

Research Paper

**HEPATOPROTECTIVE ACTION OF STEM BARK EXTRACTS OF
Newbouldia laevis IN RATS TREATED WITH CARBON TETRACHLORIDE
(CCl₄)**

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Abstract

The present work evaluates the hepatoprotective, phytochemical and antioxidant properties of stem bark extracts of *Newbouldia laevis* in rats treated with carbon tetrachloride (CCl₄). Carbon tetrachloride was used to induce hepatotoxicity. Phytochemicals, clinical chemistry, antioxidant activities and enzymes were estimated using standard methods. Saponins, tannins, flavonoids, alkaloids, volatile oils, steroids, flavonoid glycosides, balsams, saponin glycosides and resins were detected in the extracts. A significant (P<0.05) rise in the levels of transaminases, bilirubin, alkaline phosphatase, cholesterol and lipid peroxides in CCl₄ intoxicated rats were restored to normal levels in groups treated with 40,80,120 and 160mg/kg of the extracts and CCl₄ to be deleted in a dose-dependent manner. However, levels of albumin, total protein, vitamin C, vitamin E, reduced glutathione and catalase were significantly increased (P<0.05) when treated with the extract at 120 and 160mg/kg/body weight. Similarly, an increase in the activities of microsomal marker enzymes and lipids were recorded when treated with the extracts at the same dose. Histopathological observation show fatty changes, necrosis and ballooning degeneration of hepatocytes in CCl₄ treated group. These attributes were not seen in groups treated with 160mg/kg/ body weight of the extract and CCl₄, which indicates high protective effect than 40, 80, and 120mg/kg/ body weight. The lethal dose (LD₅₀) of the plant extract was greater than 5000mg/kg. The results suggest that CCl₄-induced damage in rats can be ameliorated by treatment with the stem bark extracts of *Newbouldia laevis*.

Key words: *Newbouldia laevis*; Hepatoprotective; antioxidants; Carbon Tetrachloride Histopathological.

INTRODUCTION

The liver regulates important metabolic functions and is also a frequent target of a number of toxicants such as CCl₄ [1]. Hepatic damage is associated with distortion of these metabolic functions [2]. Liver disease is still a world health problem. Treatment and prophylaxis options

for the treatment of liver ailments are limited [3]. 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 [3]. Synthetic drugs used in the treatment of liver diseases are inadequate and sometimes have side effects. This is one of the reasons for the increased interest in the use of alternative medicine for liver diseases. Many traditional remedies employ herbal drugs for the treatment of liver ailments [4]; [5]; [6] and [7]. In recent years, researches have examined the effects of plants used traditionally by indigenous healers to support treatment of liver diseases [16, 17].

The principal cause mechanism of carbon tetrachloride (CCl₄)- induced hepatic damage is lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals [8]; [9]. The body system has a proper way to prevent and neutralize the free radical- induced damage, through the help of enzymes such as superoxide dismutase (SOD), catalase and Glutathione peroxidase (GPX). These enzymes serve to defend the living system against reactive oxygen species (ROS) [10]. Antioxidants such as ascorbic acid, β -carotene and α -tocopherol offer non-enzymatic protection to the body [11]; [12]; [13]; [14].

Medicinal plant products have protective effects against liver damage due to the presence of several components which have distinct mechanism of action. Some of these components are enzymes and proteins and others are low molecular weight compounds such as vitamins, carotenoids, and flavonoids [15]. Hence many plants and their products are recommended for the treatment of liver diseases, such plants includes, *Ocimum gratissimum*, *Solanum nigrum*, *Balanites aegyptiaca*, *Khaya senegalensis* [16, 17].

Newbouldia laevis is a boundary tree called "Aduruku" in Hausa, 'Ogirisi' in Igbo and 'Akoko' in Yoruba languages [18]. It is a medium sized angiosperm of the Bignoniaceae family and is native to tropical Africa and grows from Guinea savannah to dense forests [19]. The leaves of the plant were reported to have hepatoprotective activity [20]. The stem bark is used in folk medicine locally used for the treatment of malaria, pain, several inflammatory conditions and liver diseases. The plant has anti-diabetic [21], uterine contractile [22] antihypertensive [23], analgesic and anti-inflammatory properties [24]. To the best of our knowledge, there are no reports on the hepatoprotective and antioxidant properties of stem bark extracts of *Newbouldia laevis*. Therefore, this study reports evaluates the hepatoprotective, antioxidant and phytochemical properties of stem bark extract of *Newbouldia laevis* and its possible mechanism (s) of hepatoprotective action.

MATERIALS AND METHODS

Materials and reagents

Chemicals and reagent used were of analytical grade.

Plant collection and identification

The stem bark of the plant, flowers and leaves were collected from Kebbi state, Ngaski local government, to help for identification. The plants were botanically authenticated at the Herbarium of the Botany unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

The stem bark were open- air dried under shade, cut into small pieces (using Pestle and Mortar) and were stored until required for use.

Animals

Sixty (60) 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 extract

Small pieces (400g) of stem bark were extracted with two litres of 50% methanol at room temperature overnight and were filtered through Whatman No 1 filter paper. The filtrate was

concentrated to dryness using rotary evaporator and the yield was calculated. The extract was stored in closed container until required for reconstitution in distilled water (for oral administration).

Phytochemical analysis

The methods of Harborne [25], Sofowora [26] and El-Olemy [27] were used.

Acute toxicity studies (Determination of LD₅₀)

After the acclimatization period, the acute oral toxicity study was carried out according to the method of Organization for Economic and Cultural Development [28]. Five (5) animals were randomly selected. For limit test dose, 5000mg/Kg body weight (bw) of the extract was given in a single dose. Each animal was dosed and observed one after the other. Observation time for the first 8hrs, 14hrs, 24hrs, 48hrs and then up to 14days for signs of toxicity like tremors, itching, depression, weakness, food and water refusal, salivation and death if any, were recorded. If three (3) or more animals died within 48hrs the LD₅₀ is greater than 5000mg/kg and if one (1), two (2) or none died within 48hrs the LD₅₀ is less than 5000mg/kg[28].

Hepatoprotective activity

Induction of hepatotoxicity was done according to the method of Guntapalli [29].

The animals were divided into seven (7) groups, of five (5) rats each

Group I: (Normal control) received daily dose of liquid paraffin (1ml/kg body weight *per os*) for five (5) days.

Group II: (Induction control) received 1ml/kg body weight i.p of CCl₄ (30% in liquid paraffin) from the 2nd day to the 5th day.

Group III: received Silymarin, a known antihepatotoxic drug (Sigma Chemicals Company, USA) at a dose of 100mg/kg *per os*, for five days and CCl₄ induction from the 2nd day to the last day

Groups IV, V, VI and VII: received the stem bark extracts of *Newbouldia laevis* (40, 80, 120 and 160mg/kg), respectively for 5 days followed by CCl₄ induction on the 2nd day simultaneously to the last day.

The animals were sacrificed on the sixth (6th) day under ether anesthesia, blood and liver samples were collected.

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

The serum was used for clinical chemistry estimations (aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total protein, total cholesterol, albumin, bilirubin). Some part of the liver were perfused with cold 0.86% KCl homogenized and centrifuged at 9000g for 20 minutes to obtain post mitochondrial supernatant for the estimation of enzymic and non-enzymic antioxidants. The other part of the liver was placed in 10% formalin for histopathological studies.

Liver function test

5'-Nucleotidase (5'-NT) activity was assayed by the method of Reider and Otero [30]. Alanine Amino Transferase (ALT) and aspartate amino transferase were assayed using the method of Reitman and Frankel [31] and total protein by method reported by Gomall [32]. For bilirubin, Colorimetric method of Jendrassik and Grof [33] was used, Alkaline phosphatase by the method of Sood [34] and Albumin by the dye binding technique utilizing Bromocresol green (BCG) as modified by Doumas [35]. The method of Trinder [36] was used for estimation of total cholesterol.

Assessment of antioxidant activities

The liver was perfused with 0.86% cold saline solution to completely remove the red blood cells. It was then suspended in 10% (w/v) ice-cold 0.1M phosphate buffer (pH 7.4). The liver was cut into small pieces, and some quantity was weighed and homogenized. The homogenate was used for the estimation of enzymatic and non-enzymatic antioxidants.

