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Research Paper

HEPATOPROTECTIVE EFFECT OF LEAF EXTRACTS OF *Newbouldia laevis* ON CARBONTETRACHLORIDE- INTOXICATED RATS

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Abstract

Hepatoprotective effect of leaf extracts of *Newbouldia laevis* were evaluated against carbon tetrachloride (CCl_4)-induced hepatotoxicity in *wister albino* rats using standard methods. Hepatorenal indices of the methanolic extracts were not significantly ($p<0.05$) different at doses of 400 to 2000mg/kg. The median lethal dose (LD_{50}) of the leaf extract was greater than 5000mg/kg. Histopathological studies indicated normal glomeruli, tubules and hepatocytes with mild focal area of fatty change at 2000mg/kg body weight of the extract. Significantly ($p<0.05$) increased levels of transaminases, 5' nucleotidase, alkaline phosphatase, bilirubin and cholesterol with decrease in total protein and albumin in CCl_4- intoxicated rats were observed. Normal levels of these parameters were found in rats treated with the extracts fractions (Hexane, Ethylacetate, Saturated butanol and Last water remaining methanol) and CCl_4. However, Ethylacetate fraction was the most active at lower dose (40mg/kg b.w) similar to the effect produced by silymarin and CCl_4. Hexane fraction showed lesser effect when compared with Ethylacetate but more effective when compared with Saturated butanol and Last water remaining methanol fractions. Significantly ($p<0.05$) depressed microsomal, 5' nucleotidase, aniline hydroxylase, glucose-6-phosphatase, catalase, proteins, glutathione and non-enzymic antioxidants levels were found, but significantly higher ($p<0.05$) levels of peroxide value, triglyceride and cholesterol were also observed in CCl_4 administered rats. Simultaneous administration of the extracts of *Newbouldia laevis* and CCl_4 showed reversed effects of CCl_4 on the activities of these endogenous parameters. The study suggests that hepatoprotective activity and mechanism of action exhibited by the extract against CCl_4-induced hepatic injury may be due to its ability to modulate microsomal drug-detoxifying system.

Key words: Hepatoprotective, mechanism of action, *Newbouldia laevis* leaves, Hepatorenal indices, Histopathological.

INTRODUCTION

Herbal medicines are considered to be a safe and useful approach for the treatment of chronic hepatopathy[1]. Xenobiotics detoxification is controlled mainly by the liver [2]. Plants constitute an important source of active natural products that differ widely in terms of their structure and

biological properties [2]. Plants played remarkable roles in the traditional medicine of various countries [3,4].More so, the increasing use of plants extracts in the food, cosmetic and pharmaceutical industries suggest that, in order to find active compounds, a systematic study of medicinal plants is very important [5].

The liver is highly involved in metabolic functions and is frequently a target for a vast amount of toxicants. Many studies have revealed that reactive oxygen species are correlated to the etiology of degenerative diseases, including some hepatopathies [6,7]. Reasons attributed to liver disease being a leading cause of medical emergency in Nigeria, includes poorly treated or untreated infective hepatitis, late presentation at hospitals for treatment, alcoholism and drug misuse [8].

The liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions [9]. In recent years, many researchers have examined the effects of plants used traditionally by indigenous healers to support treatment of liver diseases. There are no effective drugs that are available in modern medicine that confer protection to the liver against damage or help to regenerate hepatic cells [10, 11]. many plants species are known in medicine to be used for the treatment of liver diseases because of their hepatoprotectvie activities [12]. Plants and their products recommended for the treatment of liver diseases are documented in the literature such as *Ocimum gratissimum, Solanum nigrum, Balanites aegyptiaca, Khaya senegalensis,* and *Kolaviron* [12, 13,14] .There are claims that they offer tangible reliefs and most of their acclaimed efficacy is with the aim to offer significant reliefs [15].

The principal cause of carbon tetrachloride (CCl_4) induced hepatic damage is lipid peroxidation, and decreased activities of antioxidant enzymes and generation of free redicals [7,16]. The body system has an effective proper way to for preventing and neutralizing the free radical induced damage, this is done through the help of these enzymes, such as Superoxide Dismutase (SOD), catalase and Glutathione Peroxidase (GPX).These enzymes serve to defend the living system against reactive oxygen species (ROS) [17].

Antioxidants such as ascorbic acid, β-carotene and α-tocopherol offers non-enzymatic protection to the body [18,19,20,21] There are still exists a good number of plant species that are endowed with medicinal potentials, yet no studies have been done to provide empirical proof to support the acclaimed efficacy. Liver diseases are responsible for the increasing number of chemicals and environmental pollution [22]. Free radicals or oxidative injury are mostly responsible for most pathological conditions such as cancer, neurological disorder, arthritis, inflammation and liver diseases [23,24].

Newbouldia laevis (family, Bignoniaceae) is native to tropical Africa and grows from Guinea savannah to dense forests[25]. In Nigeria and Ghana, the stem bark, root and leaf extracts of *N. laevies* have been reported for its strong antibacterial potentials [26, 27, 28].

The protective effects of the methanolic leaves extracts fractions of *Newbouldia laevis* against carbontetrachloride induced toxicants have been previously reported [29], further studies on the Hexane, Ethylacetate, Butanol and Last water remaining methanol extracts fractions as well as the mechanism of action have not been studied. In this present research, the effects of the most potent ethylacetate fraction on the hepatic microsomal enzymes, protein, glutathione and non-enzymic antioxidants, lipid peroxidation, triglycerides and cholesterol were assessed to elucidate the anti-hepatotoxic effect of the active fraction of the leaf extract *in vivo.*

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used were of analytical grade.

Plant collection and identification

The leaves of the plant were collected from Ngaski local government area, Kebbi State, Nigeria. The plants were botanically authenticated at the Herbarium of the Botany unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

The leaf extracts were opened- air dried under shade, reduced into small pieces (using Pestle and Mortar) and was stored until required for use.

Animals

Albino rats weighing 200-300g of either sex were obtained from animal house, Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were kept in a well ventilated room under supervision in the animal house, with free access to feeds and tap-water *ad libitum.* They were kept in the same environment for two weeks to acclimatize.

Preparation of plant extracts

The small pieces of (200g) of the plant extracts were extracted with two litres of 95% methanol at room temperature overnight and filtered through Whatman No 1 filter paper. The filtrate was concentrated to dryness using rotary evaporator and the yield was calculated as (6.25% w/w). The extract was stored in closed container until required for reconstitution in distilled water (for oral administration). Part of the methanol residue was dissolved in water. The filtrate was partitioned with hexane, ethylacetate and butanol (saturated with water). The fractions obtained were screened for hepatoprotective and toxicological studies. Mechanism of action and antioxidant properties were conducted on the most potent fraction.

Phytochemical analysis

The methods of Harborne and others [30, 31, 32] were used. The extracts were evaluated for the presence of tannins, saponins, alkaloids, flavonoids, glycosides, cardiac glycosides, volatile oils, steroids, terpenoids, resins, balsams, saponin glycosides and flavonoid glycosides.

Acute toxicity studies (Determination of LD_{50})

After acclimatization period, the acute oral toxicity study was conducted in albino rats using Up and Down procedure of Organization for Economic and Cooperative Development [33]. Five randomly selected animals were used for limit test dose, 5000mg/kg body weight (b.w) of the extract was given in a single dose. Observation time was from first 8hrs, 24hrs and then up to 48hrs for signs of toxicity like tremors, aching, depression, weakness, food and water refusal, salivation and death if any, was recorded. If one (1), two (2) or none animal died, the Median lethal dose (LD_{50}) is greater than 5000mg/kg. However, if three (3) animals and above died the LD_{50} is less than 5000mg/kg.

