HAEMATOLOGICAL AND BIOCHEMICAL CHANGES INDUCED BY CARBON TETRACHLORIDE IN WISTAR RATS – AMELIORATIVE EFFECTS OF *Carica papaya* (PAWPAW) LEAF AQUEOUS EXTRACT

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Abstract

Hepatic disorders continue to be one of the leading causes of morbidity and mortality in the world. It is caused by chemicals and drugs. This study aimed to determine the effects of the aqueous leaf extract of *Carica papaya* on CCl_4-induced haematological and serum biochemical changes in Wistar rats. Twenty-five adult male Wistar rats divided into five groups (l-V) of five animals each (DW, CCl_4+DW, CCl_4+CPE200, CCl_4+CPE400, and CCl_4+VC) were used. Group I (DW), were dosed with 3 ml/kg of distilled water, while those in group II (CCl_4+DW) were administered 0.2 ml/rat of CCl_4 in Olive oil intraperitoneally and subsequently dosed with 3 ml/kg of distilled water. Animals in groups III, IV and V (CCl_4+CPE200, CCl_4+CPE400 and CCL_4+VC) were dosed with 0.2ml/rat of CCl_4 in Olive oil intraperitoneally and dosed with *C. papaya* leaf aqueous extract (CPE)200, 400 mg/kg or vitamin C (100mg/kg) respectively. The regimens were administered once daily by gavage for 21 days. At the end of the dosing period, the rats were sacrificed and the blood and sera samples analyzed for haematological and serum biochemical changes. The phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, tannins, carbohydrates, and reducing sugars. Flavonoids and tannins presence in the extract are reported to possess antioxidant properties. The acute oral toxicity of the leaf extract of *C. papaya* was greater than 5,000 mg/kg. Thus, the extract is relatively safe in the experimental model used. The result revealed that alterations in packed cell volume, concentration of haemoglobin, erythrocyte, absolute and differential leucocyte counts induced by CCl_4, exposure were significantly ($P< 0.05$) ameliorated by *C. papaya* leaf aqueous extract. The attenuation of this haematoxicity by the extract may be partly due to its antioxidant properties. The result also revealed a dose dependent increase in the concentration of albumin in the induced and treated groups in comparison with the induced, non-treated rats. Further, the result showed a significant ($P<0.05$) decrease in the activities of ALT, AST and ALP across the induced and treated rats in comparison to the induced and non-treated rats, indicating tissue protecting effect. The result equally showed a significant decrease in the MDA in the induced and treated groups when compared with the induced non-treated rats. It is concluded that *C. papaya* leaf aqueous extract could be useful in the management of hepatic disorders and many other conditions that may be associated with tissue damage owing partly to its antioxidant properties.

Keywords: Carbontetrachloride, Alteration, Haematological, Biochemical, *Carica papaya*, Amelioration.

Introduction

Historically, the use of medicinal plants is as old as mankind and medicine (Okpara, 2015; Bulama *et al*., 2017). Medicinal plants are widely used in management of diseases all over the world (Okpara *et al*., 2018). Their recognized biological actions led to their cultivation, even in antiquity in Egypt, Greece, along the Mediterranean and in China (Okpara, 2015).

Herbs are still the first line treatment for many of the world's population, being readily available, traditional, relatively safe and inexpensive (Okpara *et al*., 2017; Bulama *et al*., 2017). Oxidative stress has been strongly implicated in the pathogenesis of many diseases/conditions such as chronic diarrhoea, diabetes mellitus, cancer and liver cirrhosis. Also, many Nigerian medicinal plants are considered potential sources of antioxidant compounds (Sofowora, 1993; Adewole and Abiodun, 2012; Adewole *et al*., 2014; Okpara*et al*., 2016).

Thus, it has become imperative to identify and evaluate commonly available medicinal plants used in Nigeria to treat various human and animal disorders, including those used in the treatment of liver disorders for their bioactive principles as an alternative to currently used orthodox agents in the face of global upsurge of drug resistance and toxicity of synthetic products (Sodipo *et al*., 2009; Okpara *et al*., 2017).

Carica papaya commonly known as Papaya(pawpaw) belong to the fruits (family *Caricaceae*) it is used in folkloric medicine for the treatment of myriad of diseases and conditions including stroke, high blood pressure, diarrhoea, diabetes mellitus and liver disorders. However, there is paucity of information on the safety and possible hepatoprotective effects of *Carica payaya* leaves aqueous extract on CCl_4-induced haematological and serum biochemical changes in rats.

