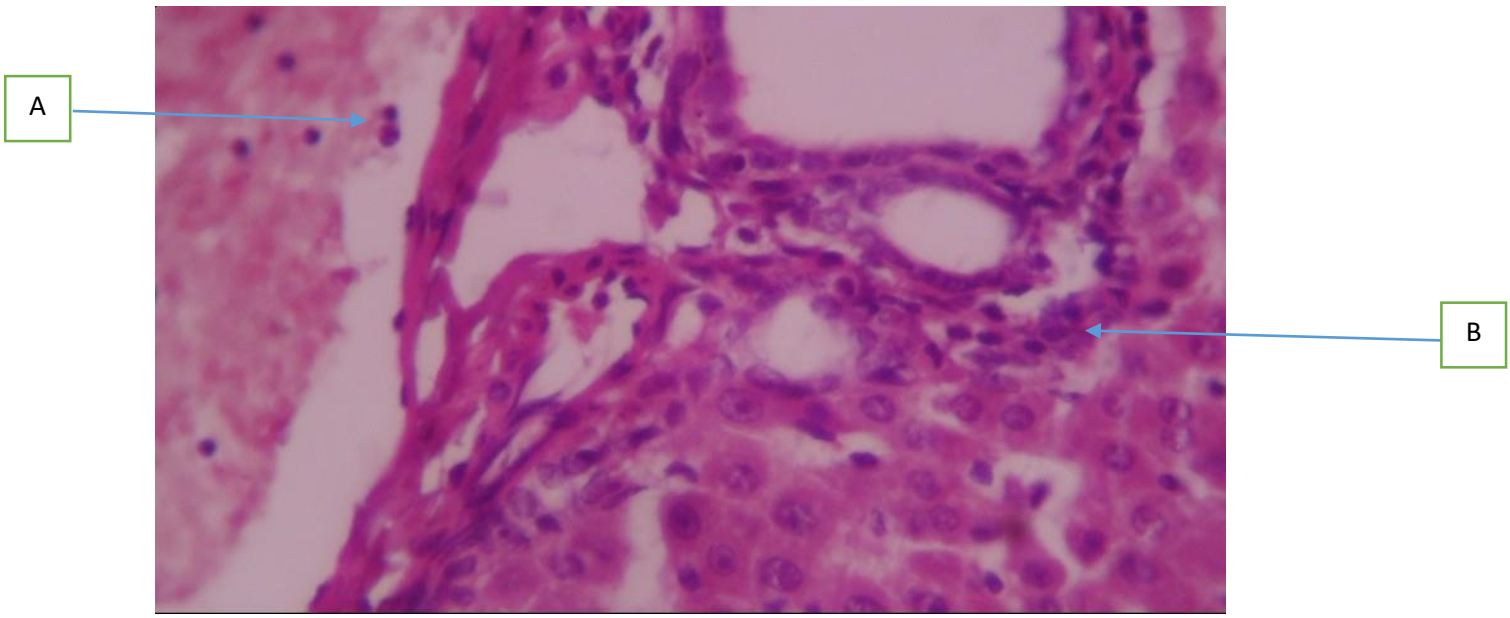

Plate 4. Rat given Streptozotocin (STZ) + 200mg Crude Extract showing: A, portal congestion and **B**, mild periportal infiltrates of inflammatory cells (H&E x 400)

Administration of 200 mg/kg of crude hydroethanol extract of *E. chlorantha* to STZ-induced diabetic rats demonstrated moderate improvement in liver histology. There was a noticeable reduction in inflammatory cell infiltration and less pronounced necrosis compared to the diabetic control, indicating the extract's potential hepatoprotective properties (H&E x 400).