Sub-chronic toxicity studies

Thirty six (36) albino (wistar) rats weighing 200-230g were divided into six (6) groups of six(6) rats each. The rats were orally administered graded doses of the LD_{50} of the methanolic leaf extracts (400, 800, 1200, 1600 and 2000 mg/kg), once daily for 28days respectively. While the control group (0.00mg/kg) received distilled water. The body weights of all the animals were taken before and weekly within the days of the treatment. After 28days, the animals were sacrificed and the collected blood was centrifuged to obtain sera for biochemical assays. The kidneys and liver of the animals were removed, weighed and preserved in 10% formalin and 0.9% of sodium chloride. Histopathological specimens of the organs were prepared as described by River *et al*.[34].

Histopathological studies

A portion of the liver and kidney tissues of the experimental rats were collected and immediately fixed in 10% formalin, dehydrated in graduated ethanol gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5µm) were prepared and then stained with Haematoxylin and Eosin (H-E) dye for photo microscopic observation under light microscope at high power magnifications (20 and x40) [35].

Experimental design for hepatoprotective activity

Induction of hepatotoxicity was done according to Guntupalli method [36] with some modifications. Experimental animals were divided into seven (7) groups of five (5) rats.

Group I: (Normal control), received daily dose of liquid paraffin (1m1/kg body Weight *Per os*) for five (5) days.

Group II: (Induced control), received 1ml/kg body weight of CCl_4 (30% in liquid paraffin, i.p.) from the 2^{nd} day to the 5^{th} day.

Group III: received silymarin a known antihepatotoxic drug (micro lab pharmaceutical company, India) at a dose of 100mg/kg *Per os*, for five (5) days and CCl_4 (i.p.) induction from the 2^{nd} day to the 5^{th} day.

Group IV, V, VI and VII: received the Hexane fraction of leaf extracts of *Newbouldia leavis* (40, 80, 120 and 160mg/kg *Per os*) respectively, for 5 days followed by CCl_4 (i.p.) induction on the 2nd day simultaneously to the 5th day.

The animals were sacrificed on the sixth day under ether anesthesia, blood and liver samples were collected.

The blood was allowed to clot and the sera were separated by centrifuging at 3000rpm for 5 minutes. The supernatant was collected using Pasteur pipette into the sample bottle.

This procedure was conducted for Ethylacetate and Saturated Butanol fractions to enable us select the most potent fraction(s).

The serum was used for biochemical estimations (Aspartate amino transferase, Alanine amino transferase, Alkaline phosphatase, Total protein, Total cholesterol, Albumin, Bilirubin). A 0.5g of the liver was perfused with cold 0.86% KCl, it was homogenized using 4.5 mL of 0.4mol/L phosphate buffer solution and centrifuged at 3500 r/min for 4 minutes. The supernatant was used for the estimation of enzymatic and non-enzymatic antioxidants. The other part of the liver was placed in 10% formaline for histopathological studies.

Experimental design for mechanism of action

This experimental design was carried out on the ethylacetate fraction (most active)**.** Induction of hepatotoxicity was done according to the method of Farombi [14] with some modifications. The animals were divided into five (5) groups, of six (6) rats each.

Group I; (Normal Control), received daily dose of liquid paraffin (1ml/ kg body weight *per os*) for fourteen days.

Group II; (Induced Control), received 1ml/kg body weight of CCl_4 (30% in liquid paraffin, i.p.), three (3) times a week for two (2) weeks.

Group III; received the leaf extracts of *Newbouldia Laevis* at 100mg/kg body weight *per os* for fourteen (14) days

Group IV and V; received the leaf extracts of *Newbouldia laevis* at 120 and 160mg/kg body weight respectively for two weeks followed by CCl_4 induction ((30% in liquid paraffin i.p.) three (3) times a week for two (2) weeks.

The animals were sacrificed, 24hr after the last day of induction. The liver was removed and rinsed in ice-cold 1.15% KCl, dried and weighed. It was homogenized in 4vols of ice-cold isotonic phosphate buffer, pH 7.4, and centrifuged at 9,000g for 20minutes to obtain the post-mitochondrial supernatant fraction. The supernatant was immediately frozen to dry ice. The following parameters were determined to postulate the antihepatotoxic effect of the most potent ethylacetate fraction.

Microsomal lipids/triglycerides estimation [37],Microsomal cholesterol estimation [38],Microsomal protein determination with folin phenol reagent [39],Aniline hydroxylase assay by spectrophotometric method [40], Microsomal 5'-nucleotidase (5'-NT), the method which employs inhibition by nickel ion was used [41, 42], Glucose 6-phosphatase activity [43].

Renal function test

Serum uric acid

Serum uric acid was determined by Fe (III) Reduction Detector method [44, 45]

Serum Urea

Serum urea was determined by Diacetyl monoxime Method using thiosemicarbazide [46].

Serum Creatinine

Colorimetric method with deproteinization was used [47].

Alanine Amino Transferase (ALT)

Serum alanine amino transferase and Aspartate Aminotransferase (AST) activitities were assayed using method of Reitman and Frankel [48].

Total Protein

Biuret method of Gomall was used [49].

Bilirubin (Total and direct)

Colorimetric method of total and conjugated bilirubin was used [50].

Alkaline Phosphatase (ALP)
Colorimetric method of Sood was employed [51].
Albumin (ALB)
The dye binding technique utilizing Bromocresol green (BCG) as modified by Doumas *et al* [52] was employed.
Triglycerides (TG)
Colorimetric method described by Tietz was used to determine the TG after enzymatic hydrolysis with lipases [53].
Total Cholesterol (CHL)
The method of Trinder was employed [54].
Assessment of antioxidant activity
The liver was perfused with 0.86% cold saline to completely remove the red blood cells. It was suspended in10% (w/v) ice-cold 0.1m phosphate buffer (pH 7.4). The liver was cut into small pieces, and some quantity weighed and homogenized. The homogenate was used for the estimation of enzymatic and non-enzymatic antioxidants.
Estimation of serum vitamin C and E
They were assayed using the method of Baker and Frank [55].
Catalase determination
This was done by the method of Beers and Sizer [56]

Reduced Glutathione (GSH) Estimation
It was estimated by the Method of Patterson and Lazarow [57].
Malondialdehyde (MDA)
Tissue supernatant (150µl) was diluted to 500µl with double deionized water. Two hundred and fifty (250 µl) of 1.34% thiobarbituric acid e was added to all the test tubes, followed by addition of an equal volume (250µl) of 4% trichloroacetic acid (TCA). The resulting mixture was shaken and incubated for 30 minutes in a hot steam water bath (temperature greater than 90^0C). The test tubes were allowed to cool to room temperature and the absorbance of the complex formed was read at 532nm [58, 59]. The absorbance was extrapolated from a standard curve generated by using a standard (1,1,3, 3-tetraethoxypropane). Results was expressed as nanomoles of MDA per milliliter of supernatant.
Statistical analysis
The data were represented in mean ± standard error of the mean. Results were analysed statistically by one way analysis of variance (ANOVA) using Instat Software, Benferoni multiple comparison test, Graph Pad Instat Software(San Diego, USA). A p-value less than 0.05 ($P < 0.05$) was considered significant.

RESULTS
Percentage yield of the ethylacetate leaf fraction was 30.75%W/W.. From Table 1, the result revealed the presence of alkaloids, saponins, glycosides, volatile oils, flavonoids aglycons, flavonoids glycosides, saponin glycosides, balsams, resins, triterpenoids, terpenoids. However, tannins, cardiac glycosides, anthraquinones and steroids were not detected.

