

THERAPEUTIC POTENTIAL OF *Gongronema latifolium* AND *Ocimum gratissimum* ON LIVER AND KIDNEY FUNCTIONS OF ALLOXAN-INDUCED DIABETIC RATS EXPOSED TO CARBONTETRACHLORIDE

ABSTRACT

Diabetes is a persisting and deadly disease which occur due to pancreatic dysfunction resulting indecrease or lack of insulin production. This study investigated thetherapeutic potential of *Gongronema latifolium*(GL) and *Ocimum gratissimum*(OG)on liver and kidney functions of alloxan-induced diabetic rats exposed to 1.0 ml/kg/bw carbontetrachloride(CCl₄), intraperitoneally. Forty-five (45) rats divided into 9 groups of 5 rats each were used. Groups 1, 2 and 3 were respectively normal control (NC), diabetic control (DC) and diabetic exposed to CCl₄ (D_{CCl4}) on the 14th day. Groups 4, 5 and 6 were diabetic treated with single GL (200 mg/kg), OG (200 ml/kg/bw) and combined GL and OG (100 ml/kg/bw) each, respectively.Groups 7, 8 and 9 were diabetic treated with single leaf GL (200 ml/kg) and exposed to 1.0ml/kg/bw of CCl₄on the 14th day, OG (200 mg/kg/bw) and exposed to 1.0ml/kg/bw of CCl₄on the 14th day and combined GL and OG (100 mg/kg/bw)each and exposed to 1.0ml/kg/bw of CCl₄on the 14th day, respectively. Treatment lasted for 14th days and 24 hours after, animals were sacrificed, blood and liver collected for analysis.Results showed significant ($p < 0.05$) elevations in AST, ALT, ALP, urea, creatinine, and blood glucose levels, along with decreased albumin levels in the DC and D_{CCl4} groups compared to NC. Conversely, treatments with GL, OG, and GLOG extracts mitigated these adverse effects, restoring biochemical parameters to near-normal levels. Combined extracts exhibited superior antioxidant and hepato-nephroprotective activities compared to single extracts. Conclusively, the extracts, singly and in combination mitigated the damaging effect of alloxan-induced diabetic rats exposed to CCl₄ by exhibiting antioxidant and hepato-nephroprotective effects, evidently shown in the analyzed parameters and histopathological results.

1.0 INTRODUCTION

Diabetes mellitus is a persisting, weakening, deadly disease and a disorder with different causes. Diabetes mellitus is an important chronic metabolic disorder (11). Disorder in carbohydrate metabolism caused diabetes mellitus and it is characterized by impairment in insulin production by the body cells (23). Chronic or persisting hyperglycemia with disturbance of carbohydrates, fat and protein metabolism together with over production and/or underproduction of glucose are the characteristics of diabetes mellitus (20). Inability of the body to produce or respond to insulin, thereby maintaining normal levels of sugar (glucose) in the blood result in diabetes (23).

Beta cells found within clusters of cells in the pancreas are responsible for the secretion of insulin which stimulate cells to take up and metabolize glucose (10). In diabetic patients, their beta cells are defective leading to low insulin secretion, or their muscle and adipose cells may not respond to the effects of insulin, resulting in a decreased ability of these cells to take up and metabolize glucose. In these conditions, there is an increase in blood glucose levels resulting in hyperglycemia (high blood sugar) (23). The commonest effect of uncontrolled diabetes is hyperglycemia, and cause damage to many body systems, especially the nerves and blood vessels (41).

Occupational chemicals can increase the severity of diabetes. Chemicals like carbon tetrachloride (CCl_4) can have adverse effects on diabetes mellitus. Degenerative liver and kidneys can be seen on exposure to high concentration of CCl_4 and long-term exposure can be severe. Exposure to CCl_4 result in reduced kidney functions and increase in blood urea, nitrogen, creatinine concentration, creatinine clearance, total protein and albumin. Metabolism of CCl_4 by cytochrome p450 result in the formation of free radical trichloromethyl and trichloromethyl peroxy which causes cell damage as well as kidney injury (26,28, 33). Direct nephrotoxic effects such as dysfunction in mitochondria, inhibition in lysosomal hydrolase, damage to phospholipids and intracellular calcium concentration increase results in proximal tubular toxicity (38). Oxidative stress caused by free radicals formed have greater effect in kidney such as kidney failure, uremia as well as kidney diseases and renal oxidative stress usually lead to the upregulation of proxy-to-enzyme-dependent reactive oxygen species (ROS) formation and antioxidants exhaustions (19, 38).

Medicinal plants have been used widely in tackling diseases due to their availability, affordability and increased safety with minimal side effects (34). *Gongronema latifolium* and *Ocimum gratissimum* have been reported to be used for several diseases such as diabetes mellitus

and this offers a great opportunity for the development of new types of therapeutics (36). Poly-herbal therapy allows for combination of secondary metabolites which would not only exert but also potentiate biological effects with minimal side effects (34). In Nigeria, *Gongronema latifolium* and *Ocimum gratissimum* are one of the many plants found to lower glycaemia in Type-1 diabetes (6). The phytochemical investigations carried out on different forms of these plants revealed the presence of different phytochemicals such as alkaloids, phenols, terpenoids, glycosides, flavonoids, steroids, saponins, and tannins which facilitate their therapeutic potentials such as analgesic, antitumor, broad spectrum antimicrobial (antibacterial, antifungal, antiparasitic and antiviral), antipyretic, antioxidant, anti-inflammatory, antiulcer, anti-sickling, anti-asthmatic, mild expectorant, hypoglycemic, hypolipidemic, hepatoprotective, digestive tonic and laxative properties. This study investigated the therapeutic potential of *Gongronema latifolium* and *Ocimum gratissimum* on some biochemical parameters of alloxan-induced diabetic rats exposed to CCl₄.

2.0 MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

Fresh but mature leaves of *Gongronema latifolium* and *Ocimum gratissimum* were purchased from Itam Market in Uyo Local Government of Akwa Ibom State. The plant leaves were taken to the herbarium unit of Botany and Ecological Science Department, Faculty of Biological Science, University of Uyo, for identification.

The fresh leaves of GL and OG after being obtained were washed to get rid of debris and air dried. The dried leaves were pulverized into powder. The powdered sample were weighed and about 70g of the powdered samples each were macerated in 95% absolute ethanol (Sigma Aldrich) for 72 hours and then filtered. The filtrates were concentrated to dryness using a water bath at 45°C. The dried extracts were refrigerated at 2-8°C until required for use.

2.2 Preparation of Alloxan and Carbon Tetrachloride

1.5ml/kg of body weight was used. 2ml of CCl₄ was mixed in 6ml of corn oil with a spatula which means a ratio of 1:3 (37). Alloxan was diluted in 100m/2ml of distilled water and administered.