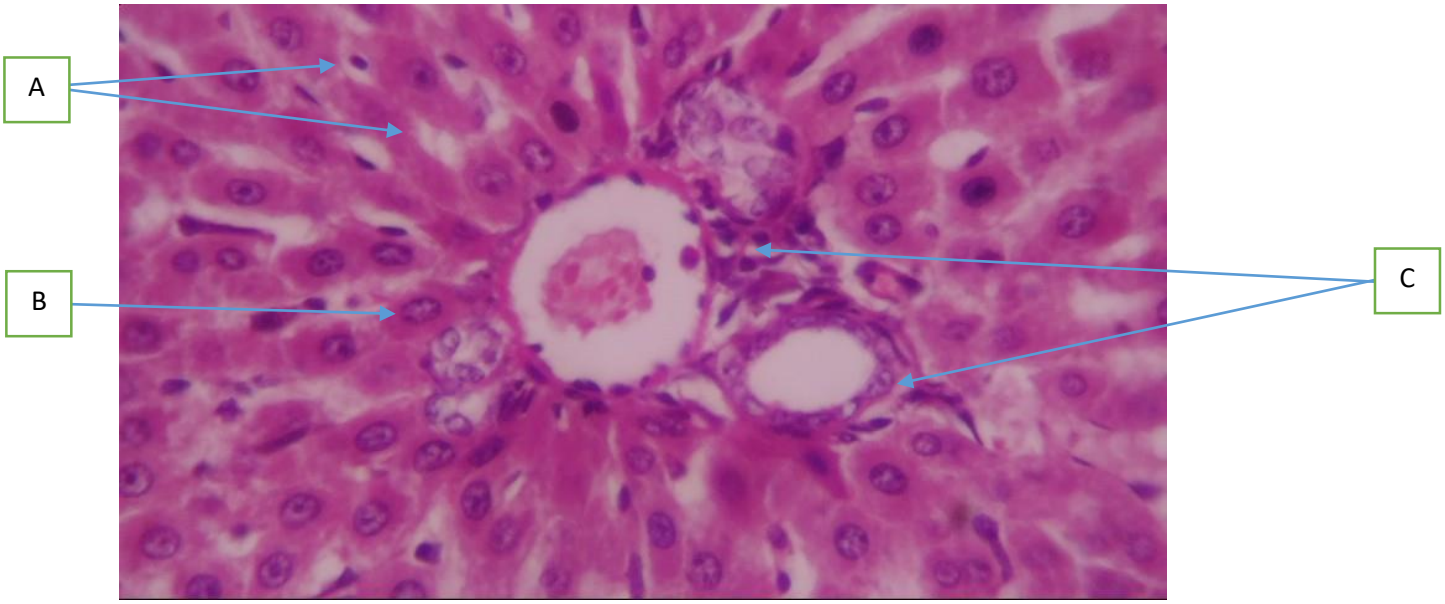

Plate 5. Rat given Streptozotocin (STZ) + 400mg Crude Extract showing: A, normal hepatocytes, **B**, kupffer cell activation and **C**, normal portal triad (H&E x 400)

Treatment with 400 mg/kg of crude hydroethanol extract of *E. chlorantha* resulted in further histological improvements. The liver sections showed significantly reduced periportal inflammation, minimal necrosis, and less portal congestion, suggesting a dose-dependent hepatoprotective effect of the extract (H&E x 400).

