

The Amelioration of Carbon Tetrachloride – Induced Toxicities by Methanolic Extract of *Buchholzia coriacea* Leave in Male Albino Rat

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Abstract: *The study was designed to investigate potential ameliorative effect of methanolic leave extract of Buchholzia coriacea on carbon-tetrachloride (CCl₄) - induced toxicities in male Albino rats. Thirty adult male Albino Rat were divided into five groups: each comprising 6 animals, Group (I) the controls received only vehicles; olive oil (0.5 ml/kg bw) and DMSO, Group II (induction controls) received single dose of CCl₄ (CCl₄ + Olive oil in 1:1 ratio; 2 ml/kg bw; i.p, Group III, IV and V received 200mg/kg bw ,400mg/kg methanolic extract of Buccholzia coriacea and 200mg/kg , Silymarin (50 mg/kg, o.p) as standard reference drug respectively after induction with a single dose of CCl₄ for seven days. Evaluations were made for Lipid peroxidation and antioxidant activities, enzyme activities and some other biochemical parameters, haematology and pathology profile using standard methods. The methanolic leave extract of Buchholzia coriacea at 200mg/kg and 400mg/kg reduced the levels of AST and ALT, however, there was a significant (P<0.05) reduction of ALP when 200mg/kg and 400mg/kg dose of the leaves extract were administered. The treatment with 200mg/kg and 400mg/kg methanolic extract of Buccholzia coriacea was able to reduce the levels lipid peroxide with corresponding increase in activity of antioxidant GR, CAT, GPX, GSH compared with group treated with single dose of CCl₄ only. The histopathology results revealed restoration of the normal or near normal histo-architecture and haematological toxicities were reversed at the doses of the plant extract tested. In conclusion, the inhibition of lipid peroxidation and concomitant increase of the endogenous antioxidant defence systems in serum and organ studied with subsequent restoration of the normal or near normal histo-architecture of the these organs aver or demonstrate the significant ameliorative and protective role of methanolic extract of Buccholzia coriacea at 200mg/kg and 400mg/kg.*

Keyword: Antioxidant activities, *Buccholzia coriacea*, Carbon tetrachloride, Lipid peroxidation and Toxicities

1. Introduction

The formation of free radicals or oxidants is a well-established physiological event in aerobic cells, which convene enzymic and non enzymic resources known as antioxidant defences. Free radical is defined as any species that contains one or more unpaired electron occupying an atomic or molecular orbital by itself such as superoxide anion radical, hydrogen peroxide, hydroxyl radicals, lipid alkoxyl and peroxy radicals etc. The free radicals have a special affinity for lipids, proteins, carbohydrates and nucleic acids (Velavan, 2011) threatening the integrity of these various biomolecules including proteins (Stadtman and Levine, 2000), lipids as well as lipoproteins involved in atherosclerosis (Yla - Herttuala, 1999) and DNA (Marnett, 2000). This high reactivity of free radicals causes oxidative damage that is potentially toxic, mutagenic, or carcinogenic.

Oxidative damage or stress refers to the imbalance between the concentrations of reactive oxygen and nitrogen species and the anti oxidative defence systems of the body (Dotan et al., 2004). Antioxidant

Antioxidants play a crucial role in maintaining optimal cellular functions and thus systemic health and wellbeing and as such an ideal antioxidant should be readily

absorbed and quench free radicals, and chelate redox metals at physiologically relevant levels. Humans have evolved highly complex antioxidant systems (enzymatic and non enzymatic) that which work synergistically and in combination with each other to protect from aging and also in pathogenesis of age related disorders such as cancer, hypertension, atherogenesis , Alzheimer's disease and Parkinson disease. Endogenous antioxidants system may not be able to alleviate or fight these degenerative diseases hence need for the consumption various fruits, vegetables, edible (Adefegha and Oboh, 2011) and medicinal plants to maintain optimal cellular functions.

Many medicinal plants are considered to have antioxidants activities and contain high content of phenolics like gallic acids and other active constituents. All the herbs and plants contain natural antioxidants compounds including flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechin, isocatechin, gallic acid, esculetin, etc. (Aquil et al., 2006). Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. The need for antioxidants becomes even more critical with increased exposure to free radicals.