2.3 Induction of Diabetes and Exposure to CCl₄

A single dose 140-180 mg/kg of alloxan was used for the induction of diabetes mellitus in an overnight fasted albino Wistar rats through intraperitoneal injection. The diabetic rats were divided randomly into 9 groups and some groups were exposed to CCl₄.

2.4 Experimental Design and Treatment of Animals

Forty-five Wistar rats were divided into nine (9) groups of five rats each. Groups 1, 2 and 3 were normal control (NC), diabetic control (DC) and diabetic control exposed to 1.0 ml/kg/bw of CCl₄ intraperitoneally on the 14th day, respectively. Groups 4, 5 and 6 were diabetic treated with single GL (200 mg/kg), OG (200 ml/kg/bw) and combined GL and OG (100 ml/kg/bw) each, respectively. Groups 7, 8 and 9 were diabetic treated with single leaf GL (200 ml/kg) and exposed to 1.0ml/kg/bw of CCl₄ on the 14th day, OG (200 mg/kg/bw) and exposed to 1.0ml/kg/bw of CCl₄ on the 14th day and combined GL and OG (100 mg/kg/bw) each and exposed to 1.0ml/kg/bw of CCl₄ on the 14th day, respectively

The plant extracts reconstituted in distilled water were administered orally and treatment lasted for 14 days. Carbon tetrachloride was administered intraperitoneally. Throughout this period, daily changes in blood glucose and body weight were measured with the use of a glucometer and animal weighing balance respectively. The animals were maintained on growers' food pellets. This is shown in Table 1.0.

Table 1.0 Experimental design

Group	Treatment
1.	Normal control Feed and water only
2.	Diabetic control Feed, water and alloxan only
3.	Diabetic CCl ₄ (1.0 mg/kg) on the 14 th day
4.	Diabetic GL(200 mg/kg/bw) for 14 days
5.	Diabetic OG (200 mg/kg/bw) C for 14 days
6.	Diabetic GL + OG (100 mg/kg/bw each) for 14 days
7.	Diabetic GL(200 mg/kg bw) for 14 days + CCl ₄ (1.0 mg/kg) on the 14 th day
8.	Diabetic OG (200 mg/kg/bw) for 14 days + CCl ₄ (1.0 mg/kg) on the 14 th day
9.	Diabetic GL + OG (100 mg/kg/bw each) for 14 days + CCl ₄ (1.0 mg/kg) on the 14 th day

GL= *Gongronema latifolium*, OC= *Ocimum gratissimum*, CCL₄ =Carbon tetrachloride, BW= Body weight

2.5 Collection, Preparation and Storage of Samples

At the end of 14th days, feeds were withdrawn, the rats fasted overnight but had free access to water. They were then euthanized under ketamin vapour(0.8ml to 1.0ml) and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles. The blood was emptied into plain tubes and allowed to clot for about 2 hours. The clotted blood was thereafter centrifuged using bench top centrifuge (MSE) at 3000rpm for 10 minutes to recover serum from clotted cells. Serum was separated with sterile dropper and stored frozen until used for biochemical analysis. The livers were surgically removed and blotted using filter paper to remove traces of blood and then weighed with analytical balance. After weighing, parts of the liver tissues were collected with paper bag and stored frozen until need for tissue homogenate preparation. The livers were suspended in 10% formal saline for fixation preparatory to histological processing.

2.6 Biochemical Analysis

2.6.1 Estimation of Aspartate Aminotransaminase (AST)

The activity of AST was estimated using Randox kit based on method described by(32)

Reagent provided

- Buffer reagent Phosphate buffer, 100nM, pH 7.4, L-aspartate, 100nM, oxoglutarate, 2Mm.
- Reagent 2 2,4- dinitrophenyl hydrazine, 1Mm

Reagent preparation: Contents are ready for use

Procedure: As per schedule below, pipette into test tubes.

Reagent	blank	Sample
Sample	-	0.2mL
Solution 1(buffer)	1mL	1mL
Distilled water	0.2mL	-

Mixed and incubated for exactly 30 minutes at 37° C

Solution 2	1mL	1mL
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Mixed and was allowed to stand for exactly 20 minutes at 20- 25° C

Sodium hydroxide	10mL	10mL
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Mixed and absorbance of sample was read against the reagent blank at 546nm after 5 minutes

2.6.2 Estimation of Alanine Aminotransaminase (ALT)

The activity of ALT was estimated using Randox kit based on method described by(32)

Reagent provided

- Buffer reagent Phosphate buffer, 100nM, pH 7.4, L-alanine, 200mol/L, a-oxoglutarate, 2mmol/L
- Reagent 2 2,4- dinitrophenyl hydrazine, 1mM

Reagent preparation: Contents are ready for use

Procedure: As per schedule below, pipette into test tubes.

Reagent	blank	Sample
Sample	-	0.2mL
Solution 1	1mL	1mL

Distilled water 0.2mL -

Mixed, incubated for exactly 30 minutes at 37° C

Solution 2 1mL 1mL

Mixed and was allowed to stand for exactly 20 minutes at 20- 25° C

Sodium hydroxide 10mL 10mL

Mixed, and absorbance of the sample was read against the reagent blank at 546nm after 5 minutes

2.6.3 Estimation of alkaline phosphatase (ALP) activity

Reagent provided

- Buffer reagent Phosphate buffer, 100mM, pH 7.4, alkaline phosphatase, 200mU/L, a-oxoglutarate, 2mmol/L
- Reagent 2 2,4- dinitrophenyl hydrazine, 1mM

Reagent preparation: Contents are ready for use

Procedure: Test tubes were labeled STD, blank and test. 0.5mL of ALP substrate was pipetted into all the test tubes and incubated at 37°c for 3mins –50ul of the Std sample and d/w was added into appropriate tubes. The mixtures were incubated for exactly 10mins at 37°c. 2.5mL of ALP color developer was pipetted into all the test tubes and mixed properly. The spectrophotometer was zero at 590nm with the blank. Absorbance were read and recorded.

$$\text{Calculation} = \frac{\text{ABS of test} \times \text{conc of STD}}{\text{ABS of STD}}$$

2.6.4 Determination of urea concentration in serum using Randox kit

Reagent provided

- Reagent 1 EDTA, 116mM, Sodium nitroprusside, 6mM, Urease, 1g/L
- Reagent 2 diluted phenol, 120mM
- Reagent 3 sodium hypochlorite (diluted), 27mM, Sodium, 0.14N
- Standard Urea, 80mg/dl (13.3 mmol/L)

Reagent Preparation:

R1: Content of vial R1a (Urease enzyme) was transferred into bottle R1b nitroprusside in EDTA and mixed gently. Stable for 2 months at 2-8°C.

R2: Sodium phenol reagent was diluted with 660mL of distilled water, bottle rinsed thoroughly and mixed. Stable for 2 months at 2-8°C when stored in dark.

R3: Sodium hypochlorite was diluted with 750mL of distilled water, bottle rinsed thoroughly and mixed. Stable for 2 months at 2-8°C when stored in dark.