The aim of this work is to carry out acute toxicity studies and to evaluate the ameliorative effects of the aqueous extract of *C. payaya* leaves on carbon tetrachloride (CCl_4)-induced haematological and serum biochemical changes in Wistar rats.

Materials and Methods

Chemical and Plants Substances

The reagents for the determination of protein albumin and the Kits for ALT, ALP, and AST used were of analytical grade and were obtained from Randox, Laboratories Company, Antrim, Northern Ireland, and United Kingdom. While the methanol and carbon tetrachloride (CCl_4) used was from the British Drug House (BDH), London. Commercial grade vitamin C tablets (Emsor Pharmaceutical Ltd, Nigeria), distilled water to make 10% stock solution.Fresh green leaves of *Carica papaya* identified and authenticated by Mr. Sam Yusuf Shwarpshakka (a taxonomist) with the Livestock Investigation Division, NVRI, Vom were collected from K – Vom, Jos South Local Government Area, Plateau State, Nigeria. Voucher specimen JO2015A was preserved in the Herbarium for reference purpose.

Preparation of Crude Aqueous Leaves Extract of *Carica papaya*

Fresh green leaves removed from apparently healthy plants in November, 2015 was air dried in the Laboratory for 21 days and grounded to a coarse powder with mortar and pestle. Four hundred and fifty-one gram(451 g) of the powdered leaves was soxhlet-

extracted with distilled water at 100ºC to give an aqueous extract with yield of 17.2% which was coded JOCPE2015 using the methods of Sodipo *et al*., (2009). The resultant extract was concentrated *in vacuo* labeled and stored in a capped specimen bottle at room temperature until required.

Phytochemical Tests

Freshly prepared aqueous extract of *C. papaya* leaves was subjected to standard phytochemical screening tests for various constituents. The extract were screened for the presence of flavonoids, alkaloids, tannins, glycosides, saponnins, resins, triterpenoids, steroids, carbohydrate and reducing sugars using various protocols (Harborne, 1998 and Evans, 2002).

Experimental Animals

Twenty-five 8-weeks old male Wistar rats weighing 130 – 134 g, and twelve Swiss albino mice – adult males (21 – 23.4 g) respectively obtained from the Animal House of Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria were used for the study. The animals (rats and mice) were kept in steel cages and were fed on standard rat's chours. Water was provided *ad libitum*. The experimental procedures were conducted in accordance with guideline on the use of Laboratory Animals (NRC, 1996).

Determination of Medium Lethal Dose (LD_{50})

The median lethal dose (LD_{50}) was determined through a two phase approach as described by Lorke (1983); the first phase involved 9 mice divided into 3 groups of 3 mice each. Each group was administered *C. papaya* leaves aqueous extract at doses of (10, 100 and 1000 mg/kg) body weight *per os,* respectively. Signs of toxicity and death were observed over a period of 48 hours. The doses of the second phase which depended on the result obtained from phase I, consisted of three mice divided into three groups of one mouse each, administered with *C. papaya* leaves extracts at 2000, 4000, and 5000 mg/kg orally respectively.

Animals and Treatments

Twenty-five healthy male Wistar rats weighing 130 – 134 g were used for the hepatoprotective study. The animals were randomly assigned into five groups (I - V) and treated as follows: (DW, CCl_4+DW, CCl_4+CPE200, CCl_4+CPE400, CCl_4+VC100) of five rats each. Liver injury was induced with CCl_4 using modified method of (Okpara, 2015).

Group I,DW – untreated control, was given 3 ml/kg body weight of distilled water *per os*.

Group II, was given 0.2 ml/rat of CCl_4 in Olive oilintraperitoneally and dosed with distilled water 3 ml/kg.

Group III, was given 0.2 ml/rat of CCl_4 in Olive oil intraperitoneally and dosed with 200 mg/kg body weight of *Carica papaya* leaf extract (CPE).

Group IV, was given 0.2 ml/rat of CCl_4 in Olive oil intraperitoneally and dosed with 400 mg/kg of CPE.

Group V, was given 0.2 ml/rat of CCl_4 in Olive oil intraperitoneally and dosed with 100 mg/kg of vitamin C (Ambali *et al*., 2011).

These regimens were administered orally by gavages once daily for 21 days. At the end of the study period, the rats were sacrificed by jugular venesction after light chloroform anaesthesia. Two milliliters (2 ml) of blood was collected from each rat in EDTA sample bottles and used to evaluate the haematological parameters. While another 2 ml of blood were collected from each rat in

non-EDTA Sample bottles and allowed to clot before been centrifuged at 1000 xg and the serum used for the evaluation of ALT, AST and ALP activities and the concentrations of albumin. While the liver tissue from each rat were assayed for the concentration of malondialdehyde (MDA) as an index of lipoperoxidation (Drapper and Hadley, 1990; Okpara, 2015).