Vitamin C

It was estimated Using 2,4 dinitrophenyl hydrazine [37] . Four milliliters (4ml) of TCA (10g/dl) solution were pipetted into three test tubes labelled blank, standard and sample. Then 1ml of the supernatant was added to the sample test tube, and mixed for 30 seconds and centrifuged for 10 minutes at 2000 rpm, then 2.4ml of the supernatant were added into another set of test tubes of equal number, followed by the addition of 0.8ml of DTC reagent and the content was then mixed. The test tubes were incubated at 40°C for 30 minutes for the reaction to take place. After the incubation period, the test tubes were transferred into a water bath at 25°C for 10 minutes. Then 4mls of cold 12M H₂SO₄ solution was added slowly with gentle mixing. The tubes were then allowed to stand at room temperature for 20 minutes. Vitamin C concentration was calculated using the relation:

$$\text{Vitamin C (mg/dl)} = \frac{\text{Abs of sample}}{\text{Abs of Standard}} \times \text{conc. Of std}$$

Estimation of serum vitamin E

It was done using the method of Baker and Frank [37].

Estimation of Catalase.

It was done using the method of Beers and Sizer [38]

Reduced glutathione was assayed by Patterson and Lazarow method [39].

- A. One milliliter (1ml) oxalated sample and 7.0ml water **were** (instead of was) mixed. Two milliliters (2ml) 25% meta phosphoric acid **were** added.

The resulting mixture was centrifuged.

- B. The test tubes were set up as follows

Reagent blank, 2 tubes were set up:

b = 1.0ML 5% metaphosphoric acid + 1.0ml 0.1M alloxan

b₀ = 1.0ml 5% metaphosphoric acid + 1.0ml H₂O

Standard, 2 tubes were set up.

S = 1.0ml standard (50µg glutathione) + 1.0ml 0.1M alloxan

S₀ = 1.0ml standard + 1.0ml H₂O

Sample, 2 tube were set up

X = 1.0ml filtrate + 1.0ml 0.1M alloxan

X₀ 1.0ml filtrate + 1.0ml H₂O

- C. All tubes were lined up in order and 1.0ml 0.5M phosphate buffer was added to the first tube, followed by 1.0ml of the equivalent NaOH solution and then mixed. This procedure was repeated with each successive tube at intervals of 30 seconds.

- D. After 6mintues, 1.0ml 1N NaOH was added to each test tube (again adding at 30 seconds interval) and mixed. The reaction was stopped and the “305” products were stable for several hours.{**changed from stabilize**}

E. At 305 nm, b was read against b_0 S against S_0 and X against X_0 .

$$\text{Mg glutathione}/100\text{ml} = \frac{A_x - A_b}{A_s - A_b} \times 0.05 \times \frac{100}{0.1}$$

Malondialdehyde (MDA)

Tissue supernatant (150 μ l) was diluted to 500 μ l with double deionized water. Two hundred and fifty **microlitres** (250 μ l) of 1.34% thiobarbituric acid **solution** were added to all tubes, followed by addition of an equal volume (250 μ l) 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°C). The tubes were allowed to cool to room temperature and the absorbance of the complex formed was read at 532nm [40]. The absorbance was extrapolated from a standard curve generated by using a standard (1,1,3, 3-tetraethoxypropane). Results were expressed as nanomoles of MDA per milliliter of supernatant.

Mechanism of action

The animals were sacrificed, 24hr after the last day. The liver was removed and rinsed in ice-cold 1.15% KCl, dried and weighed. It was homogenized in 4volumes of ice-cold isotonic phosphate buffer, pH 7.4, and centrifuged at 9000g for 20min to obtain the post-mitochondrial supernatant microsomes. The supernatant was immediately frozen on dry ice. Microsomes were resuspended in 0.15m sucrose solution. The following parameters were determined for the mechanism of action.

5'-Nucleotidase (5¹-NT) activity: assayed according to the method of Reider and Otero [30] and Subhani *et al.* [41].

Determination of cholesterol using O-phthalaldehyde: done according to the method of Rudel and Morris [42]

Determination of liver glucose 6-phosphatase : assayed by the method of Koide and Ode [43]

Determination of aniline hydroxylase [44].

Microsomes used were suspended in 50mM Tris-HCL buffer, pH 7.5. Enzyme activities were determined at 37°C in a medium containing 50mM Tris-HCL buffer, pH 7.5, 5mM MgCl₂, 0.33mM NADP⁺, 8mM isocitrate, and 15 μ g/ml isocitrate dehydrogenase (Sigma, USA), 3 μ moles NADPH generated/min/mg at 25°. Aniline hydrochloride was used as substrates. Aniline hydroxylase activity was determined by measuring p-aminophenol formation. The hydroxylation reaction was terminated by the addition of 1.5ml of 20% trichloroacetic acid to 3ml of a reaction medium containing 1mg of microsomal protein per milliliter, and the protein was removed by centrifugation at 9000g for 20min. From the supernatant fluid, 1ml was added to 1ml of 0.5M NaOH containing 1% phenol, after mixing, 1ml of M Na₂CO₃ was added. After 20 min of incubation at room temperature, the intensity of the blue color was determined at 630nm.

Histopathological studies

A portion of the tissue was collected and immediately fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5 μ m) were prepared and then stained with Haematoxylin and Eosin (H-E) dye for photo microscopic observations [45].

STATISTICAL ANALYSIS

The data were represented in mean \pm standard error of the mean. Results were analyzed statistically by one way ANOVA using Bonferroni multiple comparison, (Graph Pad Instant Software, San Diego, USA). A P < 0.05 was considered statistically significant.

RESULTS

Stem bark extracts of *Newbouldia laevis* reveal the presence of alkaloids, saponins, tannins, flavonoids, volatile oils, flavonoid glycosides, saponins glycosides, balsams, terpenoids and steroids. However, glycosides, cardiac glycosides, anthraquinones, resins and cyanogenic glycosides were not detected. Table 1 presents the effect of *Newbouldia laevis* on serum liver function indices in CCL₄-induced acute hepatic injury in rats. A significant ($P < 0.05$) rise in the levels of 5' NT (5' Nucleotidase), Alanine Transaminase (ALT), Aspartate Transaminase (AST), Total bilirubin (TBL), Direct Bilirubin (DBL), Alkaline Phosphatase (ALP) and Cholesterol (CHO) in CCL₄ intoxicated rats were restored to normal in groups treated with 40, 80, 120 and 160mg/kg of the extracts and CCL₄ in a dose-dependent manner. Levels of albumin and total protein were significantly increased when treated with the extracts at different doses. Table 2 presents the effect of *Newbouldia laevis* extracts on liver enzymatic and non-enzymatic antioxidant levels in CCL₄ treated rats. A decrease in activity of the antioxidant levels was recorded in CCL₄ treated group (Group II) and a rise in the level of lipid peroxide was seen in the same group II, whereas the standard silymarin (Group III) and different doses of the extract (Groups IV-VII) and CCL₄ showed a significant rise in the antioxidant levels with reduction in lipid peroxidation level when compared with normal control group (Group I). In table 3, the microsomal marker enzymes (Aniline Hydroxylase, 5' Nucleotidase and Glucose 6- phosphatase) activities show a significant ($p < 0.05$) decrease and a rise in lipid peroxidation in the CCL₄ treated group (Group II). Administration of *Newbouldia laevis* extracts at different dose levels (Groups III-V) reversed the decrease observed in enzyme activities and a significant rise ($p < 0.05$) in lipid peroxidation was also observed in CCL₄ treated rats from group III-V. Table 4.0, presents the apparent decrease in the levels of cholesterols, phospholipids, triglycerides and total protein in CCL₄ treated group (Group II), when compared to normal group (Group I). Treatment of animals with different doses of the extract attenuated the toxic effect of CCL₄ as observed in groups III-V.