Sample: Serum

Procedure:

Test tubes were labeled as test, standard and blank. 0.1mL of the Reagent R₁ was Pipetted into all the tubes and 10uL of the samples, standard and d/w as added into appropriate tubes. It was mixed and incubated at 37°C for 10mins. Also, 2.5mL of R₂& R₃ were Pipetted to all the tubes and were mixed and incubated at 25°C for 15mins. The absorbance was read and recorded at 546nm.

Calculation: Urea conc. (mg/dL) $\frac{A_{\text{sample}} \times \text{standard conc. (mg/dL)}}{A_{\text{standard}}}$

2.6.5 Determination of creatinine concentration in serum using Randox kit

Creatinine concentration in serum was estimated using Randox kit based on method described by (18)

Reagent provided

Reagent

Already constituted reagent kits. A (picric acid solution), B (alkaline solution) and C (standard solution) were used. Reagent A and B were mixed in equal parts, to obtain working reagent. The concentration of the standard was 2mg/dL (76.8 Nmol/L) while those of the reconstituted working reagents were as follows:

Picric acid	55.0mM
Sodium carbonate	50.0mM
Sodium hydroxide	0.4M

The working reagent which was stored in the dark was stable for up to 15 days at 25°C.

Procedure

The standard solution, sample and working reagents were pipetted into respective tubes as follows:

	Blank	Standard	Sample
Standard (mL)	-	0.2	-
Sample (mL)	-	-	0.2
Working reagent (mL)	2.0	2.0	2.0

These were mixed and then transferred to measuring cuvette. The absorbance was at 20 and 80 seconds interval at 546nm. The creatinine in the sample was then calculated.

Calculation:
$$\frac{A_{\text{sample}} \times 177}{A_{\text{standard}}} = \text{umol/l}$$

2.6.6 Determination of albumin in serum using Randox kit (Bromocresol Green)

Reagent provided

- BCG concentrate, Succinate buffer, 75mM, pH 4.2, Bromocresol green, 0.15mM, Brij.35 and preservative
- Standard Albumin 49g/L.

Reagent preparation:

Each vial of R1 reagent (13.5 mL) was diluted with distilled water and used as BCG working reagent.

Procedure:

To clean tubes, 10ul (0.01mL) of standard/ sample/ distilled 1000ul (1mL) of diluted BCG reagents was added, mixed and incubated for 5 minutes at 20 – 25°C. Absorbance of the tube content was measured at 578nm reagent blank.

Calculation:

$$\text{Albumin conc. (g/dL)} = \frac{A_{\text{sample}} \times \text{standard conc. (mg/dL)}}{A_{\text{standard}}}$$

2.6.7 Glucose estimation using Randox assay kit (GOD-PAP method based on Barham and Trinder)

Glucose was estimated using Randox kit based on method described by(1)

Reagents provided

- Buffer(R1a) Phosphate buffer, 0.01 mol/L, Ph 7.0; Phenol, 1mmol. L
- GOD-PAP reagent (R1b) 4- aminophenazone phenol, 0.77 mmol/L Glucose oxidase ≥ 1.5 KU/L; Peroxidase ≥ 1.5 Ku/L
- Standard Glucose, 100mg/dl/ 5.5 mmol/L

Reagent preparation: GOD- PAP reagent, R1a reconstituted with the entire content of one vial of R1a (100 mL) and rinsed several times to form the working reagent. Stable for 3 months that +2 – 18°C for 5 days and +15 - 25°C.

Sample: Serum

Procedure: Into set tubes of 10 uL of standard/ sample was pipetted followed by 1000 uL of working reagent mixed and incubated for ten minutes at 37°C (or 25 minutes at 15- 25°C). The absorbance was measured against the reagent blank within 60 minutes at 526nm.

$$\text{Calculation: Glucose conc. (mmol/L)} = \frac{A_{\text{sample}} \times \text{Standard conc. (mmol/ L)}}{A_{\text{Standard}}}$$

2.7 Histological Analysis

The liver of the experimental animals was surgically removed and fixed in 10% buffer formalin. They were then processed and stained with haematoxylin and eosin (H&E) for liver study according to standard procedures at Chemical Pathology Department, University of Uyo Teaching Hospital, Uyo. Changes in morphology were observed and recorded in the excised organs of the sacrificed animals. Histologic pictures were taken as micrograph

2.8 Statistical Analysis

All results were analyzed using the Statistical Package for Social Sciences (SPSS), where the data are presented as Mean \pm Standard Error of Mean (SEM). ANOVA was used to compare means, and values were considered significant at $P < 0.05$.

3.0 RESULT AND DISCUSSION

3.1 Therapeutic Potential of *Gongronema latifolium* and *Ocimum gratissimum* on Biochemical Indices of Liver Functions in Alloxan-induced Diabetic Rats Exposed to CCl_4

Tables 2.0 and 3.0 summarize the therapeutic potential of *Gongronema latifolium* and *Ocimum gratissimum* on biochemical indices of liver and kidney functions in alloxan-induced diabetic rats exposed to CCl_4 . Diabetic control (DC) and diabetic + CCl_4 (D_{CCl_4}) groups exhibited significant ($p < 0.05$) elevations in AST, ALT, ALP, liver weight, urea, creatinine, and blood glucose levels, along with decreased albumin levels compared to the normal control (NC) group. The administration of CCl_4 further exacerbated these alterations. Treatment with GL, OG, and in combination significantly restored these parameters toward normal levels. Combined extracts demonstrated superior efficacy in reducing oxidative stress markers and restoring liver and kidney function. The results suggest that the antioxidant properties of GL and OG contribute to their hepato-nephroprotective effects.

Table 2.0: Therapeutic Potential of *Gongronema latifolium* and *Ocimum gratissimum* on Biochemical Indices of Liver Functions in Alloxan-induced Diabetic Rats Exposed to CCl_4