Table 1: Phytochemical Constituents (qualitative) of Ethylacetate Fraction of Leaf extract of *Newbouldia laevis.*

ALK	SAP	TAN	GLY	VLO	FLA	FLG	CGS	SAG	ATO	BAL	RES	TRP	TER	STR
+++	+++	-	+++	+++	+++	+	-	+++	-	+++	+++	+++	+++	-

- =Absence, +=Presence in trace amount, +++ = Presence, ALK = Alkaloids, SAP = Saponins, TAN = Tannins
GLY = Glycosides, VLO = Volatile oils, FLA = Flavonoid aglycones, FLG = Flavonoid glycosides, CGS = Cardiac glycosides
SAG = Saponin glycosides, ATQ = Anthraquinones, BAL = Balsams, RES = Resins, TRP = Triterpenoids, TER = Terpenoids,

STR= Steroids, CYG = Cyanogenic glycosides

Acute toxicity study and behavioural effects

Oral administration of methanolic leaf extract of *Newbouldia laevis* at a single dose of 5000mg/kg body weight (b.w) produced no adverse behavioural changes such as excitement, restlessness, respiratory distress, slow movement, convulsions, or coma but there was death of one (1) animal (Table 3). The medium lethal dose (LD_{50}) is greater than 5000mg/k body weight (b.w).

Table 2: Effects of the Administration of Leaf Extracts of *Newbouldia laevis*

Dose (mg/kg)	Groups	No. of animals	No. of deaths
00	A	1	0
5000	B	1	0
5000	C	1	0
5000	D	1	0
5000	E	1	1
5000	F	1	0

Body Weight

The results of the mean body weights of the test animals are presented in table 3. There were increases in body weights of animals in all the groups. Percentage (%) weight of rats administered with 1600mg/kg b.w. (0.53%) was significantly ($p<0.05$) different when compared with the control group (4.82%). However, changes in the body weight of the rats were progressive.

Table 3. Mean Body Weights of Rats before and after Administration of Methanolic Extracts of *Newbouldial laevis*.

Dose (mg/kg)	Week 1	Week 4	%Weight Difference
00	199.80 ± 2.67	209.42 ± 3.33	4.82
400	169.71 ± 2.98	175.23 ± 4.28	3.25
800	180.81 ± 0.94	185.89 ± 1.00	2.81
1200	188.88 ± 1.48	193.34 ± 3.36	2.36
1600	192.49 ± 0.54	193.51 ± 1.69	0.53*
2000	197.98 ± 1.99	208.65 ± 4.35	5.39

Values are mean ± standard error of five replicates. * Significantly different ($P<0.05$) from the control using Instat soft ware, Benferoni compare all columns (San Diego, USA).

Table 4: Effect of Administration of Methanolic leaf Extract of *Newbouldia laevis* on the Weight of Organs.

Dose (mg/kg)	Kidney weight (g)	Liver weight (g)
0	0.95 ± 0.05	6.04 ± 0.31
400	1.01 ± 0.08	6.69 ± 0.61
800	0.83 ± 0.07	7.03 ± 0.54
1200	0.90 ± 0.02	6.73 ± 0.47
1600	0.98 ± 0.04	7.29 ± 0.41
2000	0.83± 0.04	8.65± 0.21*

Values are mean ± standard error of mean with five replications. *Significantly different (P<0.05) from the control using Instat soft ware, Benferoni compare all columns (San Diego, USA).

Table 4, showed the effects of the administration of methanolic leaf extracts of *Newbouldia laevis* on the weight of organs (kidney and liver). There were no significant (p>0.05) increases in kidney weight compared to the control group. There were also no significant (p>0.05) different in the weight of liver except group administered with 2000mg/kg body weight of the extract with mean weight of 8.65g. Table 5 showed the effects of the administration of leaf extracts of *Newbouldia laevis* on organ body index. There were no significant (p>0.05) difference in the organ body index in groups administered with 400-1600mg/kg with exception of 2000mg/kg (4.13%).

Table 5: Effects of the Administration of Leaf Extracts of *Newbouldia laevis* on Organ Body index (%).

Dose (mg/kg)	Kidney	Liver
0	0.46 ± 0.02	2.88 ± 0.14
400	0.58 ± 0.05	3.82 ± 0.24
800	0.45 ± 0.4	3.78 ± 0.30
1200	0.47 ± 0.02	3.48 ± 0.22
1600	0.51 ± 0.03	3.77 ± 0.22
2000	0.40 ± 0.02	4.15 ± 0.13*

Values are mean ± standard error of mean with five replications.
*Significantly different (P<0.05) from the control using Instat soft ware, Benferoni compare all pairs of columns (San Diego, USA).

Relative Body Weight

Table 6 showed the relative body weight of rats administered leaf extracts of *Newbouldia laevis*. There were no significant differences (p>0.05) in the relative body weight of all the groups when compared with control group. The relative body weight of the control group was 0.0045, while groups administered with the extracts of *N. laevis* were in the range of 0.0043 to 0.0053.

Table 6: Relative Body Weight of Rats Administered Leaf Extract of *Newbouldia laevis*

Dose (mgk^{-1})	Kidney/Body Weight Ratio
0.00	0.0045±0.000041
400	0.0050±0.000073
800	0.0042±0.000042
1200	0.0043±0.000071
1600	0.0053±0.00011
2000	0.0043±0.000083

Values are mean ± standard error of mean of five replications. All values are not significantly (P>0.05) different using Instat Soft ware, Benferoni multiple comparism, compare all pairs of columns (San Diego, USA).

Renal Function Indices

There were no significant difference (p>0.05) observed for the renal function indices (urea, uric acid and creatinine) (Table 7). These results have justified the safety of the extracts.

Liver Function Indices

The results of the effects of the administration of leaf extracts of *Newbouldia laevis* on rats after 28 days are presented in table 8. There were no significant difference in most of the liver function indices observed. However, there were increases (p>0.05) in the levels of Alkaline phosphatase, Aspartate amino transaminase, Alanine transaminase, and 5' Nucleotidase. Total bilirubin and Direct bilirubin showed no significant different (p>0.05), but there were slight increase in the serum of the tested rats administered 1600 to 2000mg/kg b.w of the extracts.

Table 7: Renal Function indices of Rats Administered with Methanolic Extract of *N. laevis*

Dose (mg/kg)	Urea (mg/l)	Uric acid (mg/dl)	Creatinine
0	25.54 ± 1.45	7.05 ± 0.75	8.14 ±2.47
400	25.16 ± 2.32	5.42 ± 0.99	5.58 ± 0.3
800	24.98 ± 0.91	4.52 ± 0.14	5.91 ±2.76
1200	41.17 ± 9.47	3.85 ± 0.17	6.11 ±0.99
1600	43.43 ± 9.47	3.53 ± 1.14	8.81 ±0.93
2000	45.68 ± 5.11	3.37 ± 1.21	12.99 ±2.8

Values are mean ± standard deviation of five replications. All values are not significantly different (P>0.05) when compare with control group using using Instat Soft ware, Benferoni compare all pairs of columns (San Diego, USA).

Table 8: Liver Function indices of Rat Administered with Methanol Extract of *Newbouldia laevis*

Dose (mg/kg)	ALP	ALT	AST	ALB	TP	TB	Direct Bilirubin	5-Nucleotidase
0	105.11 ± 10.33	14.55 ± 2.2	14.90 ± 2.58	2.30 ± 0.16	8.27 ± 0.41	2.59 ± 0.52	0.72 ± 0.18	29.37 ±1.78
400	113.16 ± 16.82	17.21 ±3.14	9.87 ± 1.31	2.40 ±0.24	9.42 ± 0.58	3.03 ± 0.32	0.51 ± 0.05	30.63 ± 6.50
800	132.25 ±20.79	28.23 ± 6.75	6.53 ±0.26	2.22 ± 0.02	10.06 ± 1.32	3.21 ± 0.05	0.55 ± 0.12	33.54 ± 3.18
1200	137.80 ±26.09	34.04 ± 13.32	7.77 ± 0.83	2.20 ± 0.05	12.25 ± 1.19	3.32 ± 1.92	0.44 ± 0.17	34.88 ±4.61
1600	140.21 ± 19.60	36.32 ±5.94	10.85 ± 3.45	2.11 ± 0.17	12.26 ± 0.70	3.41 ± 0.55	1.04 ± 0.10	35.84 ± 3.16
2000	144.67 ±7.81	28.07 ± 5.13	15.84 ±5.14	1.93 ± 0.03	10.83± 1.38	3.51 ± 0.67	1.36 ± 0.37	38.21 ±5.29

Values are mean ± standard error of five replications. All values are statistically not significant (P>0.05) compared with control using Instat Software, Benferoni compare all pairs of columns (San Diego, USA). ALB- Albumin, ALP-Alkaline phosphatase, AST- Aspartate amino transferase, ALT- Aniline amino transferase, TP- Total protein, TB- Total bilirubin, DB-Direct bilirubin, 5'NT- 5'Nucleotidase

Histopathological Results

The histopathological results of kidney of rats administered 400, 800, 1200, 1600 and 2000mg/kg of the methanol extract show normal glomeruli and tubules. However, Normal histology of hepatocytes were seen in the liver of rats administered 400, 800, 1200mg/kg but with mild and severe focal areas of fatty changes were seen in the liver of group administered with 1600 and 2000mg/kg body weight (b.w) of the leaf extracts for 28 days (Table 9).