The histopathological observations generally support the results obtained from serum enzyme assays. Photomicrograph of the liver sections of normal control (Group I), shows normal hepatocytes with no histopathological lesion (plate I). Photomicrograph of liver sections of CCL₄-intoxicated rats (Group II) shows severe micro and macro vesicular steatosis (fatty change), (plate 2). The fatty change in CCL₄-induced group was more severe than other groups. The histopathological architecture of liver sections of rats treated with the standard drug (Silymarin) in group III, shows very mild steatosis with normal hepatocytes at the background. Histopathological observations of liver sections of rats treated with *Newbouldia laevis* extracts at 40, 80, 120 and 160mg/kg, showed a more or less normal lobular pattern with a mild degree of fatty changes in groups treated with the higher doses (120 and 160mg/kg) of the extracts.

Table 1: Effect of stem bark extracts of *Newbouldia laevis* on serum liver function indices in CCL₄ - induced acute hepatic injury in rats.

Groups	5 ¹ Nucleotidase	ALT (u/l)	AST (u/l)	T.P (g/dl)	ALBU (g/dl)	TBL (μmol)	DBL (μmol)	ALP (u/l)	CHO (mmol)
Grp I	127.94±1.68	8.00±1.78	7.60±0.35	5.20±0.43	4.41±0.07	5.00±0.65	3.34±0.17	48.11±7.42	68.73±1.05
Grp II	301.23±3.64	19.50±1.47	19.70±1.16	2.68±0.44	0.34±0.28	17.17±0.62	9.35±0.47	129.71±21.06	148.82±5.60
Grp III	129.61±2.75 ^y	8.30±2.23 ^y	8.70±0.22 ^y	5.07±0.32 ^y	4.38±0.18 ^y	7.14±0.79 ^y	3.39±0.25 ^y	61.08±10.44 ^{xy}	70.20±1.34 ^y
Grp IV	286.59±3.14 ^x	18.41±1.29 ^x	12.00±0.30 ^{xy}	2.80±0.25 ^{xy}	0.97±0.14 ^x	13.01±0.42 ^{xy}	8.07±0.10 ^x	121.07±10.00 ^x	139.38±5.67 ^x
Grp V	262.68±2.85 ^x	17.18±1.35 ^x	10.99±0.26 ^{xy}	3.00±0.36 ^x	1.90±0.12 ^x	8.44±0.39 ^{xy}	5.45±0.35 ^{xy}	119.81±6.05 ^x	123.01±8.81 ^{xy}
Grp VI	195.18±2.43 ^{xy}	10.22±1.69 ^y	9.40±0.14 ^y	3.39±0.41 ^x	2.80±0.09 ^x	7.75±0.28 ^{xy}	4.98±0.14 ^y	99.91±6.90 ^{xy}	100.03±4.77 ^{xy}
Grp VII	130.54±3.72 ^y	8.81±1.82 ^y	8.92±0.27 ^y	5.17±0.37 ^y	4.20±0.56 ^y	6.62±0.46 ^y	3.44±0.56 ^y	63.28±6.14 ^{xy}	94.24±4.15 ^{xy}

Values are mean ± standard error of five (5) replicates. x= significantly different Vs group I: P<0.05; y= significantly different Vs group II: p<0.05; using analysis of variance (ANOVA), Bonferroni multiple comparison, Instant Graph Pad Software (San Diego, USA). ALT- Alanine aminotransferase, AST- Aspartate aminotransferase, T.P- Total protein, Albu- Albumin, TBL- Total bilirubin, DBL- Direct bilirubin, ALP- Alkaline phosphatase, CHO- Cholesterol.

Group I; (Normal control) received liquid paraffin (1ml/kg body weight peros)

Group II; (Negative control) received 1ml/kg body weight i.p of 30% CCL₄ in liquid paraffin

Group III; received 100mg/kg peros of silymarin and 1ml/kg body weight of 30% CCL₄ in liquid paraffin.

Group IV; received 40mg/kg body weight of the extract and 1ml/kg body weight of 30% CCL₄ in liquid paraffin.

Group V; received 80mg/kg body weight of the extract and 1ml/kg body weight of 30% CCL₄ in liquid paraffin.

Group VI; received 120mg/kg body weight of the extract and 1ml/kg body weight of 30% CCL₄ in liquid paraffin.

Group VII; received 160mg/kg body weight of the extract and 1ml/kg, body weight of 30% CCL₄ in liquid paraffin.

Table 2: Effect of stem bark extracts of *Newbouldia laevis* on liver enzymatic and non- enzymatic antioxidant levels in CCL₄ – induced hepatic injury in rats.

Groups	Vitamin E (mg/dl)	Vitamin C (mg/dl)	Catalase (u/mg of tissue)	Red Glutath (mg/100ml)	Lip Peroxides (nmoles of MDA/g tissue)
Grp I	37.33±3.80	38.07±5.55	7.00±1.22	462.05±9.88	0.201±0.08
Grp II	4.80±0.34	5.95±3.20	0.35±0.32	185.70±5.84	1.213±0.04
Grp III	32.76±0.49 ^y	31.24±6.92 ^{xy}	4.61±1.52 ^y	444.60±15.61 ^y	0.238±0.02 ^y
Grp IV	8.42±4.14 ^{xy}	7.97±1.63 ^x	0.76±0.60 ^x	216.66±6.32 ^{xy}	1.066±0.05 ^x
Grp V	9.11±4.52 ^{xy}	9.71±5.12 ^{xy}	1.93±0.84 ^x	267.63±8.22 ^{xy}	0.825±0.16 ^x
Grp VI	13.54±8.07 ^{xy}	12.97±4.01 ^{xy}	2.99±0.93 ^x	400.70±6.45 ^{xy}	0.536±0.21 ^{xy}
Grp VII	30.70±6.34 ^{xy}	29.68±4.14 ^{xy}	3.91±0.81 ^{xy}	435.81±10.65 ^{xy}	0.311±0.04 ^y

Values are mean ± standard error of five (5) replicates. x= significantly different Vs group I: P<0.05; y= significantly different Vs group II: p<0.05; using analysis of variance (ANOVA), Bonferroni multiple comparison, Instant Graph Pad Software (San Diego, USA),
Group I; (Normal control) received liquid paraffin (1ml/kg body weight peros)
Group II; (Negative control) received 1ml/kg body weight i.p of 30% CCL₄ in liquid paraffin
Group III; received 100mg/kg peros of silymarin and 1ml/kg body weight of 30% CCL₄ in liquid paraffin.
Group IV; received 40mg/kg body weight of the extract and 1ml/kg body weight of 30% CCL₄ in liquid paraffin.
Group V; received 80mg/kg body weight of the extract and 1ml/kg body weight of 30% CCL₄ in liquid paraffin.
Group VI; received 120mg/kg body weight of the extract and 1ml/kg body weight of 30% CCL₄ in liquid paraffin.
Group VII; received 160mg/kg body weight of the extract and 1ml/kg, body weight of 30% CCL₄ in liquid paraffin.

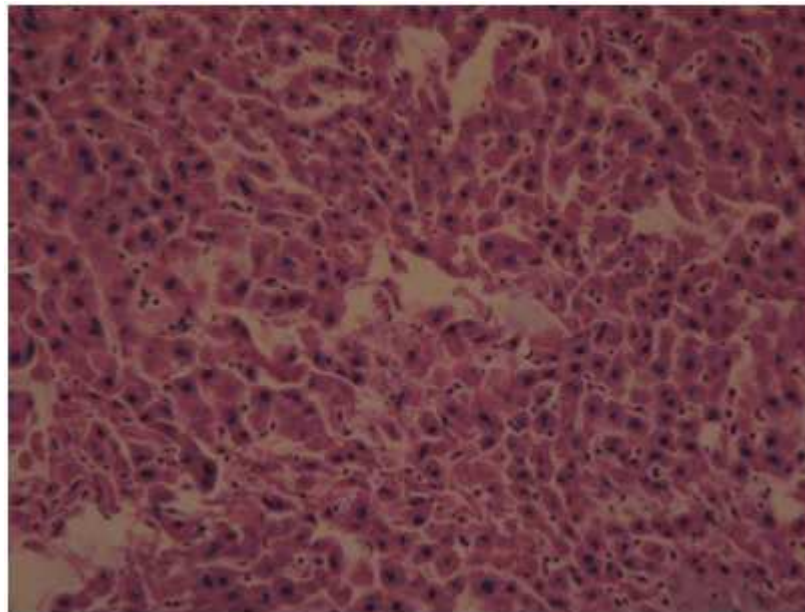


Plate 1: Liver sections of normal rats (Control) administered with liquid paraffin (1mg/kg) showing normal hepatocytes with no histopathological lesion. Hematotoxylin and Eosin (H&E) X400.