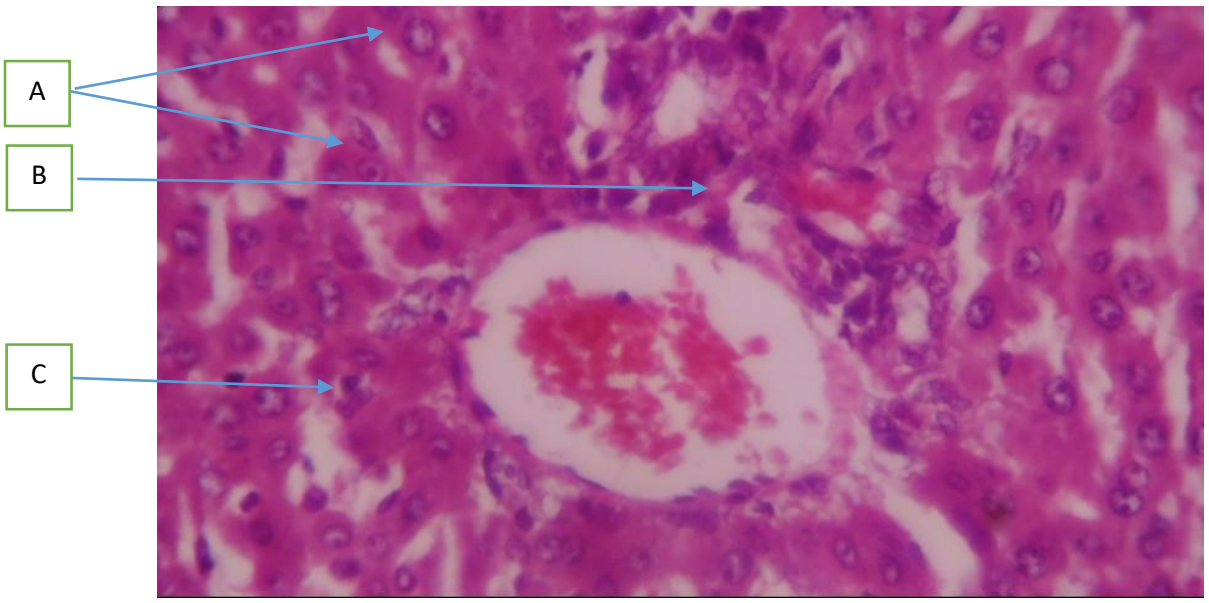

Plate 6. Rat given Streptozotocin (STZ) + 200mg Ethylacetate Extract showing: **A**, normal hepatocytes, **B**, focal periportal infiltrates of inflammatory cells and **C**, kupffer cell activation (H&E x 400)

Rats treated with 200 mg/kg of ethyl acetate extract of *E. chlorantha* exhibited moderate histological improvement. The liver sections showed decreased inflammatory infiltrates and congestion, with mild necrosis, indicating that the ethyl acetate fraction also possesses protective effects against STZ-induced liver damage (H&E x 400).