Table 9: Histopathological Results of Kidney and Liver of Rats Administered Leaf Extracts of *Newbouldia laevis*.

Dose (mgk^{-1})	Kidney	Liver
Control	Normal glomeruli and tubule seen	Normal hepatocytes seen
400	Nornal glomeruli and tubules seen	Normal hepatocytes seen
800	Normal glomeruli and tubules seen	Normal hepatocytes seen
1200	Normal glomeruli and tubules seen	Normal hepatocytes seen
1600	Normal glomeruli and tubules seen	Normal hepatocytes with mild focal area of fatty changes
2000	Normal glomeruli and tubules seen	Normal hepatocytes with mild focal area of fatty change

Histopathological Observation

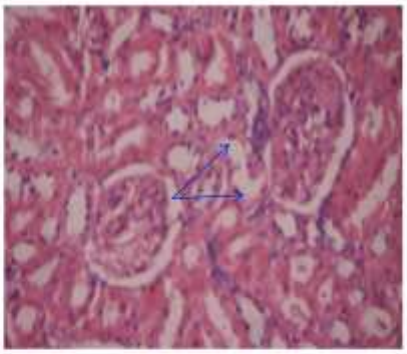

Plate 1: Photomicrograph of normal control rat showing normal tubules, glomeruli and (blue arrow) (Mag X 20)

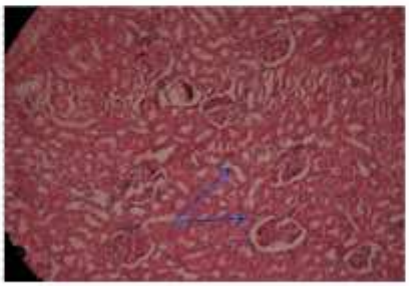

Plate 2: Photomicrograph of kidney of rat administered 400mg/kg^{-1} of methanolic leaf extracts showing normal glomeruli, tubules and (blue arrow) (Mag: x 20)

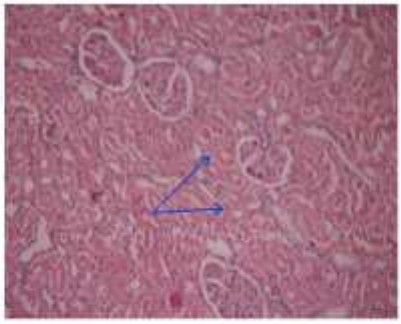

Plate 3: Photomicrograph of kidney of rat administered 300mg kg^{-1} methanolic leaf extract, showing normal glomeruli, tubules and (blue arrow) (Magx40)

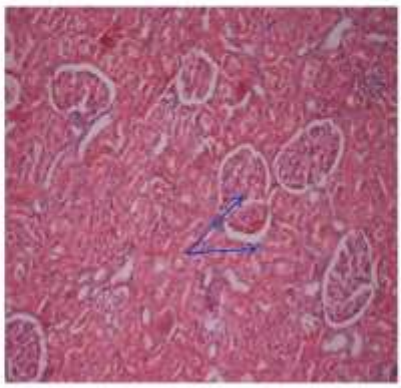

Plate 4: Photomicrograph of kidney of rat administered 1200mg kg^{-1} of methanolic leaf extract, showing normal glomeruli, tubules and (blue arrow) (Magx40)

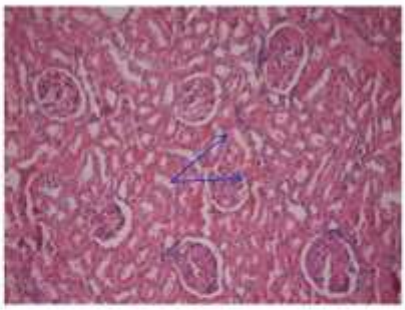

Plate 5: Photomicrograph of kidney of rat administered 1400mg kg^{-1} of the methanolic leaf extract, Showing normal glomeruli and tubules (Magx 20)

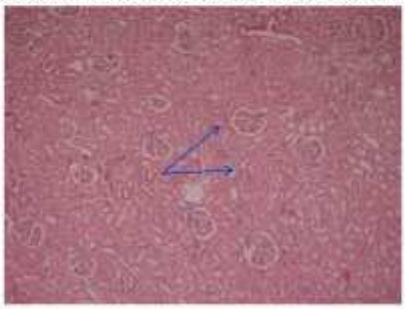

Plate 6: Photomicrograph of kidney of rat administered 2000mg kg^{-1} of the methanolic leaf extract, Showing normal glomeruli and tubules (Magx 20)

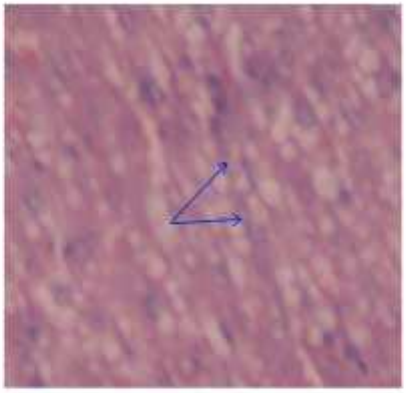

Plate 7: Photomicrograph of liver of normal (control) rat showing normal hepatocytes and (blue arrow) (Mag X 20)

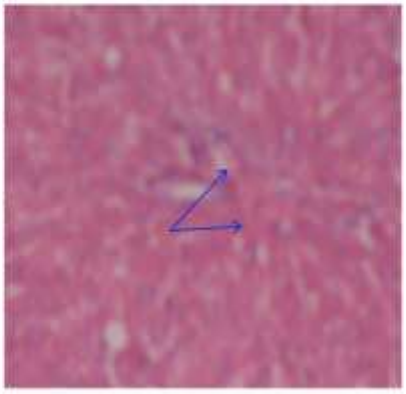

Plate 8: Photomicrograph of liver of rat administered 400mg kg^{-1} methanolic leaf extract showing normal portal tract and (blue arrow) (Mag X 20)

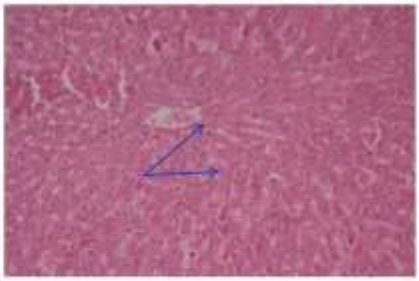

Plate 9: Photomicrograph of liver of rat treated with 800mg kg^{-1} of the methanolic leaf extract showing normal hepatocyte and (blue arrow) (Mag X 20)