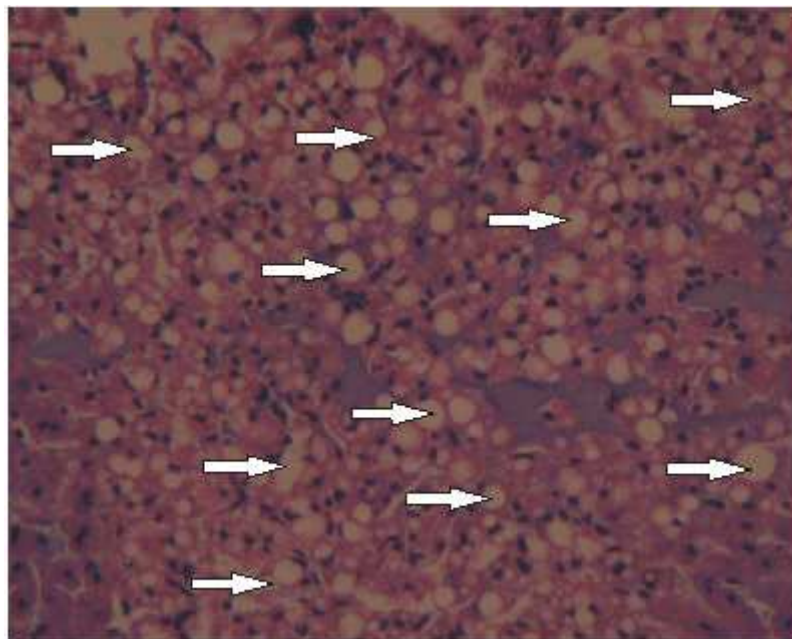


Plate 2: Liver sections of rats administered with CCL₄(1ml/kg,i.p)x4days, showing severe micro and macro vesicular steatosis(fatty change). Hematotoxylin and Eosin (H&E)X400

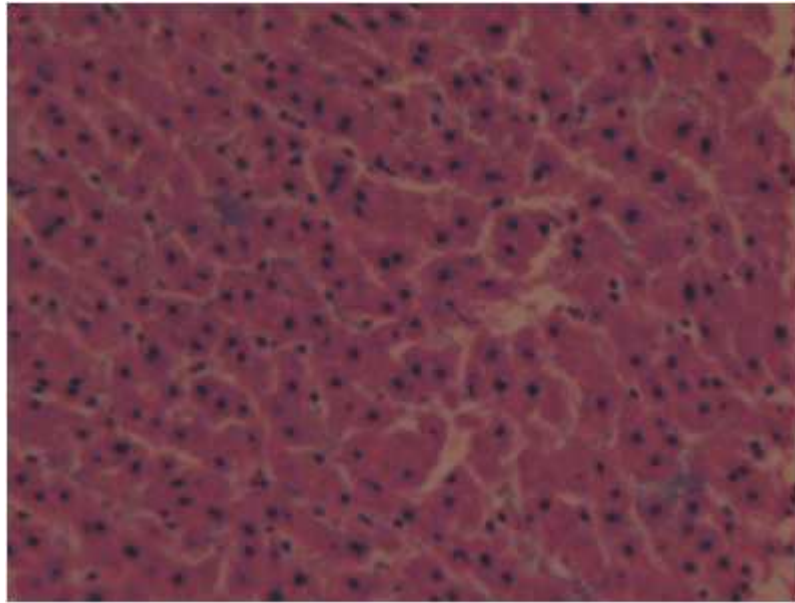


Plate 3: Liver sections of rats treated with Silymarin (100mg/kg,P.O)+CCL₄(1ml/kg, I.p)x5days, showing very mild fatty change with normal hepatocytes at the background. Hematotoxylin and Eosin (H&E)X400.

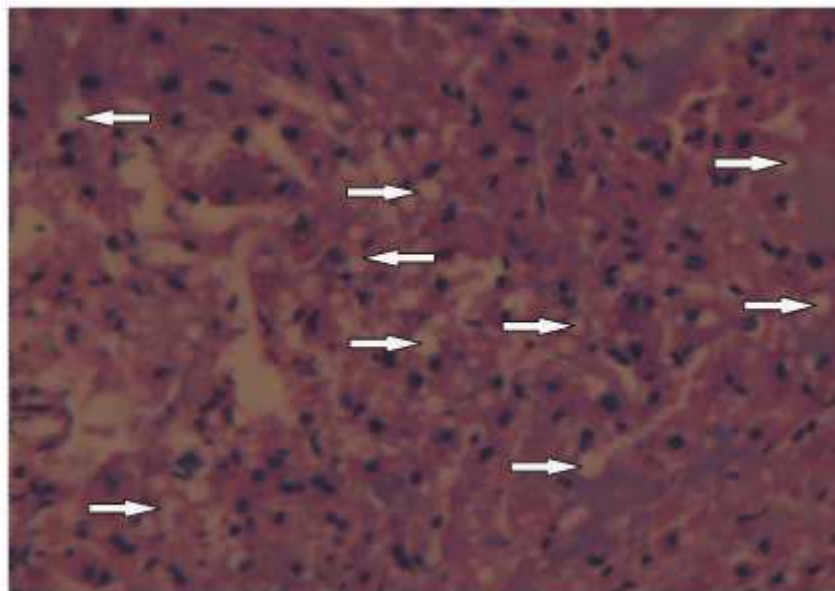


Plate 4: liver sections of rats treated with plant extract (40mg/kg p.o)+CCL₄(1ml/kg,i.p)x5days, showing macro vesicular steatosis (fatty change) and peripheral hepatocellular necrosis. Hematotoxylin and Eosin (H&E)X400.

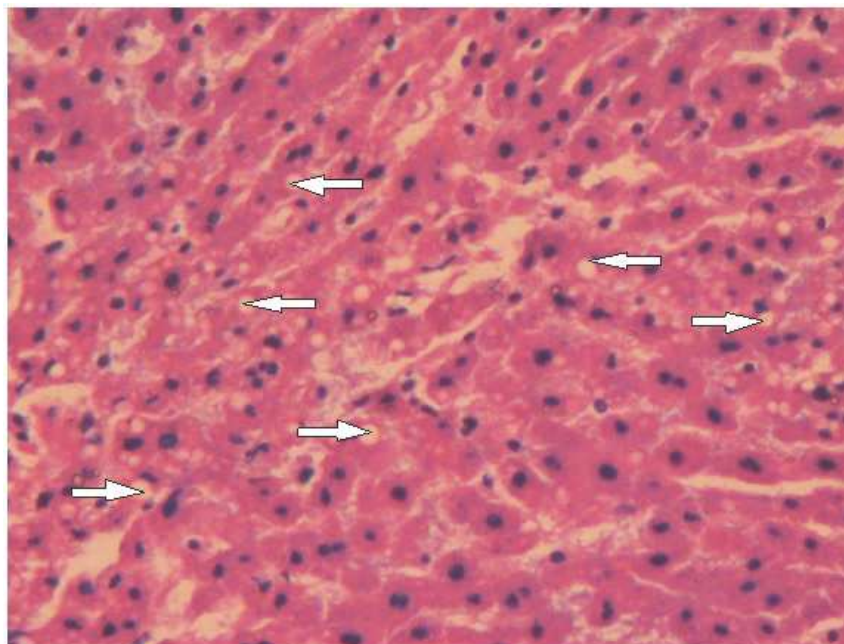


Plate 5: Liver sections of rats treated with plant extract (80mg/kg,p.o)+CCl₄ (1ml/kg,i.p) x 5days, showing moderate steatosis (fatty change). Hematoxylin and Eosin (H&E) X 400.