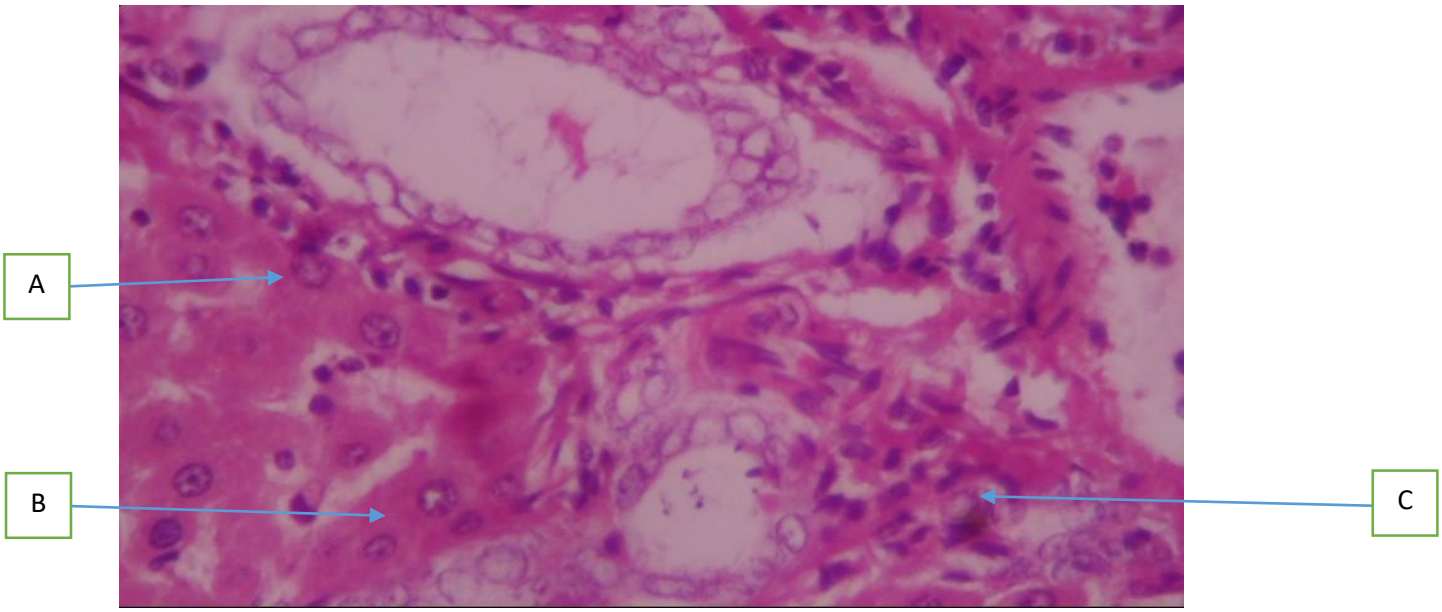

Plate 7. Rat given Streptozotocin (STZ) + 400mg Ethylacetate Extract showing: **A**, normal hepatocytes, **B**, kupffer cell activation and C, mild periportal infiltrates of inflammatory cells (H&E x 400)

Administration of 400 mg/kg of ethyl acetate extract of *E. chlorantha* showed the most significant histopathological recovery. Liver sections revealed minimal inflammatory cell infiltration, negligible necrosis, and well-preserved liver architecture, demonstrating the extract.

DISCUSSION

In the present study, we investigated the effects of *E. chlorantha* stem bark extract on hypoglycemic and pro-inflammatory markers TNF-α and NF-κB in hepatic cells of STZ-induced diabetic rats. The astronomical worldwide increase in the prevalence of diabetes has become a serious public health problem (Wild *et al.,* 2004). Diabetes is frequently accompanied by long-term microvascular and macrovascular complications, which lead to both morbidity and mortality (Fowler, 2011). Accumulating evidence shows that inflammation play a crucial intermediary role in the pathogenesis of diabetes (Donath and Shoelson, 2011). It has been reported that interleukin-6 (IL-6) and C-reactive protein (CRP) are associated with type 2 diabetes (Doi *et al.,* 2004). interleukin-6, a pleiotropic proinflammatory cytokine, is produced by a variety of cells, including activated leukocytes, endothelial cells, and adipocytes (Gabay,2006). C-reactive protein is an acute-phase plasma protein synthesized by the liver and has been shown to be a sensitive, systemic biomarker of inflammation (Donath and Shoelson, 2011). Our findings demonstrated that the administration of *E. chlorantha* extracts significantly reduced ($p<0.05$) serum glucose levels and inflammatory markers in a dose-dependent manner, thus providing insight into its potential therapeutic benefits in diabetes management The hypoglycemic effect of *E. chlorantha* stem bark extract was evident in STZ-induced diabetic rats, as shown in Table 1. Streptozotocin injection resulted in a significant ($p < 0.05$) increase in serum glucose levels compared to the normal control, consistent with previous findings by Bonner-Weir *et al*.,1981 who reported elevated blood glucose levels (>300 mg/dL) and reduced β-cell numbers following STZ administration. Our study showed that rats treated with 200 mg/kg and 400 mg/kg hydroethanol extract of *E. chlorantha* exhibited a dose-dependent reduction in serum glucose levels. The hypoglycemic effect of *E. chlorantha* was comparable to that of metformin, a standard anti-diabetic drug. Streptozotocin, a glucose analogue, selectively accumulates in pancreatic β-cells via GLUT2 transporters, causing DNA damage through its DNA-alkylating activity. This damage leads to ATP depletion, oxidative stress, and ultimately β-cell destruction, resulting in hyperglycemia. The hyperglycemic state observed in our study corroborates findings by Litherland *et al*. (2001), indicating that STZ administration at 35 mg/kg body weight induces significant diabetogenic responses. The glucose-lowering effect of *E. chlorantha* can be attributed to its phytochemical composition, which includes tannins, flavonoids, alkaloids, glycosides, and sterol compounds. These compounds are known for their hypoglycemic properties, as reported by Adesokan *et al*. (2008), Gbadamosi *et al*. (2011), and Dawodu *et al*. (2014). The effect of *E. chlorantha* on