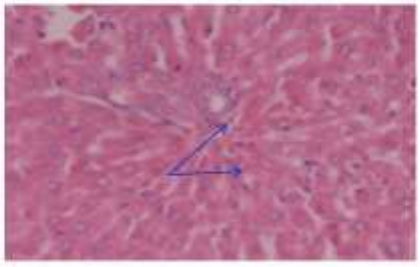

Plate 10: Photomicrograph of liver of rat treated with 1200mg kg^{-1} of the methanolic leaf extracts showing normal but with mild focal area of fatty change and (blue arrow) (Mag x 20)

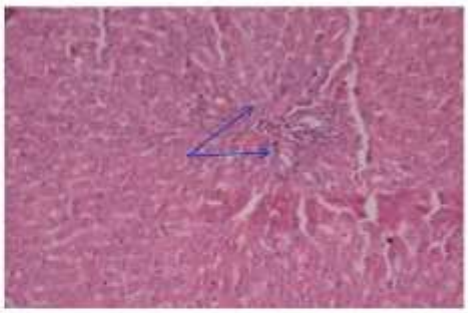

Plate 11: Photomicrograph of liver of rat treated with 1000mg kg^{-1} of the methanolic leaf extracts showing slight distortion of hepatic and focal areas fatty change and (blue arrow) (Mag x 20)

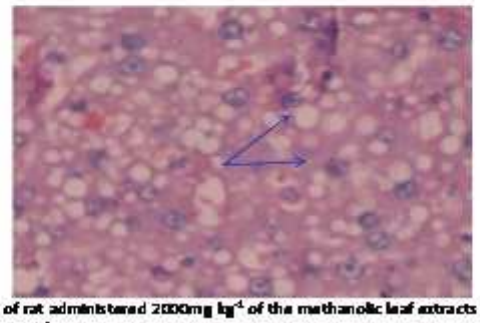

Plate 12: Photomicrograph of liver of rat administered 2000mg kg^{-1} of the methanolic leaf extracts showing severe fatty change and distortion of hepatic portal and (blue arrow) (Mag x 20)

Groups of rats administered with carbon tetrachloride exhibited significant ($p<0.05$) increase in the activities of ALP, AST, ALT, 5NT, TB and DB when compared to normal control rats. However, oral administration of the extract fractions (Hexane, Ethylacetate, Saturated butanol and Last water remaining methanol) of the plant (40, 80, 120 and 160mg/kg body weight) and silymarin and CCl_4 showed significant ($p<0.05$) decrease in serum enzymes activities. Ethylacetate extract fraction was the most effective at lower dose similar to the effects produced by silymarin and CCl_4 animal treated groups. Hexane extract fraction and CCl_4 on serum enzyme markers, showed lesser effects when compared with the Ethylacetate fraction and silymarin treated groups. The effects of Saturated butanol and Last water remaining extracts on the serum enzymatic markers were also lesser than Ethylacetate and Hexane extracts fractions.

Table 10: Liver Function indices of Rats Administered with Carbon tetrachloride and Hexane Leaf Extract Fraction of *Newbouldia laevis*

Groups	ALP	AST	ALB	ALT	TP	TB	Direct Bilirubin	5-Nucleotidase
Group I	87.78 ± 0.500	17.29 ± 0.42	3.60 ± 0.08	16.63 ± 0.18	8.69 ± 0.08	10.43 ± 0.20	9.86 ± 0.16	23.44 ± 0.18
Group II	265.78 ± 4.07^{x}	37.02 ± 0.33^{x}	1.88 ± 0.03^{x}	42.15 ± 0.90^{x}	5.69 ± 0.17^{x}	37.62 ± 0.83^{x}	18.28 ± 0.20^{x}	106.91 ± 1.71^{x}
Group III	90.92 ± 0.76^{qy}	17.07 ± 0.22^{qy}	3.45 ± 0.15^{qy}	16.28 ± 0.29^{qy}	8.89 ± 0.16^{qy}	14.88 ± 0.29^{qy}	10.30 ± 0.37^{qy}	28.66 ± 0.68^{xy}
Group IV	89.43 ± 0.43^{qyz}	$16.13 \pm 0.13^{xy\alpha}$	3.61 ± 0.08^{qyz}	$16.34 \pm 0.30^{qy\alpha}$	$9.08 \pm 0.13^{qy\alpha}$	12.83 ± 0.44^{xyz}	11.48 ± 0.10^{xyz}	21.01 ± 0.44^{qyz}
Group V	89.97 ± 0.42^{qyz}	$16.53 \pm 0.17^{qy\alpha}$	$3.63 \pm 0.17^{qy\alpha}$	$15.86 \pm 0.26^{qy\alpha}$	$9.01 \pm 0.14^{qy\alpha}$	12.80 ± 0.42^{xyz}	11.82 ± 0.14^{xyz}	22.37 ± 0.43^{qyz}
Group VI	$98.48 \pm 2.38^{xy\alpha}$	$16.73 \pm 0.11^{qy\alpha}$	$2.51 \pm 0.12^{xy\alpha}$	$16.21 \pm 0.09^{qy\alpha}$	$7.72^{c} \pm 0.08^{xy\alpha}$	$15.41 \pm 0.28^{xy\alpha}$	12.01 ± 0.08^{xyz}	23.08 ± 0.37^{qyz}
GroupVII	$99.27 \pm 2.48^{xy\alpha}$	$17.43 \pm 0.15^{qy\alpha}$	$2.33 \pm 0.10^{xy\alpha}$	$16.42 \pm 0.12^{qy\alpha}$	$6.46 \pm 0.14^{xy\alpha}$	$17.41 \pm 0.21^{xy\alpha}$	12.53 ± 0.06^{xyz}	23.30 ± 0.41^{qyz}

Values are mean ± standard error of five replicates, x= vs I: $p<0.05$; y= vs II: $p<0.05$; z= vs III :$p<0.05$; q= vs I: $p>0.05$; p= II $p>0.05$, α= vs III: $p>0.05$, using analysis of variance (ANOVA), Benferroni compare all pairs of columns, Instat Graph Pad Software (SAN Diego USA). ALB-Albumin, ALP-Alkaline phosphatase, AST- Aspartate amino transferase, ALT- Aniline amino transferase, TP- Total protein, TB- Total bilirubin, DB-Direct bilirubin, 5'NT- 5' Nucleotidase Group I:Liquid paraffin treated group, Group II:30% CCl_4 in liquid paraffin, Group III: Silymarin + CCl_4, Group IV: 40 mg/kg of the extract + CCl_4 , Group V: 80 mg/kg + CCl_4Group, VI: 120 mg/kg + CCl_4, Group VII: 160 mg/kg + CCl_4

Table 11: Liver Function indices of Rats Administered with Carbon tetrachloride and Ethylacetate Leaf Extract Fraction of *Newbouldia laevis*

Groups	ALP	AST	ALB	ALT	TP	TB	Direct Bilirubin	5'-Nucleotidase
Group I	60.34 ±0.99	20.16 ±0.47	2.96 ±0.12	18.71±0.11	9.23 ± 0.29	15.14 ± 0.52	13.53 ± 0.34	24.10 ± 0.99
Group II	252.60 ±0.34^{x}	40.93 ±0.49^{x}	0.59 ±0.22^{x}	39.02±0.42^{x}	4.74 ± 0.13^{x}	32.78 ± 0.52^{x}	19.98 ± 0.58^{x}	95.42 ± 0.65^{x}
Group III	111.50 ±0.77^{xy}	19.58 ± 0.43^{qy}	2.23 ±0.17^{qy}	19.43±0.68^{qy}	8.14 ± 0.47^{qy}	16.92 ± 0.53^{qy}	13.37 ± 0.41^{qy}	23.52 ± 0.78^{qy}
Group IV	64.76 ±2.46^{qyz}	20.88 ± 0.63^{qyz}	2.39 ±0.17$^{q\alpha z}$	17.07±0.48^{qyz}	9.62 ± 0.39$^{q\alpha y}$	14.30 ± 0.13^{qyz}	13.42 ± 0.41^{qyz}	25.76 ± 0.25$^{q\alpha y}$
Group V	65.69 ±1.64^{qyz}	21.80 ± 0.75^{qyz}	2.79 ± 0.04$^{q\alpha z}$	16.38±0.70^{qyz}	9.05 ± 0.09$^{q\alpha y}$	16.36 ± 0.24^{xyz}	13.86 ± 0.19^{qyz}	23.51 ±0.58$^{q\alpha y}$
Group VI	69.57 ±0.45^{xyz}	23.43 ± 0.31^{qyz}	2.69 ± 0.36$^{q\alpha z}$	18.03±0.53^{qyz}	9.20 ± 0.07$^{q\alpha y}$	16.65 ± 0.24^{qyz}	13.41 ± 0.21^{qyz}	24.46 ± 0.35$^{q\alpha y}$
GroupVII	71.43 ±1.52^{xyz}	23.57 ± 0.38^{qyz}	2.57 ± 0.04$^{q\alpha z}$	19.15 ± 0.26^{qyz}	9.04 ± 0.02$^{q\alpha y}$	17.50 ± 0.46^{xyz}	13.53 ± 0.21^{qyz}	24.95 ± 0.50$^{q\alpha y}$