Groups and Treatments	AST(U/L)	ALT(U/L)	ALP(U/L)	Liver Weight (Mg)	Total Protein
1 – Normal Control	22.33 \pm 0.33	13.17 \pm 0.55	42.67 \pm 0.88	5.97 \pm 0.07	56.00 \pm 2.08
2 – Diabetic Control	43.33 \pm 0.88 ^a	32.67 \pm 0.88 ^a	54.67 \pm 2.40 ^a	6.58 \pm 0.32 ^a	61.33 \pm 2.03
3 – Diabetic + CCl_4	52.67 \pm 1.20 ^{ab}	46.67 \pm 0.88 ^{ab}	63.00 \pm 1.15 ^{ab}	7.57 \pm 0.20 ^{ab}	56.00 \pm 2.08
4 – Diabetic + GL	34.33 \pm 0.88 ^{abc}	29.00 \pm 0.00 ^{abc}	43.33 \pm 0.88 ^{bc}	6.58 \pm 0.32 ^{abc}	57.33 \pm 1.45 ^c
5 – Diabetic + OG	34.00 \pm 0.58 ^{abc}	23.20 \pm 1.27 ^{abcd}	41.33 \pm 0.88 ^{bc}	6.40 \pm 0.1 ^{bc}	59.00 \pm 2.08 ^c
6 – Diabetic + GL + OG	24.33 \pm 1.76 ^{bcde}	25.77 \pm 0.43 ^{abcde}	36.00 \pm 1.53 ^{abcde}	5.62 \pm 0.2 ^{bc}	55.00 \pm 1.15 ^{ac}
7 – Diabetic + CCl_4 + GL	40.67 \pm 0.33 ^{acdef}	32.70 \pm 0.81 ^{acdef}	48.33 \pm 0.88 ^{abcdef}	5.97 \pm 0.07 ^{aef}	57.33 \pm 1.45 ^c
8 – Diabetic + CCl_4 + OG	34.67 \pm 1.76 ^{abcfg}	27.90 \pm 0.52 ^{abceg}	39.67 \pm 2.73 ^{bcg}	5.62 \pm 0.27 ^{aef}	57.33 \pm 1.45 ^c

9 – Diabetic + CCl ₄ + GL+ OG	27.33±1.76 ^{abcdegh}	24.40 ± 0.35 ^{abcdgh}	36.00±0.58 ^{abcdeg}	5.22 ± 0.41 ^{abc}	57.33 ± 1.45 ^c
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Data are presented as Mean ± Standard Error of Mean (SEM). Mean of groups were compared with each other and considered significantly different at $p < 0.05$. 'a' = significantly different when compared with Group 1; 'b' = significantly different when compared with Group 2; 'c' = significantly different when compared with Group 3; 'd' = significantly different when compared with Group 4; 'e' = significantly different when compared with Group 5; 'f' = significantly different when compared with Group 6; 'g' = significantly different when compared with Group 7; 'h' = significantly different when compared with Group 8.

Table 3.0: Therapeutic Potential of *Gongronema latifolium* and *Ocimum gratissimum* on Biochemical Indices of Kidney Functions in Alloxan-induced Diabetic Rats Exposed to CCl₄

Groups and Treatments	Urea(mg/dL)	Creatinine(μmol/L)	Glucose (mg/dL)	Albumin
1 – Normal Control	4.80 ± 0.21	71.33 ± 2.03	3.83 ± 0.09	36.00 ± 1.53
2 – Diabetic Control	6.27 ± 0.15 ^a	89.67 ± 1.45 ^a	5.03 ± 0.41 ^a	27.00 ± 2.08
3 – Diabetic + CCl ₄	7.70 ± 0.15 ^{ab}	96.67 ± 2.73 ^{ab}	6.03 ± 0.20 ^{ab}	23.33 ± 2.73 ^a
4 – Diabetic + GL	5.57 ± 0.07 ^{abc}	77.33 ± 1.45 ^{bc}	4.53 ± 0.09 ^{ac}	32.67 ± 3.93 ^c
5 – Diabetic + OG	5.27 ± 0.18 ^{bc}	76.33 ± 2.96 ^{bc}	4.30 ± 0.06 ^{bc}	31.33 ± 3.76 ^c
6 – Diabetic + GL + OG	4.43 ± 0.12 ^{bcd}	74.33 ± 2.33 ^{bc}	3.60± 0.38 ^{bcd}	32.67 ± 1.45 ^c
7 – Diabetic + CCl ₄ + GL	6.17 ± 0.15 ^{cdef}	93.00 ± 2.08 ^{ad}	5.30±0.15 ^{acdef}	26.67 ± 0.88 ^a
8 – Diabetic + CCl ₄ + OG	5.70 ± 0.12 ^{abcf}	84.67 ± 1.45 ^{acdefg}	5.17 ± 0.09 ^{acef}	25.33 ± 1.45 ^{adf}
9 – Diabetic + CCl ₄ + GL + OG	5.33 ± 0.38 ^{bcfg}	77.67 ± 2.19 ^{bcdgh}	4.97 ± 0.20 ^{acef}	25.33 ± 1.45 ^{adf}

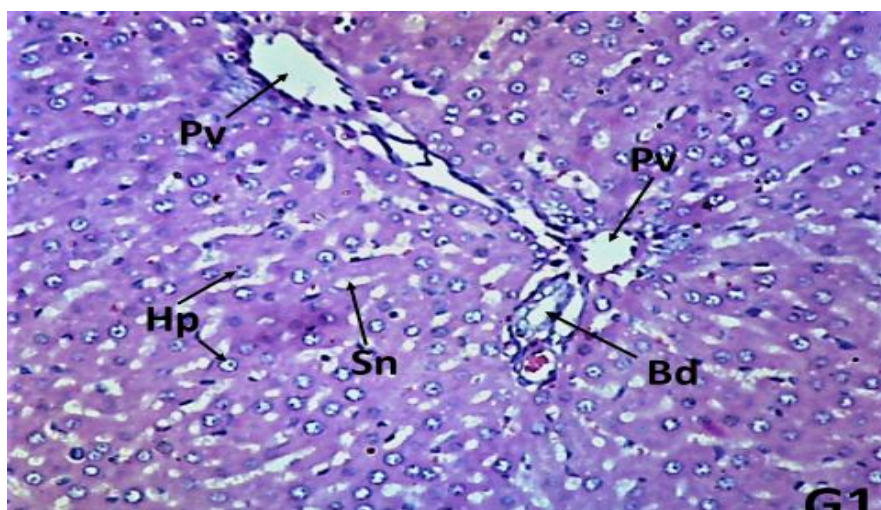
Data are presented as Mean ± Standard Error of Mean (SEM). Mean of groups were compared with each other and considered significantly different at $p < 0.05$. 'a' = significantly different when compared with Group 1; 'b' = significantly different when compared with Group 2; 'c' = significantly different when compared with Group 3; 'd' = significantly different when compared with Group 4; 'e' = significantly different when compared with Group 5; 'f' = significantly different when compared with Group 6; 'g' = significantly different when compared with Group 7; 'h' = significantly different when compared with Group 8.

3.2 Histopathological Studies of Rat Liver in CCl₄- induced Hepatotoxicity

Figure 1.0, histopathological examination of the liver section of the normal control group showed normal hepatic architecture with portal vein (Pv), and Bile duct (Bd), within the portal area, well protected hepatocytes (Hp), and arrays of sinusoidal spaces (Sn) within the hepatic lobules (H&E x 100).