Buchholzia coriacea was named after R.W buchholz, It belongs to the family capparidaceae. The seed of *Buchholzia coriacea* has medicinal values. *Buchholzia coriacea* leaves are large, obovate, oblanceolate to elliptic, shortly acuminate or acute at apex, cuneate at base, 15-30×5-11 cm, thinly coriaceous, glabrous, with very prominent mid rib below; it has about 10 lateral nerves, each running directly into the one above and forming distinct loops close to the margin, its stalk is about 10-15 cm long, swollen for about 1 cm at both ends and pale green.

The ethnomedicinal uses and ethnopharmacological properties of *buchholzia coriacea* include infusions of the leaves are applied to the eyes against filarial nematodes, and powdered or pulped leaves are applied to treat fever, ulcers, boils and haemorrhoids. Some of the scientific work done on *Buchholzia coriacea* leaves Engler include these among others: Antipyretic Properties (Chinedu et al., 2012), Antispasmodic and antidiarrhoea properties (Chinedu et al., 2012).

The objectives of this study is to evaluate the ameliorative activity of methanolic extract of *Buchholzia coriacea* leaves Engler in carbon tetrachloride induced toxicity or damage in male Albino rats.

2. Materials and Methods

2.1 Collection of Plant Material

The Fresh leaves of *Buchholzia coriacea* was obtained from Ganboro reserve at Lanke camp about 4km from Idanre, Ondo State, Nigeria and was identified by a botanist, Mr Omotayo F.O and voucher number given as UHAE 2014/82a at the Department of Botany, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria

2.2.0 Extracts Preparation

The leaves were washed and air dried after which 700g of dried sample were extracted with 2400mls of 95% methanol at 25°C for 48h and concentrated using a rotary evaporator under reduced pressure at 40°C to yield the methanolic extract.

2.3.0 Phytochemical Analysis

Phytochemical tests were carried out using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

2.3.1 Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.3.2 Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of

distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.3.3 Test for flavonoids: A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

2.3.4 Test for steroids: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue indicating the presence of steroids.

2.3.5 Test for terpenoids (Salkowski test): Five ml of the extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.3.6 Test for cardiac glycosides (Keller-Killani test): 5ml of the extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.3.7 Test for Anthraquinones: 0.5g of the extract was boiled with 10ml H₂SO₄ and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour change.

2.3.8 Test for Alkaloids: 0.5g of the powdered extracts was stirred in 5ml of 1% HCl aq on a steam bath for 5mins. The mixture was then filtered using Whatman's no1 filter paper. To the filtrate, 2-4 drops of Dragendoff's reagent was added to 1ml of the filtrate. An orange colour was observed indicating the presence of alkaloids.

2.4 In vivo studies

Studies were carried out using male albino rats weighing 180±10g purchased from the Animal House Unit, Department of Science Technology, Federal Polytechnic, Ado Ekiti, Ekiti, Nigeria and handled appropriately in accordance with the criteria of "Guide for the care and use of laboratory animals". The animals were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions. They had free access to standard diet and fresh water ad libitum. Thirty rats were divided randomly into 5 groups, each comprising 6 animals. Group (I) the controls received only vehicles; olive oil (0.5 ml/kg bw) and DMSO (0.5 ml/kg bw) and fed with a normal diet for 7 days. Group II (induction controls) received single dose of

CCl₄ (CCl₄ + Olive oil in 1:1 ratio; 2 ml/kg bw; i.p) on day 1 of the experiment. Group III, IV and V received Silymarin (50mg/kg, oral), (Wills and Asha 2006) methanolic leave extract (200 mg/kg bw; oral) and (400mg/kg; oral) once in a day for 7 days respectively along with the intraperitoneal administration of CCl₄ on day 1. At day 7, 24 hours of the last treatment all the animals were sacrificed through cervical dislocation and blood obtained via cardiac puncture for biochemical and haematological parameters. The liver and kidney were removed and placed at 4°C after perfusion with ice cold saline which were cut and homogenised for antioxidant assay. Section of the liver and kidney were also cut for histopathology analysis.

2.5 Determination of in vivo antioxidant activity

10% homogenate of liver tissue was prepared in 100 mM KH₂PO₄ buffer containing 1 mM EDTA (pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for the following experiments as described below

2.5.1 Measurement of lipid peroxidation (LPO)

The assay for membrane lipid peroxidation was done by the method of (Wright *et al.*, 1981) with some modification. The reaction mixture in a total volume of 3.0ml contained 1.0ml tissue homogenate, 1.0ml of TCA 10%, and 1.0ml TBA0.6%. all the test tube were place in boiling water bath for about 45min. The test tube was shifted to ice bath and then centrifuged at 2500× g for 10min. The amount of malondialdehyde form in each of the samples was observed by measuring the optical density of the supernatant at 532nm.