Values are mean ± standard error of five replications, x= vs I: $p<0.05$; y= vs II: $p<0.05$; q= vs I: $p>0.05$; p= vs II $p>0.05$, using analysis of variance (ANOVA). Benferroni compare all pairs of columns Instat Graph Pad Software (SAN Diego USA)., ALB-Albumin,ALP-Alkaline phosphatase, AST-Aspartate amino transferase, ALT- Aniline amino transferase, TP- Total protein, TB- Total bilirubin, DB-Direct bilirubin, 5'NT- 5'Nucleotidase. Group I:Liquid paraffin treated group, Group II:30% CCl_4 in liquid paraffin, Group III: Silymarin + CCl_4, Group IV: 40 mg/kg of the extract + CCl_4 , Group V: 80 mg/kg + CCl_4Group, VI: 120 mg/kg + CCl_4, Group VII: 160 mg/kg + CCl_4

Table 12 : Liver Function Indices of Rats Administered with Carbon tetrachloride and Saturated Butanol Leaf Extract Fraction of *Newbouldia laevis*

Groups	ALP	AST	ALB	ALT	TP	TB	Direct Bilirubin	5'-Nucleotidase
Group I	96.52 ± 1.50	19.86 ± 0.61	3.43 ± 0.11	18.71 ± 0.54	9.42 ± 0.18	**14.04 ± 0.50**	**12.60 ± 0.62**	47.64 ± 0.94
Group II	286.73 ± 0.87^{x}	42.40 ± 0.75^{x}	1.03 ± 0.08^{x}	47.64 ± 0.44^{x}	4.25 ± 0.17^{x}	**48.49 ± 0.40^{x}**	**21.30 ± 0.22^{x}**	100.11 ± 0.11^{x}
Group III	100.02 ± 1.85^{qy}	20.07 ± 0.19^{qy}	3.38 ± 0.06^{qy}	18.52 ± 0.09^{qy}	8.62 ± 0.10^{qy}	**19.33 ± 0.21^{qy}**	**12.24 ± 0.69^{qy}**	48.09 ± 0.50^{qy}
Group IV	104.75 ± 2.5^{xyz}	29.40 ± 0.80^{xyz}	3.28 ± 0.11$^{qy\alpha}$	22.05 ± 0.56$^{qy\alpha}$	6.33 ± 0.35^{xyz}	16.88 ± 0.34^{xyz}	15.30 ± 0.51^{qyz}	56.50 ± 0.16^{xyz}
Group V	115.31 ± 1.01^{xyz}	32.95 ± 0.56^{xyz}	3.05 ± 0.07^{qyz}	24.89 ± 0.31^{xyz}	4.84 ± 0.20^{xpz}	18.47 ± 0.36^{xyz}	16.21 ± 0.51^{xyz}	52.31 ± 0.28^{xyz}
Group VI	117.28 ± 0.99^{xyz}	35.99 ± 0.65^{xyz}	2.71 ± 0.17^{xyz}	31.99^{c} ± 2.22^{xyz}	3.91 ± 0.43^{xpz}	34.90^{c} ± 1.39^{xyz}	17.75 ± 0.23$^{x\alpha y}$	78.56 ± 1.45^{xyz}
GroupVII	120.77 ± 0.33$^{xy\alpha}$	36.45 ± 0.46^{xyz}	2.40 ± 0.19^{xyz}	40.40 ±0.77^{xyz}	3.83 ± 0.24^{xpz}	37.89 ± 0.50^{xyz}	18.49 ± 0.05$^{x\alpha y}$	81.08 ± 1.53^{xyz}

Values are mean ± standard error of five replicates, x= vs I: $p<0.05$; y= vs II: $p<0.05$; q= vs I: $p>0.05$; p= vs II $p>0.05$, using analysis of variance (ANOVA). Benferroni compare all pairs of columns, Instat Graph Pad Software (SAN Diego USA). ALB- Albumin, ALP-Alkaline phosphatase, AST- Aspartate amino transferase, ALT- Aniline amino transferase, TP- Total protein, TB- Total bilirubin, DB-Direct bilirubin, 5'NT- 5'Nucleotidase. Group I:Liquid paraffin treated group, Group II:30% CCl_4 in liquid paraffin, Group III: Silymarin + CCl_4, Group IV: 40 mg/kg of the extract + CCl_4 , Group V: 80 mg/kg + CCl_4Group, VI: 120 mg/kg + CCl_4, Group VII: 160 mg/kg + CCl_4

Table 13: Liver Function indices of Rat Administered with Carbon tetrachloride and Last-water Remaining Leaf Fraction of *Newbouldia laevis*

Groups	ALP	AST	ALB	ALT	TP	TB	Direct Bilirubin	5'-Nucleotidase
Group I	94.30 ± 0.34	15.53 ± 0.16	5.12 ± 0.28	15.13 ± 0.28	7.38 ± 0.20	21.17 ± 0.32	17.54 ± 0.15	38.51 ± 0.63
Group II	217.77 ± 0.54^{x}	40.51 ± 0.26^{x}	2.51 ± 0.16^{x}	38.38 ± 0.27^{x}	2.52 ± 0.13^{x}	29.94 ± 0.39^{x}	22.24 ± 0.43^{x}	117.60 ± 1.06^{x}
Group III	89.64 ± 0.17^{qy}	15.33 ± 0.32^{qy}	4.96 ± 0.04^{qy}	16.31 ± 0.20^{qy}	6.83 ± 0.30^{qy}	19.19 ± 0.36^{qy}	16.26 ± 0.21^{qy}	$39.67 \pm 0.23^{qy\alpha}$
Group IV	107.80 ± 0.99^{xyz}	29.97 ± 0.10^{xyz}	2.65 ± 0.10^{xpz}	28.27 ± 0.25^{xyz}	4.47 ± 0.20^{xyz}	$20.55 \pm 0.21^{qy\alpha}$	18.33 ± 0.28^{qyz}	42.55 ± 1.75^{qy}
Group V	122.91 ± 3.05^{xyz}	37.55 ± 1.03^{xpz}	$2.37 \pm 0.09^{xp\alpha}$	36.25 ± 1.06^{xpz}	3.38 ± 0.06^{xpz}	21.41 ± 0.34^{qyz}	19.92 ± 0.6^{xyz}	63.61 ± 1.83^{xyz}
Group VI	126.02 ± 1.71^{xyz}	42.68 ± 0.92^{xpz}	$2.15 \pm 0.05^{xp\alpha}$	43.62 ± 0.88^{xyz}	2.69 ± 0.15^{xpz}	33.25 ± 0.76^{xyz}	23.22 ± 0.20^{xpz}	86.26 ± 1.74^{xyz}
Group VII	175.33 ± 2.83^{xyz}	47.04 ± 0.33^{xyz}	$1.79 \pm 0.15^{xy\alpha}$	49.83 ± 0.58^{xyz}	2.47 ± 0.20^{xpz}	41.71 ± 0.88^{xyz}	23.85 ± 0.14^{xyz}	99.18 ± 0.36^{xyz}