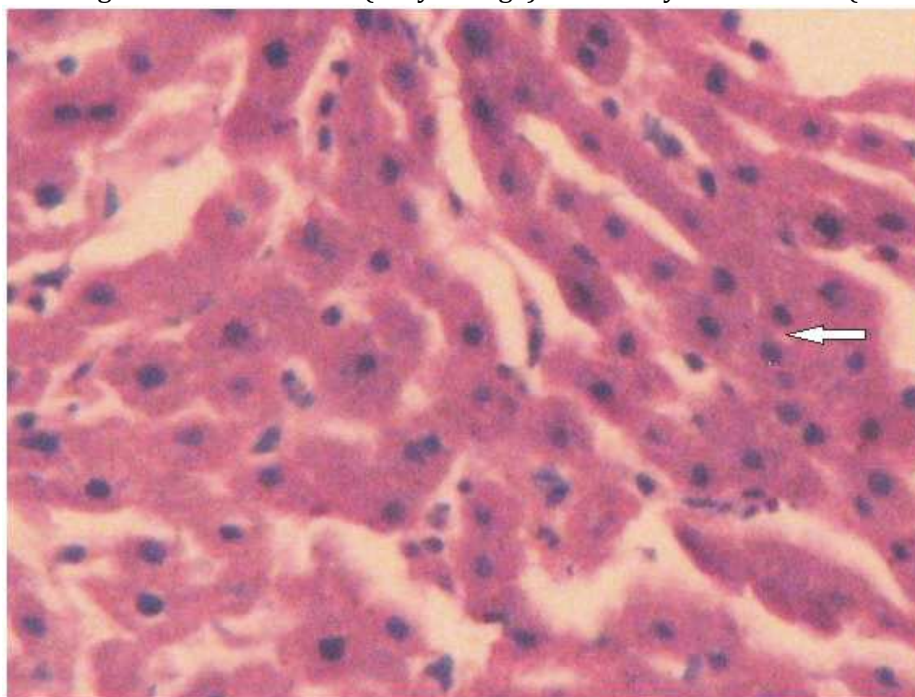


Plate 6: Liver sections of rats treated with plant extract (120mg/kg,p.o)+CCl₄ (1ml/kg,i.p) x 5days, showing mild steatosis (fatty change) at portal areas but no lesion. Hematoxylin and Eosin (H&E) X 400.

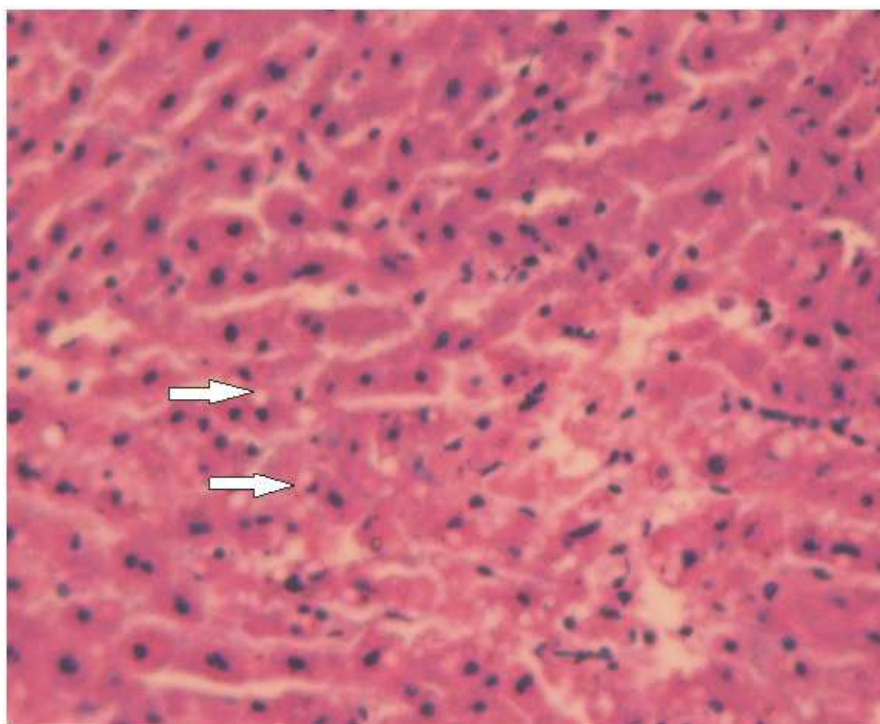


Plate 7: Liver sections of rats treated with plant extract (160mg/kg,p.o) +CCl₄ (1ml/kg,i.p) x 5days, showing very mild steatosis with no significant lesion. Hematotoxylin and Eosin (H&E) X 400.

Table 3: Effect of stem bark extracts of *Newbouldia laevis* on microsomal marker enzymes and lipid peroxidation in CCl₄-induced hepatic injury in rats.

Groups	Aniline Hydroxylase 5 ¹ (nmol min ⁻¹ mg ⁻¹ pro(Umg ⁻¹ protein)	Nucleotidase (Umg ⁻¹ protein)	Glucose 6-phosphatase (Umg ⁻¹ protein)	Malondialdehyde (nmol mg ⁻¹ protein)
Grp I	2.50±0.04	25.30±0.84	5.16±1.03	75.91±0.53
Grp II	0.79±0.50	10.05±3.38	0.26±0.21	105.76±6.15
Grp III	1.07±0.43 ^y	23.55±1.41 ^y	4.06±0.93 ^y	96.31±4.07 ^{xy}
Grp IV	1.22±0.07 ^y	19.78±1.08 ^{xy}	0.79±0.73 ^x	98.96±4.68 ^{xy}
Grp V	2.02±0.22 ^y	20.51±1.75 ^{xy}	3.13±0.96 ^y	94.48±4.40 ^{xy}

Values are mean ± standard error of five (5) replicates. x= significantly different Vs group I: P<0.05; y= significantly different Vs group II: p<0.05; using analysis of variance (ANOVA), Bonferroni multiple comparison, Instant Graph Pad Software (San Diego, USA).

Group I; (Normal control) received liquid paraffin (1ml/kg body weight peros)

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Group III; received 100mg/kg peros of the extract

Group IV; received 120mg/kg body weight of the extract and 1ml/kg body weight of 30% CCl₄ in liquid paraffin.

Group V; received 160mg/kg body weight of the extract and 1ml/kg body weight of 30% CCl₄ in liquid paraffin.

Table 4: Effects of stem bark extracts of *Newbouldia laevis* on microsomal cholesterol, lipids, triglycerides and total proteins in CCl₄-induced hepatic injury in rats.

Groups	Cholesterol ($\mu\text{g mg}^{-1}$ protein)	Phospholipid ($\mu\text{g mg}^{-1}$ protein)	Triglycerides ($\mu\text{g mg}^{-1}$ protein)	Total Protein ($\mu\text{g mg}^{-1}$ protein)
Grp I	36.94 \pm 4.33	194.18 \pm 1.72	37.95 \pm 2.19	4.20 \pm 0.31
Grp II	17.43 \pm 1.99	74.11 \pm 1.47	27.76 \pm 1.01	2.27 \pm 0.23
Grp III	34.29 \pm 3.53 ^y	191.55 \pm 3.32 ^y	33.95 \pm 1.81 ^{xy}	3.96 \pm 0.40 ^{xy}
Grp IV	30.44 \pm 1.96 ^{xy}	183.17 \pm 3.22 ^{xy}	29.63 \pm 0.97 ^x	2.45 \pm 0.19 ^x
Grp V	32.16 \pm 2.23 ^{xy}	189.81 \pm 0.99 ^{xy}	32.75 \pm 1.86 ^{xy}	3.78 \pm 0.91 ^{xy}

Values are mean \pm standard error of five (5) replicates. x= significantly different Vs group I: P<0.05; y= significantly different Vs group II: p<0.05; using analysis of variance (ANOVA), Bonferroni multiple comparison, Instant Graph Pad Software (San Diego, USA).

Group I; (Normal control) received liquid paraffin (1ml/kg body weight peros)

Group II; (Negative control) received 1ml/kg body weight i.p of 30% CCl₄ in liquid paraffin

Group III; received 100mg/kg peros of the extract

Group IV; received 120mg/kg body weight of the extract and 1ml/kg body weight of 30% CCl₄ in liquid paraffin.

Group V; received 160mg/kg body weight of the extract and 1ml/kg body weight of 30% CCl₄ in liquid paraffin

DISCUSSION

Acute toxicity test at 5000mg/kg of stem bark extract of *Newbouldia laevis* produced no mortality after 48 hours of observation. The lethal dosage (LD₅₀) of the aqueous stem bark extracts was greater than 5000mg/kg body weight, with no negative behavioral changes, such as restlessness, excitement, convulsions or coma at 5000mg/kg body weight. Apparent reduction in body weight of rats was observed which may be due to reduced food or fluid intake and loss of appetite [46; 47]. However, regardless of the above side effects, the very high values of the LD₅₀ indicate that the extract of *Newbouldia laevis* is practically non-toxic.