2.5.2 Measurement of reduced glutathione (GSH) level

The GSH content in colon was determined by the method of (Jollow *et al.*, 1974) in which 1.0ml of PMS fraction (10%) was mixed with 1.0ml of sulphosalicylic acid (4%). The sample were incubated at 4°C for at least 1h and then subjected to centrifugation at 1200g for 15min at 4°C. The assay mixture contained 0.4ml filtered aliquot, 2.2ml phosphate buffer (0.1M, pH7.8) and 0.4ml DTNB (10Mm) in total volume of 3.0ml. The yellow colour developed was read immediately at 412nm on spectrophotometer.

2.5.3 Measurement of glutathione reductase (GR) activity

The GR activity was measured by the method of (Carberg and Mannervik, 1975). The assay system consisted of

1.6ml phosphate buffer (0.1M, pH 7.6), 0.1ml EDTA (0.5mM), 0.05ml oxidized glutathione, 0.1ml NADPH (0.1mM) and 0.1ml of 10% PMS in a total volume of 2.0ml. The enzyme activity was assessed at 25°C by measuring the disappearance of NADPH at 340nm and was calculated.

2.5.4 Measurement of catalase (CAT) activity

The catalase activity was measured by the method of (Claiborne 1985). In brief, the assay mixture consist of 2.0ml phosphate buffer (0.1M, pH 7.4), 0.95ml hydrogen peroxide (0.019M) and 0.05ml of PMS (10%) in a final volume of 3.0ml. changes in absorbance were recorded at 240nm.

2.5.5 Measurement of glutathione reductase (GR) activity

The GR activity was measured by the method of (Carberg and Mannervik 1975). The assay system consisted of 1.6ml phosphate buffer (0.1M, pH 7.6), 0.1ml EDTA (0.5mM), 0.05ml oxidized glutathione, 0.1ml NADPH (0.1mM) and 0.1ml of 10% PMS in a total volume of 2.0ml. The enzyme activity was assessed at 25°C by measuring the disappearance of NADPH at 340nm.

3. Results

Phytochemical screening of *Buchholzia coriacea*

Phytochemical	Leaves
Anthraquinone	+
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Saponins	+
Steroids	+
coumerin	+
Quinone	-
Tannins	+
Phlobatannins	+
Terpenoids	+
reducing sugar	-

Note: positive (+), negative (-).

The plant extract revealed the presence of the following secondary metabolites alkaloids, anthraquinone, cardiac glycosides, flavonoids, glycosides, saponins, tannins, alkaloids, coumerin, terpenoids, phlobatannins, steroids.

Table 1: Effect of methanolic leave extract of *Buchholzia coriacea* on some Biochemical indices in carbon tetrachloride induced damage in male Wistar rats.

	CONTROL			UNTREATED			STANDARD			200mg/kg			400mg/kg		
	Liver	Kidney	Serum	Liver	kidney	serum	Liver	Kidney	Serum	Liver	Kidney	Serum	Liver	Kidney	Serum
AST	73.33±3.667	121.00±7.327	81.25±3.250	229.7±0.720*	228.0±21.47*	190.1±4.133*	160.40±13.84*	151.1±2.613*	99.20±3.200*	172±18.58*	128.2±16.88*	77.22±7.217*	133.20±4.833*	110.5±0.4533*	59.47±8.767**
ALP	87.40±7.910	145.3±4.953	103.3±2.018	148.5±9.648*	187.7±8.801*	141.0±10.21*	104.2±7.36	167.2±9.007	97.61±6.253*	110.1±1.103*	117.4±13.76*	114.2±6.97*	126.6±1.840*	139.8±2.390*	103.1±3.957
ALT	61.76±3.20	59.25±2.93	68.43±5.7	112.6±15.76*	99.73±0.587*	122.6±15.76*	80.21±1.387	82.13±1.17*	86.88±1.72	93.10±2.550	70.46±2.873**	103.10±2.55	71.01±1.353*	65.01±1.647**	81.38±1.57**
TP	13.40±0.5881	18.08±0.707	8.660±0.2797	21.54±4.117	30.73±1.094	10.75±0.5285	22.30±3.213	20.21±1.814	9.927±1.242	64.14±3.036**	39.93±4.501	12.88±0.8599	93.98±5.823**	58.40±5.211	17.79±0.7322**

Result were expressed in mean \pm S.E.M (n=5). One way ANOVA was used to test mean difference significance using Tukey's Multiple comparison using Grap prism software, a represent $p < 0.05$ compare with control group of rat, b represent $p < 0.05$ compare with CCl_4 induced group of rat, c represent $p < 0.05$ compare with standard treated group of rat.