the pro-inflammatory marker TNF-α in STZ-induced diabetic rats is depicted in Figure 2. TNF-α, produced primarily by activated monocytes and lymphocytes, plays a crucial role in the inflammatory response. STZ-induced diabetes led to increased TNF-α expression, indicating an inflammatory state. Treatment with metformin, hydroethanol extract (200 mg/kg and 400 mg/kg), and ethyl acetate fraction of *E. chlorantha* stem bark significantly downregulated TNF-α expression in a dose-dependent manner. This aligns with studies by Oriakhi and Orumwensodia,2021 who reported the anti-inflammatory effects of garlic acid and catechin on TNF-α expression and Monika *et al*., 2023, who showed that catechin, epicatechin, curcumin, garlic, pomegranate peel and neem extracts of Indian origin showed enhanced anti-inflammatory potential in human primary acute and chronic wound derived fibroblasts by decreasing TGF-β and TNF-α expression

The expression of NF-κB, a key regulator of inflammatory responses, was also assessed. STZ-induced diabetic rats showed a significant increase ($p < 0.05$) in NF-κB expression compared to the normal control group (Figure 3). NF-κB controls the transcription of various pro-inflammatory cytokines, chemokines, and adhesion molecules. In our study, treatment with metformin, hydroethanol extract (400 mg/kg), and ethyl acetate fraction of *E. chlorantha* stem bark significantly downregulated NF-κB expression. However, the 200 mg/kg dose of hydroethanol extract showed higher NF-κB expression, indicating a dose-dependent effect.

Polyphenolic compounds in *E. chlorantha* are known to inhibit inflammatory pathways by downregulating NF-κB and STAT3, thus reducing the expression of pro-inflammatory cytokines like TNF-α, IL-1β, IL-6, and COX-2. This anti-inflammatory activity is similar to that of metformin, which also significantly reduces NF-κB expression.

The histopathological analysis of the liver in STZ-induced diabetic rats treated with various extracts of *E. chlorantha* and metformin was considered in this study. The liver sections from the different groups were stained with haematoxylin and eosin (H&E) and examined under a microscope. Normal Control Group: The liver tissues showed normal architecture with well-preserved hepatocytes, clear cell boundaries, and normal sinusoidal spaces.

In the diabetic control Group, the liver sections exhibited significant histopathological changes, including hepatocyte degeneration, necrosis, and inflammatory cell infiltration. There was also evidence of fatty changes and congestion in the sinusoids. In the metformin treated group: Liver tissues showed marked improvement with reduced signs of hepatocyte degeneration and necrosis. The architecture appeared more similar to the normal control group with minor inflammatory cell infiltration. The histopathological analysis indicates that *Enatia chlorantha* extracts, especially at higher doses, have protective and regenerative effects on liver tissues in STZ-induced diabetic rats. The improvements were comparable to the standard antidiabetic drug, metformin, suggesting the potential therapeutic benefits of *Enatia chlorantha* in managing diabetes-induced histopathological changes

Conclusion

The study showed that *E. chlorantha stem* bark extract has significant hypoglycemic and anti-inflammatory effects in STZ-induced diabetic rats. These effects are dose-dependent and comparable to those of metformin. The findings support the traditional use *E. chlorantha* in managing diabetes and its complications and highlight its potential as a source of bioactive compounds for developing new therapeutic agents.. Further research is required to elucidate the molecular mechanisms underlying these effect

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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