The phytochemical constituents of stem bark extracts of *Newbouldia laevis*, show the presence of saponins, tannins, flavonoids, alkaloids, volatile oils, steroids, balsams, flavonoid glycosides, saponin glycosides and terpenoids. However, resin, glycosides, anthraquinones and cardiac glycosides were not detected. Compounds like flavonoids, triterpenes, steroids, alkaloids and polyphenolics have been reported to have hepatoprotective and antioxidant activities [48; 49;50;51]. The quantity yield of flavonoids, alkaloids and tannins were found to be 8.2, 3.34 and 1.40% respectively.

CCl₄ is a hepatotoxic compound and it has to be biotransformed to be eliminated from the body. Biotransformation can result in either a decrease or increase (or no change) in toxicity. CCl₄ is metabolized by the cytochrome P-450 in the liver by abstraction of one of the four chlorine atoms. This results in formation of highly reactive trichloromethane radical, which initiates a cascade of lipid peroxidation by removing a hydrogen atom from membrane phospholipids

CCl₄ induces liver damage through its toxic metabolite CCl₃ (Trichloromethyl radical), which is bioactivated by cytochrome P₄₅₀ in the presence of oxygen to give trichloromethyl peroxy radicals. This radical binds covalently to macromolecules and causes peroxidative degradation of lipid membrane of the adipose tissue [52].

ALT, AST and ALP are considered markers for liver functions [53;54]. ALT is located primarily in the cytosol of hepatocytes and is a more sensitive marker of hepatocellular damage than AST, which is found in the cytoplasm and mitochondria in different tissues

such as heart, skeletal muscles, liver, kidneys and pancreas and erythrocytes [55]. Hepatic injury leads to the elevation of the serum marker enzymes, which are released from the liver into the blood. The increased levels of ALT, AST, ALP, Cholesterol and bilirubin are conventional indicators of liver injury [56]. The present study reveal a significant increase in the activities of serum AST, ALT, ALP, 5' nucleotidase and bilirubin levels on exposure to CCl₄, indicating hepatocellular injury [47]. The increase in AST and ALT levels in the CCl₄ treated group may be due to the release of enzymes from the cells of the damaged liver, or a change in the membrane permeability of the cells. A rise in ALP is an indication of hepatobiliary and bone disease, while increase in 5' nucleotidase is specific to hepatobiliary diseases. Elevation of plasma concentration of bilirubin is an indication of liver cell damage, because bilirubin measures the binding, conjugating and excretory capacity of hepatocytes.

Albumin and protein can act as criteria for assessing the synthetic capacity of the liver, since nearly all of them are synthesized in hepatocytes. Decrease in serum proteins and albumin, and an increase in cholesterol levels on exposure to CCl₄ is an indication of hepatocellular injury. Administration of the stem bark extracts of *Newbouldia laevis* and CCl₄ attenuated the rise in the levels of serum marker enzymes and increased the level of protein and albumin in a dose-dependent manner which reflects the hepatoprotective activity. Stimulation of protein synthesis accelerates the regeneration process and the production of liver cells [57], which caused subsequent recovery towards normalization like that of Silymarin treated animals. Our results indicate that the extract at different dose levels offers hepatoprotection with group VII (160mg/kg) being more effective, followed by group VI (120mg/kg and CCl₄), and this may be due to its rich content of phytochemicals, which might have scavenged the free radicals, thereby initiating hepatoprotective activity.

Decreased activities of enzymatic and non-enzymatic antioxidants and elevated level of lipid peroxidation were seen in CCl₄ treated group, where as the standard silymarin with CCl₄ and extracts with CCl₄ treated groups reflected a significant rise in the antioxidant levels with reduction in lipid peroxidation level when compared with the CCl₄-treated control group. The *Newbouldia laevis* extract therefore possesses treated group showed a dose-dependent hepatoprotective activity that was close to that of the standard silymarin (100mg/kg.p.o and CCl₄).

Thus the antioxidant activity or inhibition of the generation of free radicals is important in the protection against CCl₄ induced hepatic injury. A set of endogenous antioxidant enzymes such as catalase, glutathione peroxides and superoxide dismutase act to effectively defend, prevent and neutralize the free radical induced damage. These enzymes constitute a mutually supportive team of defense against reactive oxygen species (ROS) [10]. In CCl₄-induced hepatotoxicity, the balance between reactive oxygen species production and antioxidant defenses may not be maintained, hence "oxidative stress" results, which through a series of events deregulates the cellular functions of the hepatocytes leading to hepatic necrosis. The reduced activities of catalase and glutathione observed in Table 3, signify the hepatic damage in rats administered with CCl₄. However, the extract treated groups significantly (p<0.05) reversed the decrease in the level of these enzymes, indicating antioxidant activity of *Newbouldia laevis* extract.

Glutathione is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic glutathione has been shown to be associated with an enhanced toxicity to chemicals, including CCl₄ [58]. In this research work, a decrease in hepatic glutathione level was observed in the CCl₄ treated group. The resulting increase in

glutathione level in rats treated with the plant extract and CCl_4 may be due to *de novo* glutathione synthesis or regeneration. A significant ($p < 0.05$) decrease in the levels of vitamin E and vitamin C, was observed in CCl_4 treated group when compared to the normal control and extract plus CCl_4 treated groups. This result is in accordance with that of Narasimhanaidu. and Ponnaian [59], which showed that under condition of severe oxidative stress, the massive production of reactive oxygen species may also lead to the depletion of protective endogenous and exogenous antioxidants such as glutathione, vitamin E and vitamin C in rats. Administration of the plant extract and CCl_4 at different dose increases the levels of non-enzymatic antioxidant in a dose- dependent manner. Glutathione plays a role in recycling of antioxidants, such as vitamin E and C that have become oxidized through its reducing power.

Peroxidation of lipids is a complex process mediated via a free radical mechanism and is implicated in innumerable pathological conditions. Under normal physiological conditions, low concentrations of lipid peroxidation products are always seen in tissues and cells. In pathological conditions, more peroxidation products are formed [60]. Increased level of lipid peroxidation products in the liver homogenate of CCl_4 intoxicated rats is an indication of excessive membrane damage and alteration of cellular functions. In the present study, a significant ($p < 0.05$) rise in lipid peroxidation in the liver of rats treated with CCl_4 was observed. The increase in malonaldehyde levels in the liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent the formation of excessive free radicals [61]. But treatment with *Newbouldia laevis* extracts significantly reversed these changes. Thus the possible mechanism of hepatoprotection by the extract may be via its antioxidant action due to the high content of some phytochemicals such as flavonoids, steroids, polyphenols and alkaloids. It is obvious that the extract treated groups showed a dose-dependent activity with group VII showing high activity comparable to that of the standard drug (Silymarin).

The active component in silymarin is silibinin, a flavonoid complex which has multiple actions as a hepatoprotective agent. The antioxidant property and cell- regenerating functions are considered as most important [62]. The plant extract at 160mg/kg, p.o offers high hepatoprotective activity probably due to its rich phytochemical content in flavonoids and alkaloids. The possible mechanism of action of *Newbouldia laevis* may be due to its free radical scavenging activity and cell-regenerating activity by stimulating protein synthesis. The possible mechanism by which *Newbouldia laevis* protects against tissue damage was also ascertained in this study. The apparent decrease in the activities of aniline hydroxylase, glucose 6-phosphatase and 5' nucleotidase was observed in CCl_4 treated rats, decreased enzyme activity may be attributed to the increase in radicals that affects the inner mitochondrial membrane and intracellular calcium stores, which may lead to structural and functional disorganization, overall loss in energy production, irreversible damage and loss of enzymatic activity [63; 64; 65]. Administration of *Newbouldia laevis* extracts at different dose levels reversed the decrease observed in enzyme activity which was comparable to that of the normal control (Group I).