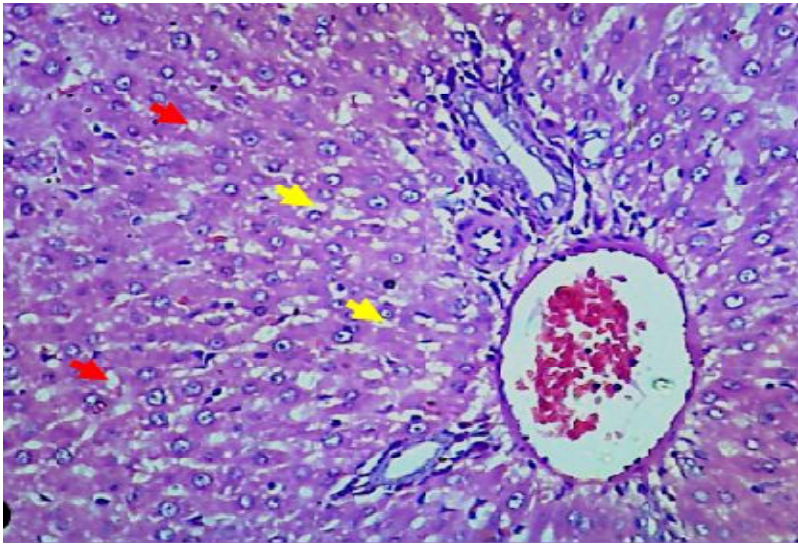
Figure 2.0 also showed the photomicrograph of a section of a group 2 (diabetic control) liver tissue showing atrophying hepatic architecture with wide spread macro and micro vesicular steatosis (red arrow) and areas of vacuolated hepatocytes (yellow arrow) all within the hepatic lobules.

Cellular abnormalities such as atrophic hepatic architecture with wide spread clusters of balloon hepatodegeneration (black arrow), macro and micro vesicular steatosis (red arrow) and areas of vacuolated hepatocytes (yellow arrow) all within the hepatic lobules was seen in the histopathology of liver tissue of group 3 (CCl₄ exposed group) when compared to the control groups. Also, the histopathology of the diabetic groups (4,5 and 6) which were treated with single and combined leaf extracts (200mg/kg and 100mg/kg) of *Gongronema latifolium* and *Ocimum gratissimum* showed a normal hepatic histo-structure with portal vein, and bile duct within the portal area, well protected hepatocytes, and wide proliferating kupfer cells in the arrays of sinusoidal spaces within the hepatic lobules (Figure 4.0 – 6.0) when compared to group 2. Also, abnormalities such as liver tissue showing atrophying hepatic architecture with wide spread clusters of balloon hepato-degeneration (black arrow), and areas of macro and micro vesicular steatosis (red arrow) within the hepatic lobules was still observed in CCl₄ exposed group treated with both single (200mg/kg each) and combined 100mg/kg each) of *Gongronema latifolium* and *Ocimum gratissimum* (Figure 7.0 -9.0).



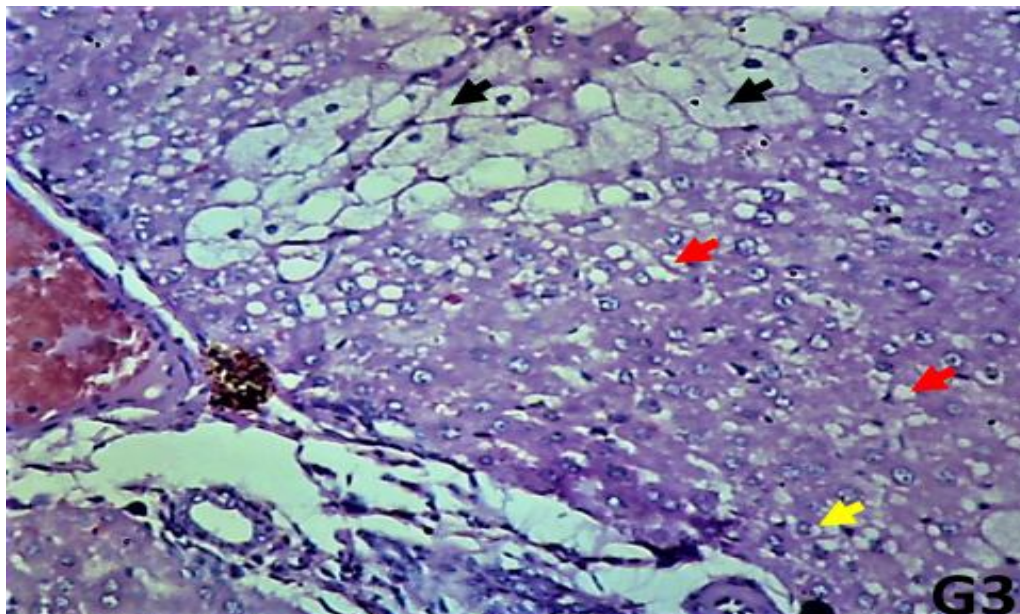
Photomicrograph of a section of a group 1 liver tissue showing normal hepatic architecture with portal vein (Pv), and Bile duct (Bd), within the portal area, well protected hepatocytes (Hp), and arrays of sinusoidal spaces (Sn) within the hepatic lobules (H&E x 100).

Figure 1.0: Histological section of liver of rat in the normal control group.



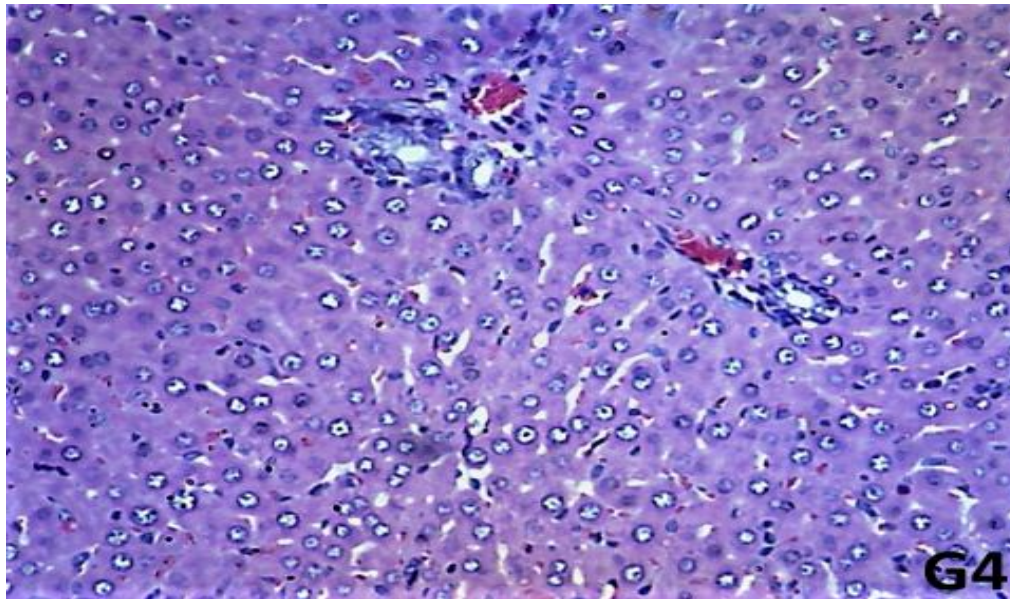
Photomicrograph of a section of a group 2 liver tissue showing atrophying hepatic architecture with wide spread macro and micro vesicular steatosis (red arrow) and areas of vacuolated hepatocytes (yellow arrow) all within the hepatic lobules (H&E x 100).