Values are mean ± standard error of five replicates, x= vs I: $p<0.05$; y= vs II: $p<0.05$; q= vs I: $p>0.05$; p= vs II $p>0.05$, using analysis of variance (ANOVA). Benferroni compare all pairs of columns Instat Graph Pad Software (SAN Diego USA). ALB- Albumin, ALP-Alkaline phosphatase, AST-Aspartate amino transferase, ALT- Aniline amino transferase, TP- Total protein, TB- Total bilirubin, DB-Direct bilirubin, 5'NT- 5'Nucleotidase. Group I:Liquid paraffin treated group, Group II:30% CCl_4 in liquid paraffin, Group III: Silymarin + CCl_4, Group IV: 40 mg/kg of the extract + CCl_4 , Group V: 80 mg/kg + CCl_4Group, VI: 120 mg/kg + CCl_4, Group VII: 160 mg/kg + CCl_4

Effects of Ethylacetate of leaf extract fraction *Newbouldia laevis* on liver enzymatic and non enzymatic antioxidants in CCl_4 induced hepatic injury in rats was assessed in liver homogenate by assesing Vitamins C and E, Catalase, Reduced glutathione and Lipid peroxides (Table 14). Increased ($p<0.05$) levels of Vitamins C and E, Catalase and reduced glutathione were observed when compared with the CCl_4 treated group. There were high levels of lipid peroxide value in CCl_4 treated group but with minimal levels in all the Ethylacetate fraction and CCl_4 treated groups. Effects of Ethylacetate extract fraction were evaluated on microsomal Triglycerides, Cholesterol, Protein, 5'Nucleotidase, Glucose-6-phoshatase and Aniline hydroxylase (Table 15). Reduced ($p<0.05$) levels of Triglycerides and Cholesterol were observed when compared with the CCl_4. Reduction ($p<0.05$) in Total protein were also observed in CCl_4 group when compared with group administered Ethylacetate and CCl_4. However, decrease in 5' Nuccleotidase, Glucose-6-phosphatase and Aniline hydroxylase were observed in group treated with CCl_4 only.

Table 14: Effect of Ethylacetate Leaf Extract Fraction of *Newbouldia laevis* on Liver Enzymatic and Non-enzymatic Antioxidants in Carbon tetrachloride- induced Hepatic Injury in Rats.

Treatment Groups	Vitamin C (mg/dl)	Vitamin E (mg/dl)	Catalase	Reduced Glutathione	Lipid Peroxides
Group I	54.44 ± 0.38	5.37 ± 0.15	2.27 ± 0.09	208.86 ± 0.58	0.30 ± 0.01
Group II	32.17 ± 0.27^{x}	0.73 ± 0.01^{x}	1.62 ± 0.11^{x}	117.09 ± 0.28^{x}	2.36 ± 0.08^{x}
Group III	54.28 ± 0.55^{qy}	5.13 ± 0.06^{qy}	2.46 ± 0.06^{qy}	205.93 ± 1.36^{qy}	0.25 ± 0.01^{qy}
Group IV	55.91 ± 0.99^{qy}	4.99 ± 0.11^{qy}	2.14 ± 0.06^{qy}	203.62 ± 0.26^{xy}	0.15 ± 0.01^{qy}
Group V	63.57 ± 0.28^{xy}	2.32 ± 0.11^{xy}	2.17 ± 0.08^{qy}	195.76 ± 0.46^{xy}	0.11 ± 0.01^{xy}

Values are mean ± standard error of the mean of four replicates, x= vs I: $p<0.05$; y= vs II: $p<0.05$; q= vs I: $p>0.05$; p= vs II $p>0.05$, using analysis of variance (ANOVA). Benferroni compare all pairs of columns Instat Graph Pad Software (SAN Diego USA).Group I: Liquid paraffin treated group, Group II: 30% CCl_4,Group III: 100 mg/kg of extract + CCl_4, Group IV:120 mg/kg of extract + CCl_4
Group V: 160 mg/kg of extract + CCl_4

Table 15: Effect of Ethylacetate Extract Fraction of Leaves of *N. laevis* on the Activities of Microsomal: Triglyceride, Cholesterol, Protein, 5'-Nucleotidase, Glucose-6-Phosphatase and Aniline Hydroxylase in Carbon tetrachloride treated Rats.

Treatment

Groups	Triglycerides	Cholesterol	Total Protein	5'-Nucleotidase	Glucose-6-Phosphatase	Aniline Hydroxylase
Group I	2.31 ± 0.12	3.21 ± 0.08	2.80 ± 0.02	25.02 ± 0.24	1.78 ± 0.02	2.16 ± 0.08
Group II	6.50 ± 0.17^{x}	6.97 ± 0.26^{x}	0.28 ± 0.02^{x}	20.48 ± 0.10^{x}	0.81 ± 0.05^{x}	0.61 ± 0.13^{x}
Group III	2.21 ± 0.06^{qy}	3.16 ± 0.03^{qy}	2.61 ± 0.17^{qy}	25.41 ± 0.41^{qy}	1.89 ± 0.04^{qy}	2.37 ± 0.05^{qy}
Group IV	2.71 ± 0.05^{qy}	3.77 ± 0.03^{qy}	2.44 ± 0.08^{qy}	25.72 ± 0.23^{qy}	2.03 ± 0.05^{xy}	2.69 ± 0.04^{xy}
Group V	4.71 ± 0.14^{xy}	4.51 ± 0.09^{xy}	1.75 ± 0.09^{xy}	26.01 ± 0.10^{qy}	2.38 ± 0.04^{xy}	2.92 ± 0.03^{xy}

Values are mean ± standard error of the mean of four replicates, x= vs I: $p<0.05$; y= vs II: $p<0.05$; q= vs I: $p>0.05$; p= vs II $p>0.05$, using analysis of variance (ANOVA). Benferroni compare all pairs of columns Instat Graph Pad Software (SAN Diego USA). .Group I: Liquid paraffin treated group, Group II: 30% CCl_4,Group III: 100 mg/kg of extract + CCl_4, Group IV:120 mg/kg of extract + CCl_4, Group V: 160 mg/kg of extract + CCl_4

DISCUSSION

The median lethal dosage (LD_{50}) of the aqueous leaves extracts was greater than 5000mg/kg body weight (b.w). There was slight increase ($p>0.05$) in the body weight of the rats administered with methanol extract (Table 3). The increase body weight compared with the control group might be as a result of the weight of some of the individual organs and not a sign of toxicity of the plant extract. A significant ($p<0.05$) percentage weight (0.53%) in the group administered 1600mg/kg b.w. of the extract was observed and might be due to the accumulation of antinutritional agents in the extracts [60]. The non significant organ and organ body index and kidney body weight ratio implies that the extract is safe at these concentrations (400, 800, 1200, 1600 and 2000mg/kg) (Table 4, 5 and 6).

The phytochemicals detected may contribute to the medicinal uses of the plant and have been previously reported to have some medicinal ingredients which supports its usage [61,62].

There were no significant differences ($p>0.05$) observed for the renal and hepatic function indices (Tables 7 and 8). This has justified the safety of the extracts with efficacy at lower doses. It is probable that extract at the concentrations employed may suppress the activities of the liver function enzymes by preventing damages to the liver which in turn obstructs the influx of the enzymes into the serum, as observed in table 8. Albumin and total protein in the serum were not significantly ($p>0.05$) different when compared with the control, an evidence of synthetic functions of proteins in order to replenish the lost. Gradual increase in the level of total bilirubin and direct bilirubin at higher concentration of the extract (1600-2000mg/kg), may indicate impaired excretory function of the liver with concomitant increase in the bile production.