In this study, a significant ($p < 0.05$) rise in malondialdehyde (lipid peroxidation) was observed in CCl_4 treated animals which corroborates the findings of Farombi [66], pretreatment with the plant extracts ameliorates the toxic onslaught of CCl_4 by enhancing the metabolic detoxifying enzymes and antioxidants in a dose dependent manner. Administration of the extract alone did not significantly affect the drug oxidizing enzymes.

The microsomal lipids in the liver of the CCl₄ treated rats were found to be considerably low compared to the normal control. It has been hypothesized that one of the principal causes of CCl₄-induced liver injury is lipid peroxidation by free radical derivatives of CCl₄ [67]. However, microsomal drug oxidizing system is made up of three compounds; cytochrome P₄₅₀, cytochrome C reductase and phospholipids. Interference with any of these components may cause membrane perturbation and lead to altered membrane function [66]. In animals challenged with CCl₄ the contents of cytochrome P₄₅₀ substantially reduced [67]. Trichloromethyl peroxy radical, a derivative of CCl₄ attacks lipid in the membrane of endoplasmic reticulum [67]. These radicals further propagate chain reaction leading to lipid peroxidation in cellular membranes, destruction of cells and finally death [67]. This may increase membrane fluidity as a result of ROS (reactive oxygen species) involvement, which results in the leakage of enzymes and lipid constituents into the circulation [68]. However, treatment of animals with stem bark extracts of the plant at the two highest doses mitigated the toxic effect of CCl₄ by decreasing the level of lipid peroxidation thereby maintaining the activity of the enzymes and the hepatocytes in general.

CONCLUSION

Newbouldia laevis contains phytochemicals and antioxidants who mediate its hepatoprotective action by probably preventing the initiation and propagation of lipid peroxidation processes. Further studies on structural elucidation of the active components of *Newbouldia laevis* are recommended.

REFERENCES

1. Chatterjee, T.K. (2000). Medical plants with hepatoprotective properties. In herbal options, 3rd (ed), Calcutta Books and Allied publisher Ltd, pp: 135-137.
2. Wolf, P.L. (1999). Biochemical diagnosis of liver disease, Indian J. clin. Biochem. pp: (18): 59-90.
3. Adesunkanmi, A.R., Akinkuolie, A. A. and Badru, O.S. (2002). A five year analysis of death in accident and emergency room of a semi-urban Hospital. West Afr. J. Med., 21 (2): 299-104.
4. Dhuley, G.P. and Naik, S.R. (1997). Protective effect of Rhinax, a herbal formulation against CCl₄-induced liver injury and survival in rats. J. Ethnopharmacol., 56: 87-96.
5. Venkateswaran, L., Pari, P., Viswanathan, M. and Menon, V.P. (1997). Protective effect of a herbal formulation against erythromycin estolate-induced hepatotoxicity in rats. J. Ethnopharmacol. 55: 553-558.
6. Latha, M.G., Rajesh, M. and Latha, M.S. (1999). Hepatoprotective effect of an ayurvedic medicine. Indian. Drg. Pp: 470-473.
7. Mitra, S.K., Seshadri, S.J., Venkataranganna, M.V., Gopumadhavan, S., Venkatesh, U.V. and Sarma, D.N. (2000). Effect of HD- 03 a herbal formulation in galactosamine induced hepatopathy in rats. Indian. J. Physiol. Pharmacol. 44: 82-86.
8. Castro, J. A., Ferrya, G.C., Casio, S. R., Sasame, H., Fenos, O.M. and Gillete, J.R. (1974). Prevention of Carbon tetrachloride induced necrosis by inhibitors of drugs metabolism. Further studies on the mechanism of their action. Biochem. Pharmacol., 23: 295-307.
9. Poli, G. (1993). Liver damage due to free radicals. Br. Med. Bull. 49: 20-604.

10. Venukumar, M.R., and Latha, M.S. (2002). Antioxidant activity of *Curculigo orchoides* in carbon tetrachloride induced hepatopathy in rats. *Ind. J. clin. Biochem*, 17:80-87.
11. Ames, B.N., Cathear, T.R., Schwiers, E. and Hochstein, P. (1981). Uric acid provides an antioxidant defense in humans against oxidant and radicals caused aging and cancer. A hypothesis, *pro. NaH. Acal. Sci., USA*. 78:8658-8662.
12. Lehninger, A. L., Nelson, A. L., and Cox, M.M. (1993). *Principles of Biochemistry* Worth publishers, Inc. New York 2nd edition pp: 437-439.
13. Tella, I.O and Ojo, O.O., (2005). Effects of *Azadirachta Indica*, *Tamarindus India* and *Eucalyptus camaldulensis* on paracetamol induced-lipid peroxidation in rats. *J. Sustain Deve. Agric. Envin. Vol. 1(8): 420-994*
14. Tella, I.O., Ojo, O.O., and Nadro, M.S. (2006). Protection of rats by extract of some common Nigerian trees against acetaminophen-induced hepatotoxicity. *Afr. J. Bio. technol. Vol. 5(5): 7 55-760*.
15. Zhang, H.Y. and Wang, L.F. (2002). Theoretical elucidation on structure antioxidants activity relationships for indolinomic hydroxylamines. *Bio. Org. Med.Chem., Lett; 12: 225-227*.
16. Baytop, T. (1999). *Turkiye de Bitkiler Tedavl. Phytoterapy in Turkey*, 2nd edition. Nobel Tip Kitabeuleri Ltd., Sti, Istanbul.
17. Arulkumaran, K.S., Rajasekaran, R., Ramasamy, M., Jegadeesan, S., Kavimani, A. and Somasundaram, D. (2009). *Cassia Roxburghii* Seeds protects liver against toxic effects of ethanol and carbon tetrachloride in rats. *Int. J. Pharm. Tech. Rest., 1: 237-246*.
18. Hutchinson, J. and Dalziel, J.M. (1963). *Flora of West Tropical Africa Vol. 11 Crown agents for oversea government and administration 4, millbank, London, PP: 435-336*.
19. Arbonnier, M. (2004). *Trees, shrubs and lianas of West African Dry Zones (IRAD, Margraf publishers GMBH MNHN, Cote D' Ivorie. P:194*.
20. Hassan, S. W., Salawu, K., Ladan, M. J., Hassan, L. G., Umar, R. A. and Fatihu, M. Y. (2010). Hepatoprotective, Antioxidant and Phytochemical properties of leaf extracts of *Newbouldia laevis*. *International J. of Pharm. Tech. Research 2 (1) ; 573-584*.
21. Tanko, Y., Okasha, M. A., Sale, M. I. A., Mohammed, A., Yerima, M., Yaro, A. H. and Isa, A. I. (2008). Antidiabetic effect of ethanolic flower extracts of *Newbouldia laevis* (Bignoniaceae) on blood glucose levels of streptozocin-induced diabetic wister rats. *Research Journal of Medical sciences 2 (2) ; 62-65*.
22. Bafar, E and Sanni, U. (2009). Uterine contractile effects of the aqueous and ethanol leaf extracts of *Newbouldia Laevis* (Bignoniaceae) *in-vitro*. *Indian J. of Pharmaceutical sciences 71 (2) : 124-127*.
23. Enye, J. C., Onubeze, D. P. M., Chineke, H. N., Nweke, I. (2013). The antihypertensive properties of methanolic extracts of *Newbouldia laevis* on anaesthetized cats. *Journal of Dental and Medical sciences. Volume 8, (6) ; 35-39*.
24. Usman, H., Yaro, A. H. and Garba, M. M. (2008). Analgesic and anti-inflammatory screening of *Newbouldia laevis* flower in rodents. *Trends in medical research. 3: 10-15*.
25. Harborne, J.B. (1973). *Phytochemical methods. A guide to Modern Techniques of Plant Analysis 2nd edition. Chapman and Hall London pp: 1-10*.
26. Sofowora, A. (1991). *Medicinal plants and traditional medicine. John Wiley and Sons. Pp : 66-79*.