Figure 2.0: Histological section of liver of rat in diabetic control group.



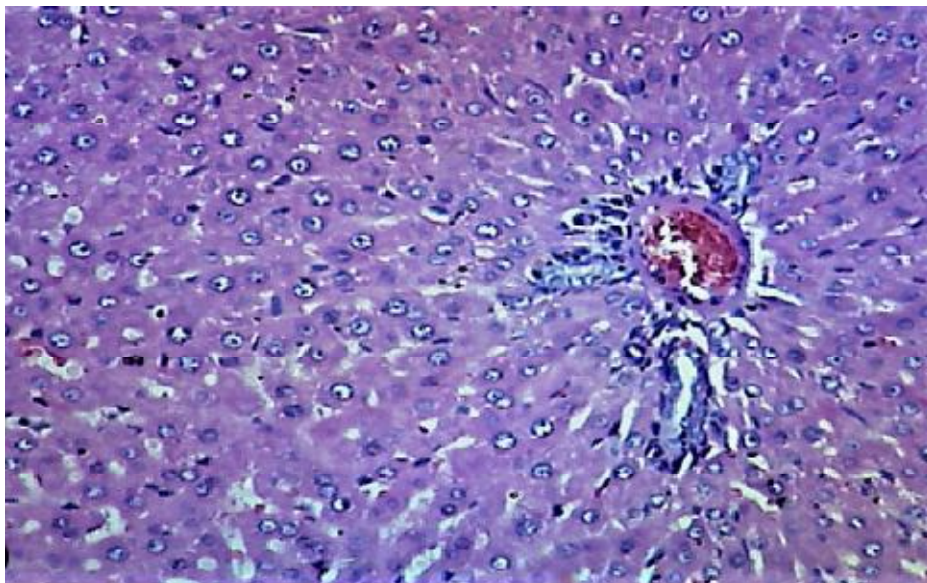
Photomicrograph of a section of a group 3 liver tissue showing atrophying hepatic architecture with wide spread clusters of balloon hepato-degeneration (black arrow), macro and micro vesicular steatosis (red arrow) and areas of vacuolated hepatocytes (yellow arrow) all within the hepatic lobules (H&E x 100).

Figure 3.0: Histological section of liver of diabetic exposed to CCl₄



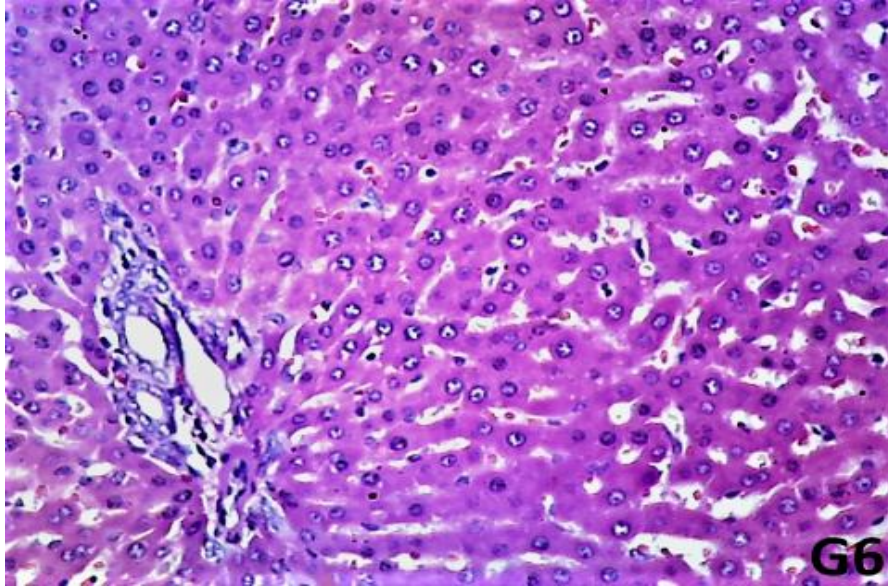
Photomicrograph of a section of a group 4 liver tissue showing normal hepatic histo-structure with portal vein, and bile duct within the portal area, well protected hepatocytes, wide proliferating kupfer cells in the arrays of sinusoidal spaces within the hepatic lobules (H&E x 100).

Figure 4.0: Histological section of liver of diabetic rat treated with single leaf extract (200 mg/kg) of *Gongronema latifolium*



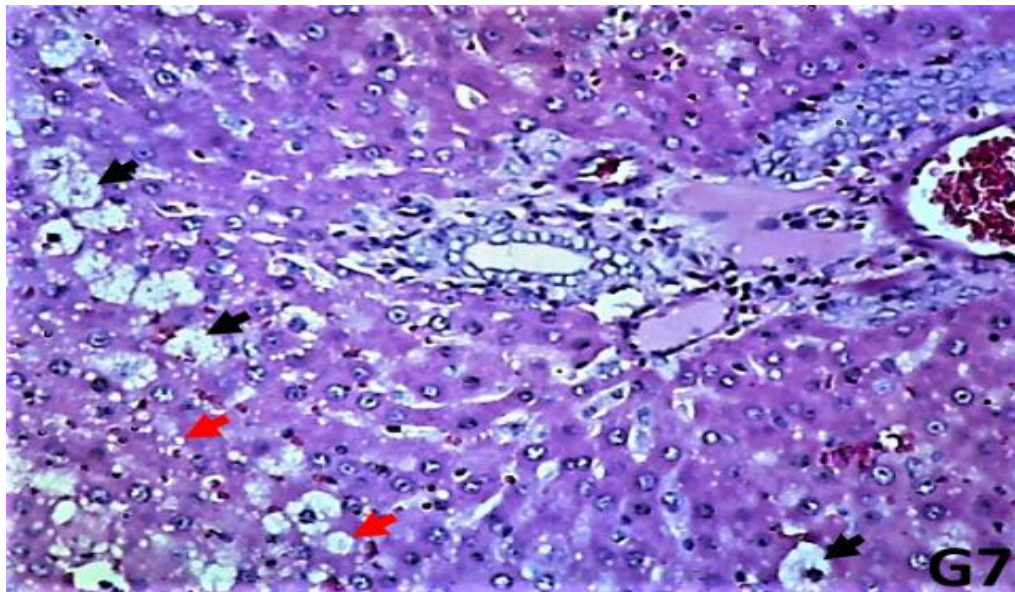
Photomicrograph of a section of a group 5 liver tissue showing normal hepatic histo-structure with portal vein, and Bile duct, within the portal area, well protected hepatocytes, and arrays of sinusoidal spaces within the hepatic lobules (H&E x 100).