Histopathology of the liver and kidney revealed normal glomeruli, tubules and hepatocyte but there was mild and severe fatty changes in the liver of rats treated with highest dose of the extract. These changes might be as a result of both the kidney and liver as the major sites for xenobiotics degradation, excretions and other biotransformation. The liver might have been exposed to certain substances present in the extracts at higher concentration.

Liver injury inflicted by CCl_4 resulted a significant ($P<0.05$) rise in marker enzymes, ALT, AST, ALP and 5'-Nucleotidase but a decrease in the level of total proteins and albumin were observed as reported previously (10,11,112 and 13).

It is obvious from the results, that the leaf extracts demonstrated dose-dependent hepatoprotection, but with the highest activity at the lowest concentration ($40mgkg^{-1}$). Thus, administration of ethylacetate extract and CCl_4 has mitigated the toxic effect of CCl_4 by maintaining the activity of the liver marker enzymes and non-enzymes parameters. The ethylacetate fraction was the most effective and at lower dose similar to the effects produced by silymarins and CCl_4 group. However, Hexane extract fraction and CCl_4 on serum enzyme marker showed lesser effect when compared with the Ethylacetate extract fraction and silymarin treated group. Total and direct bilirubin were increased as the case may be in CCl_4 and silymarin treated groups, but were moderately reduced in the extracts treated group.

The effects of saturated butanol and last water remaining extracts on the serum liver enzymatic markers were also lesser than ethylacetate and hexane extract fractions. It is clear from the result that ethylacetate extracts has better hepatoprotective ability when compared with other extracts: hexane, saturated butanol and last water remaining methanol fractions. The efficacy of this extract may be due to the presence of bioactive components responsible for stabilizing the effects emanating from CCl_4.

The study revealed significant increases in the activities of ALP, AST, ALT, TBL, DBL, and 5'NT with decrease in TP and ALB on exposure to CCl_4, indicating hepatocellular injury. Thus, administration of *N. laevis* leaf extract fractions and CCl_4 at different doses regulates the abnormal increase in the level of these enzymes; as increase in the proteins level indicate hepatoprotective activity. Induction of protein synthesis speed up the regeneration and production of liver cells [63]. There was spontaneous recovery towards normalization like that of silymarin and CCl_4 treatment.

The hepatotoxicity of CCl_4 result from its reductive dehalogenation by cytochrome P-$_{450}$ into the trichloromethyl free radical [64]. This radical and the corresponding peroxy radical create lipid radicals, thereby initiating a process of lipid peroxidation. Peroxidation is well known to decrease activities of enzymes associated with membranes. In this study, the results (Table 15), revealed decreased ($p<0.05$) microsomal activity of Glucose-6-phosphatase and Aniline hydroxylase in CCl_4-pretreated group. Previous studies by [65;66,67] showed that there are usually decrease in the activities of membrane associated enzymes, Glucose-6-phosphatase and Cytochrome P-$_{450}$ (aniline hydroxylase been a member of this family) in lipid peroxidation process. According to Hassan *et al*.[29] the level of lipids peroxidation is a measure of membrane damage and alteration in both structure and function of cellular membranes. Significant ($p<0.05$) rise of lipid peroxidation and triglycerides in the liver microsome of rats treated with CCl_4 were observed. Increase in MDA might be as a result of damage to tissues and failure of antioxidants defense mechanisms to prevent the formation of excessive free radicals [68], this defensive failure may be applicable to triglycerides being a storage form of lipid. Decrease aniline hydroxylase, as observed in CCl_4 group, support the investigation carried out on the effects of CCl_4 induction on cyt-P-$_{450}$ hydroxylating and oxidizing enzyme system by Letteron *et al*. and Kumaravelu *et al*. [69,70]. It is possible that the biological significance of this plant extracts especially the ethylacetate extract, is in the activation of aniline hydroxylase both in phase I and II metabolism [71]. The active components of the extract of *N. laevis* may regulate aniline hydroxylase in mixed function oxidase systems, which are responsible for removal of xenobiotics and catalyzing transformation of toxic aniline to a non-toxic derivatives, p-aminophenol.[72]

The results, revealed decrease in 5'-Nucleotidase and glucose-6-phosphatase, a typical 'microsomal marker enzyme'. Decrease in the activities of 'marker enzyme' may be probably due to increase formation of malondialdehyde following CCl_4–treatment [70]. The ability of *N. laevis* to counter the effects imposed through CCl_4-induced hepatotoxicity on the rats by preserving microsomal marker enzymes and the attenuation of the accumulation of lipid peroxidation products indicate its ability to exhibit *in vivo* antioxidant property that protects biomembranes against oxidative damage.

The hepatoprotective property of this plant could be attributed to the presence of endogenous substances with antioxidant and detoxifying properties. This detoxifying effect is explained by the induction of phase II antioxidant enzymes. Antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 induced hepatopathy [16].

Reduction in the microsomal activities of catalase and reduced glutathione, elucidate extent of the damage to the liver of the experimental rats administered with CCl_4. Contrary to the group treated with ethylacetate extract fraction, significantly ($p<0.05$) raised levels of these enzyme, confirmed antioxidants in the extracts. This is in conformity with the findings of Omale *et al*. [73], that increase in the activities of this endogenous substance (glutathione) in the extract treated groups suggests stabilization of plasma membranes and the repair of hepatic tissue damage inflicted by CCl_4. The depletion of GSH in the CCl_4 treated group may be explained by the increased utilization of GSH required for the removal of ROS and lipid damaged products. Hassan *et al*., [29] have reported decrease in hepatic tissue GSH level in CCl_4 treated groups with increase in hepatic GSH level in the rats treated with the extracts and CCl_4, probably due to *de novo* GSH synthesis or GSH regeneration.

It is probable that the antioxidant vitamins present in the extract initiate the stimulation and reversal of the heatotoxic effects. These results are in line with the findings of Narasimhanaidu and Pannaian [74], who hypothesized that under severe oxidative stress, there are heavy productions of reactive species which may result to the depletion of protective physiological moieties; glutathione, vitamins C and E and ceruloplasmin in rats. Glutathione also, through its enormous reducing power contributes to the recyclying of other antioxidants such as vitamins C and E, that have been oxidized [75].

A significant ($p<0.05$) rise in cholesterol level in CCl_4 treated groups was observed. The most important determinants of membrane fluidity include cholesterol-phospholipid ratio. Increase in membrane cholesterol highly correlates with decrease in membrane fluidity with subsequent alteration in membrane functions, including changes in membrane receptor, enzyme accessibility and their activation [76,77]. The ability of ethylacetate leaf extract fraction of *N. laevis* to reverse this abnormalities is evident in the CCl_4 and the extract treated groups that showed decrease ($p<0.05$) in microsomal cholesterol. Organochlorines, following microsomal metabolism by the $P\text{-}_{450}$ oxidase system undergo covalent binding to hepatic microsomal protein [78]. Our findings of significant ($p<0.05$) decrease in microsomal protein levels in CCl_4 treated group but with appreciable increase in the extract and CCl_4 treated groups confirm the findings of Narasimhanaidu and Ponnaian [78], that protein may be damaged directly by specific interaction of oxidants or free radicals with particularly susceptible amino acids. Overall, our results suggest that the leaf extracts of *N. laevis* posses natural antioxidants that mitigate damages inflicted on the liver through *in vivo* mechanistic action.

CONCLUSION

The hepatoprotective effect of leaf extracts of *N. laevis* against CCl_4-induced liver damaged may be due to its bioactive components mediated *in vivo* through reversal of the toxic on-slaught of CCl_4 by maintaining the activities of the enzymes to minimal levels. The leaf extract of the plant is recommended at lower dosage for use in treatment of liver diseases due to toxicity. Structural elucidation of the active component(s) of this plant is recommended for future research.

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