Haematological Analysis

Two milliliters (2 ml) of blood samples collected from each animal were placed in sterile sample bottle containing anticoagulant (EDTA 1 mg/ml) for the analysis of haematological parameters (RBC, WBC, PCV, Hb (Coles, 2005).

Evaluation of Serum Enzymes Activities

The serum samples were analysed for the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) as described by Rec, (1972), AST was assayed by monitoring the concentration of oxaloacetic hydrazine formed with 2,4-dinitrophenyl hydrazine colorimetrically at 540 nm. α-oxaglutarate + L-aspartate = glutamate + oxaloacetate. ALT was measured by monitoring the concentration of pyruvatehydrozone formed with 2, 4-dinitrophenyldrazine at 540 nm (Bulama *et al*., 2017). α − oxaglutarate + L-alanine = L-glutarate+ pyruvate. The ALP activity was assayed by hydrolysis of *P*-nitrophenyl phosphatate as described by Rec (1972). Phenols released by enzymatic hydrolysis from phenyl phosphatate at temperature of 37^{o}C and pH of 9.8 were estimated colorimetrically at 405 nm. *P*-nitrophenyl phosphate + water = phosphate of *P*-nitrophenol (Rec, 1972; Reitman and Frankel, 1975).

Evaluation of Hepatic Lipoperoxidation

The MDA concentration of the liver was assayed using the double heating method of Draper and Hadley (1990); Ambali *et al*., (2011). Briefly, 0.3 g of liver tissue was homogenized in 30 ml of cold phosphate buffered saline and centrifuged at 3000 xg for 10 min. The supernatant from each homogenate was divided into two parts for MDA and protein concentration, respectively. The protein concentration was determined using the method described by Lowry *et al*., (1954). For the determination of MDA concentration 0.25 ml of the supernatant was mixed with 0.5 ml of 10% trichloroacetic acid and then heated in a boiling water bath for 15 min. After cooling under running water for 5 min, the mixture was centrifuged at 1600 xg for 10 min. 1 ml of the supernatant was then added to 0.5 ml of 6.7 g/L TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was cooled under running tap water and the absorbance was then measured at 532nm using a UV. Spectrophotometer (T80 UVVIS Spectrometer Ltd., UK). The MDA concentration was calculated by the absorbance co-efficient
MDA – TBA complex 1.56 x 105/cm and expressed in nmol/mg of protein.

Serum Albumin Determination

This was assayed based on its quantitative binding (dye binding method) to the indicator 3, 3, 5, 5 – tetrabromo-M-cresol sulphonaphthalein (bromocresol green BCG). The albumin – BCG complex absorbs maximally at 378 nm, the absorbance been proportional to the concentration of albumin in the sample.

Data Analysis

Data were expressed as means± SEM for five rats. All data were analysed by one way analysis of variance (ANOVA) to determine the extent of variation between the groups. Values < 0.05 were considered significant using the statistical package (GraphPadInstat, Version 2000).

Results

Table I: Median lethal dose (LD_{50}) for *Carica papaya* leaves aqueous extract – Phase I.

Group	Dose (mg/kg) (*per os*)	No. Dead/No. Dosed
1.	10	0/3
2.	100	0/3
3.	1000	0/3

Table 2: Median lethal dose LD_{50} for *C. papaya* leaves aqueous extract – Phase II

Group	Dose (mg/kg) *per os*	No Dead/No. Dosed
1.	200	0/1
2.	4000	0/1
3.	5000	0/1

Thus, based on the method of Lorke (1983), LD_{50} was above 5000 mg/kg in the experimental animal (mice) *per os* (Table I and 2).

Table 3 Effects of Treatment on Erythrocyte Parameters of Rats Exposed to Carbon Tetrachloride (n=5; Mean ± SEM)

Group	RBC ($X10^{12}$/L)	PCV (%)	Hb (g/dL)
DW	11.02 ± 0.32^{a}	42.55 ± 0.56^{a}	13.81 ± 0.33^{a}
CCl_4+DW	7.14 ± 0.06^{b}	23.80 ± 0.37^{b}	9.60 ± 0.18^{b}
CCl_4+CPE200	9.80 ± 0.14^{a}	39.68 ± 0.12^{a}	12.08 ± 0.75^{a}
CCl_4+CPE400	10.46 ± 0.32^{a}	40.43 ± 0.25^{a}	12.51 ± 0.80^{a}
CCl_4+VC	10.57 ± 0.19^{a}	41.67 ± 0.28^{a}	13.04 ± 0.57^{a}

Values are mean ± standard error of the mean (SEM)
Means denoted by different superscripts on same columns and significantly different ($P< 0.05$).