Table 2: Effect of methanolic leave extract of *Buchholzia coriacea* on Lipid peroxidation, enzymatic and non enzymatic levels in carbon tetrachloride induced damage in male Wistar rats.

	Control			Untreated			Standard			200mg/kg			400mg/kg		
	Liver	Kidney	Serum	Liver	Kidney	Serum	Liver	Kidney	Serum	Liver	Kidney	Serum	Liver	Kidney	Serum
GR	40.63 \pm 6.770	51.80 \pm 1.701	34.04 \pm 1.995	26.11 \pm 2.637	26.47 \pm 2.948*	20.71 \pm 1.060*	61.52 \pm 11.960**	37.28 \pm 3.549	41.69 \pm 4.214*	51.64 \pm 1.866 ^b	65.35 \pm 10.82 ^{bc}	47.87 \pm 3.345*	66.92 \pm 5.706**	43.05 \pm 1.582	43.16 \pm 1.53 ^b
LPO	15.37 \pm 1.428	11.8 \pm 2.28	7.543 \pm 0.9348	39.28 \pm 0.988*	43.9 \pm 0.390	11.47 \pm 1.633	18.26 \pm 3.896*	11.6 \pm 2.85	11.85 \pm 1.320	23.19 \pm 1.702	5.27 \pm 0.099**	23.52 \pm 2.773**	36.84 \pm 8.217*	3.00 \pm 0.348**	22.33 \pm 1.76**
GSH	61.04 \pm 2.004	69.85 \pm 6.908	61.42 \pm 5.446	32.91 \pm 1.703*	36.17 \pm 2.335*	19.64 \pm 0.79*	1.91 \pm 6.175*	70.46 \pm 2.442*	74.56 \pm 2.685*	75.33 \pm 2.603*	68.84 \pm 1.928*	82.67 \pm 1.202*	65.33 \pm 2.028*	80.65 \pm 0.948*	80.33 \pm 0.88**
GPX	27.36 \pm 1.44	19.80 \pm 0.12	14.75 \pm 0.75	18.30 \pm 1.56*	8.31 \pm 0.31*	8.03 \pm 0.46*	27.43 \pm 1.37*	13.74 \pm 1.43**	11.13 \pm 20.52**	33.64 \pm 1.36*	12.68 \pm 0.43**	12.16 \pm 0.28**	34.53 \pm 1.69**	13.98 \pm 0.39**	13.66 \pm 0.28**
CAT	13.96 \pm 0.12	50.55 \pm 0.89	53.80 \pm 0.89	7.80 \pm 0.11*	7.80 \pm 0.51*	41.73 \pm 0.77*	20.03 \pm 0.27**	60.97 \pm 0.39**	60.88 \pm 0.22**	22.20 \pm 0.92**	53.77 \pm 0.92**	62.91 \pm 1.65**	25.20 \pm 0.17**	57.57 \pm 1.68**	68.51 \pm 0.36**

Result were expressed in mean \pm S.E.M (n=5). One way ANOVA was used to test mean difference significance using Tukey's Multiple comparison using Grap prism software, a represent $p < 0.05$ compare with control group of rat, b represent $p < 0.05$ compare with CCl_4 induced group of rat, c represent $p < 0.05$ compare with standard treated group of rat.

Table 3: Effect of methanolic leave extract of *Buchholzia coriacea* on the haematological profile in carbon tetrachloride induced damage in male Wistar rats.