Figure 5.0: Histological section of liver of diabetic rat treated with single leaf extract (200 mg/kg) of *Ocimum gratissimum*



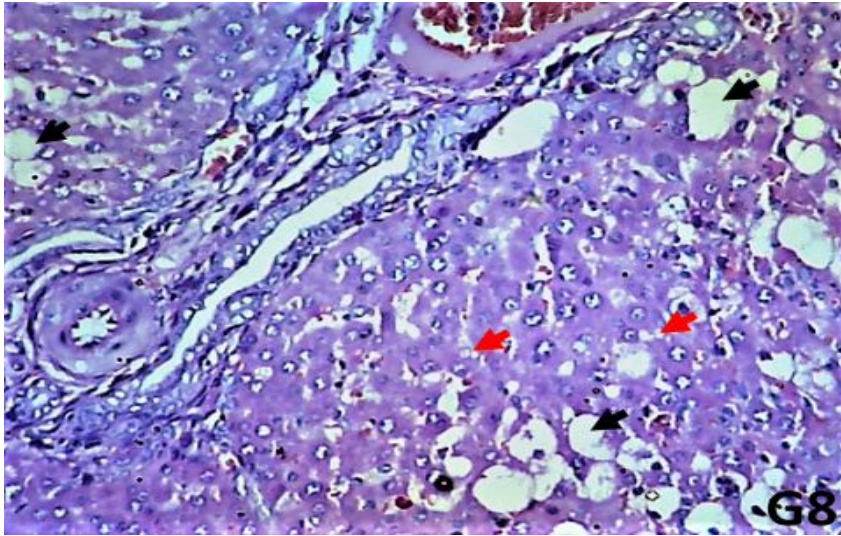
Photomicrograph of a section of a group 6 liver tissue showing normal hepatic histo-structure with portal vein, and Bile duct, within the portal area, well protected hepatocytes, and arrays of sinusoidal spaces within the hepatic lobules (H&E x 100).

Figure 6.0: Histological section of liver of diabetic rat treated with combined leaf extracts (100 mg/kg each) of *Gongronema latifolium* and *Ocimum gratissimum*



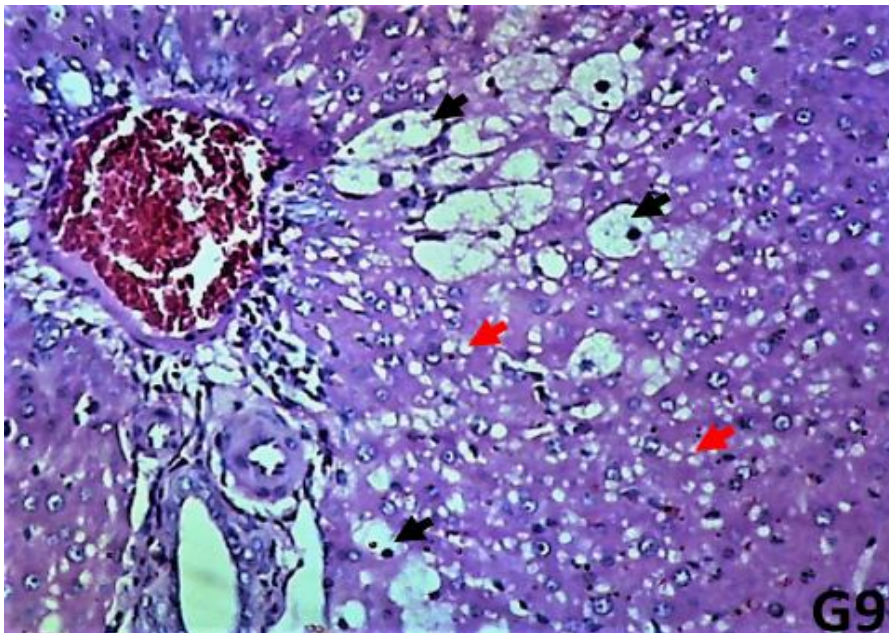
Photomicrograph of a section of a group 7 liver tissue showing atrophying hepatic architecture with wide spread clusters of balloon hepato-degeneration (black arrow), and areas of macro and micro vesicular steatosis (red arrow) within the hepatic lobules (H&E x 100).

Figure 7.0: Histological section of liver of diabetic rat exposed to CCl_4 and treated with single leaf extracts (200 mg/kg each) of *Gongronema latifolium*.



Photomicrograph of a section of a group 8 liver tissue showing atrophying hepatic architecture with wide spread clusters of balloon hepato-degeneration (black arrow), and areas of macro vesicular steatosis (red arrow) all within the hepatic lobules (H&E x 100).

Figure 8.0: Histological section of liver of diabetic rat exposed to CCl₄ and treated with single leaf extracts (200 mg/kg each) of *Ocimum gratissimum*



Photomicrograph of a section of a group 9 liver tissue showing atrophying hepatic architecture with wide spread clusters of balloon hepato-degeneration (black arrow), and wide spread macro vesicular steatosis (red arrow) within the hepatic lobules (H&E x 100).

Figure 9.0: Histological section of liver of diabetic rat exposed to CCl₄ treated with combined leaf extracts (100 mg/kg each) of *Gongronema latifolium* and *Ocimum gratissimum*.

DISCUSSION

Liver and kidney are important organs of the body. Their main function is to remove toxin from the body's blood supply. Liver and kidney function tests are groups of tests carried out to know their state. Liver enzymes include aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Changes in the levels of the liver enzymes is an indication of hepatic or liver injury. Kidney function can be assessed by testing for urea, creatinine, albumin and total protein

In this study, the levels of AST, ALT, ALP, total protein, liver weight, urea, creatinine was significantly high ($P < 0.05$) and albumin level was lowered in groups 2 and 3. This alteration was significant in group 3 when compared to group 2, explaining a synergistic effect of diabetes and CCl_4 . This corresponds with most experimental studies. There is a strong correlation between liver enzymes and hyperglycemia (40).

The liver plays a role in glycemic homeostasis regulation by means of glycogenesis and gluconeogenesis (16). The precise mechanism of the relationship between elevated serum levels of liver enzymes with the risk of diabetes remains unclear. Most studies link increased liver enzymes to non-alcoholic fatty liver disease (NAFLD) (22,30). Hyperglycemia, hyperinsulinemia and reduced insulin signaling which can lead to diabetes may be caused by high free fatty acids in the liver (5). Liver inflammation through increased proinflammatory adipocytokines as well as decrease in anti-inflammatory adiponectin can also result in diabetes (29). Most studies suggest that the association between diabetes and increased serum ALP is due to the fact that ALP showed a negative relation with adiponectin, a secreted hormone from adipose tissues (24). Decrease serum levels of adiponectin is a risk factor for progression to diabetes (8). The increase in the serum ALT level in response to diabetes is due to the prolonged half-life of ALT or its greater specificity for liver injuries (6).