27. El-Olemyl, M.M., Fraid, J. A. and Abdulfattah, A. A. (1994). Experimental photochemistry. A laboratory manual Afifi, Abdel Fattah, A comp. IV King Saud University Press., UK, Pp: 1-134.
28. OECD, (2001). Guideline for testing chemicals. Acute toxicity-up and down procedure. No. 425, pp: 1-26.
29. Guntapalli, M.M., Chandana, V.R., Palpu, P. and Anine, S. (2005). Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn. J. Ethnopharmacol., 103:484-490.
30. Reider, S.V., and Otero, M.A. (1969). A simplified procedure for the assay of 5¹-Nucleotidase. Clin. Chem., 15: 9-727-729.
31. Reitman, S., and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. chlin. Pathol. 28: 56-63.
32. Gomall, A. G., Bardawill, C. S. and David, M.M. (1949). Determination of Serum proteins by means of the biuret reaction. J. Biol. Chem. 177 (2): 751-766.
33. Jendrassik, L. and Grof P. (1938). Simplified photometric methods for the determination of bilirubin. Biochem. Zchr., 297; 81-89.
34. Sood, R. (1999). Medical laboratory technology . Method and interpretation.sed. Jaypee brothers medical publishers ltd. pp: 488-490.
35. Doumas, B.T., Watson, W. and Briggs, H.G., (1971). Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acda., 3:187-96.
36. Trinder, P. (1969). A calorimetric method of determination of serum and plasma cholesterol. Ann. Biochm., 6: 24-27.
37. Baker, H. and Frank, O. (1968). Clinical Vitaminology, Method and interpretation. Inter-science publishers. New York. 23(7):45-49.
38. Beers, R.F. and Sizer, I.W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase J.Biol.Chem., 195: 130-140.
39. Patterson, J.W., and Lazarow, A. (1955). Methods of biochemical analysis. Glick A. ed New York, Inter Science, 2:259.
40. Hartman, P. E. (1983). Putative Mutagens and Carcinogens in Foods, Iv. Malondialdehyde. Environ.Mutaen. 5 (4): 603-607.
41. Subhani, T.F., Muhammed, A., Abdalla, J., Vivian, D., Muhammed, N.A., Falyaz, S. (2009). 5¹- Nucleotidase oxidative stress and antioxidant status in alcohol consumers and cirrhotic patients. J. Biochem. Med., 19 (3): 277-286.
42. Rudel, L.L., and Morris, M.D. (1973). Determination of cholesterol using O-phthaldehyde. J. Lip. Res., 14: p: 364.
43. Koide, H., and Ode, T. (1959). The pathological occurrence of glucose 6- phosphatase in serum liver diseases. Clin. Chem. Acta. 4: 554-561.
44. Schenkman, J.B., Remmer, H., Estabrook, R.W. (1957). Spectral studies of drug interaction with hepatic microsomal cytochrome. Mol. Pharmacol. 3: 23-113.
45. Drury, R. A., Wallington, E. A. and Cameron, R. (1967). Carletons histological technique. 4th ed. Oxford University press. New York. Pp48-66.
46. Joseph, P.K., Rao,K.R. and Sundaresh, C.S. (1989). Toxic effect of garlic extract and garlic oil in rats .Ind. J. Exptal. Biol.27:977-979.
47. Hassan, S.W., Umar, R.A., Ebbo, A.A. and Matazu, I.K. (2005). Phytochemical, antibacterial and toxicity of *Parkinsonia aculaeta*. (fabacea). Nig. J. Biochem. Mol. Biol. 20 (2):89-97.

48. Dicarlo, G. N., Mascolo, A., Izzo, A. and Capasso, F. (1999). Flavonoids, old and new aspects of a class of natural therapeutic drugs life sci., 65 (4):337.
49. Ahmed, S.A., Rahman, A., Alam, M., Saleem, M. A. and Sultana, S. (2000). Evaluation of the efficacy of *lawsonia alba* in the alleviation of carbon tetrachloride induced oxidative stress. J. Ethnopharmacol., 69: (2) 157-164.
50. Kumar, G.G., Sharmila, B.P., Vanitha, P., Sundararajan, M., and Rajasekara, M. P. (2004). Hepatoprotective activity of *Trianthema Pertulacastrum* l. against paracetamol and thioacetamide intoxication in albino rats J.Ethnopharmacol., 92 (1): 37-40.
51. Sanmugapriya, E. and Venkataraman, S. (2006). Studies on Hepatoprotective and antioxidant actions of *strychnos potatorum* linn seeds on CCL₄ induced acute hepatic injury in experimental rules. J. Ethnopharmacol., 105: 154-160.
52. Avijeet, J., Manish, S., Lokesh, D., Anurekha, J., Rout, S.P., Gupta, V.B. and Krishna, K.L. (2008). Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb leaves. J. Ethnopharmacol., 115 (1): 61-66.
53. Tolman, K. G., and Rej, R. (1999). Liver function in Tietz text book of clinical chemistry, Burtis, C.A and E.R Ashwood (eds). W.B Saunders company, Philadelphia, pp: 1125-1177.
54. Hilaly, J.Z., El Israili, H. and Lyoussi, B. (2004). Acute and chronic toxicological studies of *Ajuga Iva* in experimental animals. J. Ethnopharmacol., 91(1):43-50.
55. Aniagu, S.O., Nwinyi, F.C., Olambi, B., Akumka, D.D., Ajoku, G.A., Izobe, K.S., Agala, P., Agbani, E.O., Enwerem, N.M., Iheagwara, C. and Gamaaniel, K.S. (2004). Is *Berlina grandiflora* (Leguminosae) toxic in rats? Phytomed., 11 (4):352-360.
56. Achliya, G.S., Wadodkar, S.G. and Dorle, A.K. (2004). Evaluation of hepatoprotective effect of *Amalkadi ghrita* against carbon tetrachloride induced hepatic damage in rats. J. Ethnopharmacol., 90 (2-3):229-232.
57. Maheswari, M.U., and Rao, P. (2005). Antihepatotoxic effect of grape seed oil in rat. Indian. J. pharmacol., pp:18-34.
58. Hewawasam, R.P., Jayatilaka, K.A., Pathirana, C. and Nudduwa, K.B. (2003). Protective effect of *Astera cantha longifolia* extracts on mouse liver injury induced by carbon tetrachloride and paracetamol. J. Pharm. Pharmacol. 55:1413-1418.
59. Narasimhanaidu, K. and Ponnaian, S.M., (2006). Antihyperglycemic and antioxidant effect of Rutin, a polyphenolic flavonoid in streptozocin-induced Diabetic wister rats. J. Basic. Clin. Pharmacol. Toxicol., 98: 97-103.
60. Zahnu, H., and Tappel, A.L. (1960). Mechanism of Vit E action for lipid peroxidation in Vit E deficient rabbit. Arch. Biol. Chem., 88:113-117.
61. Ashok-shenoy, k., Samayaji, S.N. and Bairy, K.L. (2001). Hepatoprotective effect of Ginkgo biloba against carbon tetrachloride induced hepatic injury in rats. India J. Pharmacol., 33:260-266.
62. Kren, V. and Walterova, D., (2005). Silibinin and silymarin- new effects and applications. Biomed papers. 149 (1). 29-41.
63. Jewell, S.A., Bellomo, G., Thor, H., Orrenius, S., Smith, M.T. (1982). Bleb formation in hepatocytes during drug metabolism is caused by disturbances in thiol and calcium ion homeostasis. Science; 217:1257-1259.
64. Rusu, M.A., Tamas, M., Puica, C., Roman, I., Sabadas, M. (2005). The hepatoprotective action of ten herbal extracts in CCL₄ intoxicated liver. Phytother. Res., 19:744-749.

65. Jadon, A., Bhadauria, M., Shukla, S. (2007). Protective effect of *Terminalia belerica* Roxb and gallic acid against carbon tetrachloride induced damage in albino rats. J.Ethnopharmacol.,109: 214-218.
66. Farombi, E.O, (1999): Mechanisms for the hepatoprotective action of Kolaviron; studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride- treated rats. J. Pharmacol.Res., vol.42, 1: 2000.
67. Malgerzata, K., Jadwiga, J. L., Malgorzata, E., Teresa, A., Irena, M., and Wieslawa, B. (2007). Protective effect of *Aquilegia vulgaris* (L) on carbon tetrachloride-induced oxidative stress in rats. Vol 45, pp: 702-711.
68. Hamed, M.A. (2011). Metabolic profile of rats after one hour of intoxication with a single oral dose of ethanol. J. pharmacol. Toxicol., 6:158-165.