Group	PCV	HB	RBC	WBC	MCH	MCHC	MCV	N	L	E	M
Control	45.0 \pm 1.73	15.0 \pm 0.577	7.50 \pm 0.289	7.43 \pm 1.16	20.0 \pm 0.000	33.3 \pm 0.000	60.0 \pm 0.000	29.7 \pm 4.33	77.0 \pm 6.43	0.000 \pm 0.000	0.333 \pm 0.333
Untreated	24.0 \pm 11.6	8.01 \pm 3.88	4.00 \pm 1.93	15.3 \pm 3.46	20.0 \pm 0.0176	33.3 \pm 0.0105	40.0 \pm 10.0	15.0 \pm 6.77	31.3 \pm 2.67*	0.000 \pm 0.000	0.000 \pm 0.000
Standard	41.3 \pm 1.86	13.8 \pm 0.623	6.89 \pm 0.309	7.50 \pm 1.22	20.0 \pm 0.0167	33.3 \pm 0.0277	60.0 \pm 0.000999	26.0 \pm 4.36	73.7 \pm 4.67*	0.000 \pm 0.000	0.333 \pm 0.333
200mg/kg	45.0 \pm 2.89	14.8 \pm 0.814	7.50 \pm 0.481	5.77 \pm 0.784 *	20.0 \pm 0.0267	33.1 \pm 0.0434	60.0 \pm 0.000999	26.0 \pm 6.03	68.7 \pm 2.19*	0.00 \pm 0.000	0.667 \pm 0.333
400mg/kg	51.3 \pm 4.10*	17.1 \pm 1.37*	8.56 \pm 0.683*	6.60 \pm 1.05*	20.0 \pm 0.0120	33.5 \pm 0.176	60.0 \pm 0.000	22.3 \pm 1.45	77.7 \pm 1.45*	0.000 \pm 0.000	0.660 \pm 0.000

Result were expressed in mean \pm S.E.M (n=5). One way ANOVA was used to test mean difference significance using Tukey's Multiple comparison using Grap prism software, a represent $p < 0.05$ compare with control group of rat, b represent $p < 0.05$ compare with CCl_4 induced group of rat, c represent $p < 0.05$ compare with standard treated group of rat.

3.1 Discussion

The biochemical mechanism that leads to oxidative stress cannot be overlooked because of its importance to the status or integrity of the system. Carbon tetrachloride (CCl_4) an oxidant or causative agent of oxidative stress has been implicated as direct cause of many pathological changes in changes liver, kidney, testes, lungs, nervous system and blood tissue by producing free radicals (Abraham *et al.*, 1999).

It has been hypothesized that CCl_4 toxicity is due to reactive free radical (CCl_3) trichloro methyl radical intermediate that resulted in severe cell damage (Johnson and Kroening, 1998) which is generated by its reductive metabolism by hepatic cytochrome P450. This reactive intermediate is considered to cause lipid peroxidation and disruption of cellular membranes (De Groot *et al.*, 1989). This corroborate that hepatotoxicity principle hinges on lipid peroxidative degradation of biomembranes (Kaplowitz *et al.*, 1986). This is evident by an elevation in the serum marker enzymes namely ALT, ALP, AST and reduction in protein level.

In the assessment of damage to the liver by CCl_4 , enzyme levels, AST, ALT is largely used as a major diagnostic determinant. Administration of CCl_4 resulted to a significant ($P < 0.05$) increase of enzyme levels such as

AST, ALT, ALP and no significant difference in total protein when compared to control. The methanolic leave extract of *Bochholzia coriacea* at 200mg/kg and 400mg/kg reduced the levels of AST and ALT, however, there was a significant ($P < 0.05$) reduction of ALP when 200mg/kg and 400mg/kg dose of the leaves extract were administered compared to control.

The efficacy of any hepatoprotective/nephroprotective drug hinges on its capacity of either reducing the toxic effect or restoring the normal physiology that has been disrupted by the toxin. The standard drug, Silymarin at 50mg/kg and the plant extract ameliorate CCl_4 induced elevated enzyme levels in tested groups, conferring protection of structure of liver cell membrane or regeneration of damaged liver cells and other affected organ.

In this study, the inability of antioxidant defense mechanism in the system to prevent formation of excess free radicals is assayed for by increased levels of lipid peroxidation which is clearly evident in rat treated with a single dose of CCl_4 which is associated with clear decrease in the activity of antioxidants GR, CAT, GPX, GSH in the liver, kidney and serum compared with the control groups. This support other works of CCl_4 induced oxidative stress. The treatment with 200mg/kg and 400mg/kg methanolic extract of *Buccholzia coriacea* was able to reduce the

levels lipid peroxide in dose dependent manner with corresponding increase in activity of antioxidant GR, CAT, GPX, GSH compared with group treated with single dose of CCl₄ only.