Elevation of acute phase proteins, globulins and fibrinogen may be because of an increase in total proteins and this is compounded by a decrease in the fractional synthetic rate of albumin due to insulin resistance/deficiency (27). Report by Hardikar *et al* shows that low levels of albumin in the body may be due to some other etiology which leads to an over estimation of HbA1c in diabetics (17). The results of this study are in line with the above findings. Decrease in

serum albumin could be due to the competition between serum albumin and hemoglobin for glycation (27).

A significantly high ($P < 0.05$) liver weights in groups 2 and 3 compared to the control group was also observed in this study. The mechanism behind this may be due to an increased triglyceride accumulation resulting in liver enlargement which could be due to the increased influx of fatty acids into the liver induced by hyperinsulinemia and the low capacity of excretion of lipoprotein secretion from liver resulting from a deficiency of apolipoprotein B synthesis caused by diabetes and CCl_4 (44).

Increase in urea and creatinine is an indication of renal damage as diabetes or prolonged hyperglycemia and CCl_4 can cause irreversible damage to the kidney nephrons, thereby impairing the main functions of kidney. Increase in serum creatinine and blood urea is because of reduction in glomerular filtration rate, as creatinine is a marker of glomerular filtration and indicating reduced filtration capacity of the kidney (2).

Treatment with both combined and single leaf extracts of *Gongronema latifolium* and *Ocimum gratissimum* significantly reduced ($P < 0.05$) the levels of these enzymes to near normal. Reduction in the level of ALP, indicates biliary function stabilization and increase in total protein by these plant extracts suggests the regeneration of endoplasmic reticulum leading to protein synthesis (37). Furthermore, AST and ALT reduction by these leaf extracts towards normal values is an indication of regeneration process from hepatocellular damage by phytochemicals present in these plants (39). Antioxidant compounds are capable of scavenging free radicals or stimulate antioxidant defense system thereby reducing ROS which are indicators of oxidative stress and decreases the activities of ALT, AST, ALP, urea, creatinine and blood glucose (14). The mitigative effects of these plants extracts was higher in groups treated with combined leaf extracts, explaining the advantage of poly-herbal therapy.

Histology is the microscopic study of tissues and organs through sectioning, staining and examining them under the microscope.

From the findings of this study, the histopathological study of liver tissue of group 2 (diabetic group) showed atrophying hepatic architecture with wide spread macro and micro vesicular steatosis and areas of vacuolated hepatocytes all within the hepatic lobules. Histopathology of the liver tissues of group 3 animals showed severe cellular abnormalities such as atrophic hepatic architecture with wide spread clusters of balloon hepatodegeneration, macro and micro vesicular steatosis (red arrow) and areas of vacuolated hepatocytes all within the

hepatic lobules when compared to group 2. Also, the histopathology of the diabetic groups (4,5 and 6) which were treated with single and combined leaf extracts (200mg/kg and 100mg/kg) of *Gongronema latifolium* and *Ocimum gratissimum* showed a normal hepatic histo-structure with portal vein and bile duct within the portal area, well protected hepatocytes, and wide proliferating kupfer cells in the arrays of sinusoidal spaces within the hepatic lobules (Figure 4.0-6.0). Cell abnormalities such as liver tissue showing atrophying hepatic architecture with wide spread clusters of balloon hepatodegeneration and areas of macro and micro vesicular steatosis within the hepatic lobules was still observed in CCl₄ exposed group treated with both single (200mg/kg each) and combined (100mg/kg each) of *Gongronema latifolium* and *Ocimum gratissimum* (Figure 7.0 -9.0).

This study corresponds with most experimental studies (7, 31). Micro-vesicular and Macro-vesicular steatosis is due to the accumulation of fat in the liver vesicle resulting in the displacement of the liver cytoplasm (21). The mechanism behind these conditions is a disruption in lipid movement through the cell (42). This can be due to underlying conditions such as diabetes mellitus, hypertension and protein malnutrition (4). Toxins such as alcohols, carbon tetrachloride, aspirin and diphtheria toxin also interfere with cellular mechanisms involving lipid metabolism. Macro-vesicular steatosis is caused by excessive supply of lipids due to insulin resistance caused by hyperglycemia. Insulin resistance is due to the disruption of beta cell of the islet of Langerhans found in the pancreas (12). Micro-vesicular steatosis occurs due to the accumulation of small intracytoplasmic fat vacuoles within hepatocytes (35).

Nuclear vacuolation in hepatocyte is due to liver damage caused by diabetes mellitus and carbon tetrachloride. Insulin resistance in the liver causes hyperglycemia and further distortion of glucose metabolism(21). Accumulation of fats in the liver will cause the vacuolation of the hepatocytes. Vacuolation of hepatocytes by CCl₄ is due to the formation of free radicals which causes peroxidation as it enters the hepatocytes leading to liver disruption and damage in liver function (43).

Hepatodegeneration caused by CCl₄ is due to oxidative stress cause by the free radicals formed. CCl₄ damages the liver through reductive halogenation, covalent binding of free radicals, inhibition of protein synthesis, fat accumulation, calcium homeostasis loss, apoptosis and fibrosis (3). Kupper cell activation, lipid peroxidation, reactive aldehydes and nucleic acid

hypomethylationcouplewith proinflammatory mediator productions are also mechanisms by which CCl₄ damages the liver (38).

In diabetic groups treated with different doses of single and combined leaf extract of *Gongronema latifolium* and *Ocimum gratissimum*,it was seen that the liver structures were restored to normal. The potentiating effects of *Gongronema latifolium* and *Ocimum gratissimum* to restore the structure is due to its antioxidants and anti-inflammatory effects (9). The restoration of liver structures was more significant (P<0.05) in groups treated with combined leaf extracts compared to the ones treated with single leaf extract. This also explains the co-administrative effects of poly-herbal therapy. There was no noticeablechanges in the liver structure of experimental animals exposed to CCl₄ and treated with both combined and single leaf extracts of *Gongronema latifolium* and *Ocimum gratissimum*.

CONCLUSION

These findings hold clinical relevance, suggesting that *Gongronema latifolium* and *Ocimum gratissimum* may offer potential therapeutic benefits for individuals with diabetes, particularly in mitigating liver and kidney function abnormalities which are caused by toxic environmental exposures like CCl₄. However, further research is warranted to elucidate the underlying mechanisms and to translate these findings into clinical applications.

RECOMMENDATIONS

Based on the findings of this study, further advance research can be recommended in this area such as conductingmore in-depth investigations into the underlying mechanisms by which these plantsexert their potential protective effects on liver and kidney.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

It is not applicable.

ETHICS APPROVAL

Ethical clearance (number: UU_FBMSREC_2024_001) was obtained from the Health Research Ethics Committee of the University of Uyo.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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