The protective and ameliorative roles in organism's system result from biological activities of some antioxidant enzymes such as, GSH GPx SOD and CAT as they are responsible for scavenging free radicals. The reduced glutathione (GSH) is a non enzymatic biological antioxidant and a tripeptide, present in the liver that provides major defense for the body by scavenging reactive oxygen species (ROS) generated from exposure to carbon tetrachloride (Arivazhagan *et al.*, 2000). The decreased concentration of GSH in the liver might be due to NADPH reduction or GSH used up to remove the peroxides (Yadav *et al.*, 1997). The reactivation of glutathione reductase by the plant extract was reflected in decreased level of lipid peroxidation, this corroborated with the earlier report of Bhandarkar and Khan (2004).

Catalase an antioxidant enzyme well distributed in many tissues helps to decompose hydrogen peroxide and offers protection to tissue from highly reactive hydroxyl radical (Chance and Greenstein, 1992). The decrease in CCl₄ treated group is harmful because of the accumulation of superoxide and hydrogen peroxide which was reversed in other groups treated with the plant extract indicating protective ability of the plant *Bucchozia coriacea*. GSH-Px detoxifies peroxides by reacting with GSH and converting it to GSSG, which is reduced to GSH by GSR (Maritim *et al.*, 2003). This GSH-dependent enzyme offer a second line of defense because they detoxify noxious by-products generated by ROS and also help to avert the dissemination of free radical (Gumienjczk, 2005). Glutathione reductase is a ubiquitous enzyme, which brings about the reduction of glutathione (GSSG) in oxidized state to glutathione (GSH). Glutathione reductase is important for the glutathione redox cycle that regulates adequate levels of reduced cellular GSH. (Dolphin *et al.*, 1989).

Previous study on the effects of CCl₄ on haematological parameters show that acute CCl₄ toxicity led to transient decrease in the Hb concentration and reticulocyte count as well as PCV and RBC counts by extension (Moritz and Pancow, 1989) which is similar to our observation in the study. The administration of *B.corriacea* extract in this study showed increase PCV, RBC, Hb, platelets values at 400mg/kg dose compare with treated group with single dose of CCl₄. This shows that *B.corriacea* extract significantly reduced the damage which could also suggest that it may have haematinic effect.

The plant extract was active at restoring the lymphocyte and neutrophil counts significantly to their normal values especially at the dose of 400mk/kg which could have been a pointer for acute hepatotoxicity with necrotic and apoptotic hepatocellular injury and impairment of liver function (Kovalovich *et al.*, 2001) in CCl₄ group.

The histopathological study revealed normal liver and kidney architecture in the control group, small sized glomerulus, tubular necrosis/vacuolation degeneration of tubule and epithelia desquamation in kidney while the liver showed necrotic hepatocytes, dilated sinusoids, enlarged/ congested central vein and portal area in group treated with single dose of CCl₄. The administration of 200mg/kg plant extract showed normal glomerulus, showed no sign of tubular necrosis, vacoulation and epithelia desquamation while 400mg/kg showed tubular necrosis and epithelia desquamation. Reduced /more preserved hepatocytes necrosis, mildly dilated sinusoids and congested portal area which at both 200mg/kg and 400mg/kg doses of the plant extract.

Phytochemical studies revealed the presence of alkaloid, anthraquinones, cardiac glycosides, flavonoids, saponin tannins among others in methanolic extract of *B.corriacea*. Phenolic compounds available in the plant kingdom are majorly accountable for the antioxidant potential of plants. Hagerman *et al.* (1998) have reported that the ability of high molecular weight phenolics to reduce free radicals and its effectiveness on the molecular weight, the number of aromatic rings and nature of hydroxyl group's substitution than the specific functional groups.

In addition, the report on green leafy vegetables, soft fruits and medicinal plants exhibited higher levels of flavonoids (Sultan and Anwar, 2009). Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Van Acker *et al.*, 1996) The flavonoids which is one of the active component of the plant *Bucchozia coriacea* is probably responsible for the antioxidant activities which confers a protective and ameliorative roles observed in this study.

In conclusion, the inhibition of lipid peroxidation and concomitant increase of the endogenous antioxidant defense systems in serum and organ studied with subsequent restoration of the normal or near normal histo-architecture of the these organs aver or demonstrate the significant ameliorative and protective role of methanolic extract of *Bucchozia coriacea* at 200mg/kg and 400mg/kg. Further studies are ongoing to identify the active component(s) and underlying mechanism (s) responsible for the beneficial effect of this plant.

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