Table 4: Effects of Treatment on Total and Differential Leucocyte Counts in Rats Exposed to Carbon Tetrachloride (n = 5; means ± SEM)

Group	Dose (mg/kg (*p.o*)	TWBC (X109/L)	Neurosis (X109/L)	Lymp (X109/L)
DW	3 ml/kg	5.37 ± 0.38[a]	3.42 ± 0.10[a]	1.95 ± 0.17[a]
CCl_4+DW	3 ml/kg	2.52± 0.03 [b]	1.46 ± 0.04[b]	1.06 ± 0.04[b]
CCl_4+CPE200	200	4.73± 0.20 [a]	2.89 ± 0.11[a]	1.84 ± 0.05[a]
CCl_4+CPE400	400	4.88 ± 0.07[a]	3.07 ± 0.04[a]	1.89 ± 0.02[a]
CCl_4+VC	100	4.89 ± 0.13[a]	3.14 ± 0.03[a]	1.90 ± 0.04[a]

Values are mean ± standard error of the mean (SEM)
Means denoted by different superscripts in same column are significantly different (P< 0.05)

Table 5: Effects of Treatments on Liver Enzymes Activity and Albumin Concentration in Rats Exposed to Carbon Tetrachloride and Treated with CPE or Vitamin C (n=5; Mean ± SEM)

Group	Dose mg/kg (*p.o*)	AST (i.u/L)	ALT (i.u/L)	ALP (i.u/L)	ALB (g/L)
DW	3 ml/kg	36.88 ± 0.10[a]	19.07 ± 0.03[a]	50.14 ± 0.21[a]	52.24 ± 0.17[a]
CCl_4+DW	3 ml/kg	135.38 ± 0.72[b]	104.52 ± 0.81[b]	124.140 ± 0.13[b]	28.31 ± 0.15[b]
CCl_4+CP200	200 mg/kg	61.26 ± 0.18[a]	22.60 ± 0.11[a]	66.54 ± 0.32[a]	40.14 ± 0.30[a]
CCl_4+CP400	400 mg/kg	66.21 ± 0.35[a]	22.04 ± 0.13[a]	62.30 ± 0.83[a]	44.36 ± 0.74[a]
CCl_4+VC	100 mg/kg	51.74 ± 0.20[a]	20.18 ± 0.20[a]	58.43 ± 0.51[a]	46.37 ± 0.41[a]

Values are mean ± standard error of the mean (SEM)
Means denoted by different superscripts are significantly different (P< 0.05)

Table 6: Effects of Treatments on Malondialehyde Concentrations in Rats Exposed to Carbon Tetrachloride (n=5; Mean ± SEM)

	Group DW	CCl_4+DW	CCl_4+CPE200	CCl_4+CPE400	CCl_4+VC
MDA Concentration ng/mgLiver Tissue	0.38 ± 0.01[a]	0.89 ± 0.11[b]	0.44 ± 0.13[a]	0.42 ± 0.35a	0.40 ± 0.63[a]

Values are mean ± standard error of the mean (SEM) n=5
Means denoted by different superscripts are significantly different (P< 0.05).

Results

Preparation of *C. papaya* Crude Aqueous Extract

The initial weight of the fresh *C. papaya* leaves was 1.25 kg, while the final dry weight was 451 g, representing 63.2% moisture content and 26.8% dry matter w/w. The 451 g of the sample yielded 75.9 g crude extract constituting about 16.5% yield sample by weight.

Median Lethal Dose (LD_{50}) determination

Toxic signs of restlessness, inco-ordination, clonic convulsion, dyspnoea and rough hair coat were observed in rats administered 5000 mg/mg, however, no death was recorded.

Table 3: Phytochemical Constituents of *Carica papaya* Leaf Aqueous Extract

Phytochemical	Inference
Glycosides	+ve
Flavonoids	+ve
Saponnins	-ve
Triterpenoids	-ve
Resins	-ve
Alkaloids	+ve
Tannins	+ve
Carbohydrate	+ve
Reducing sugars	+ve

Key
+ve = Present
-ve = absent

Phytochemical analysis of the aqueous crude extract of *Carica papaya* leaves gave positive results for glycosides, flavonoids, tannins, alkaloids, carbohydrate and reducing sugars. While resins, saponnins and triterpenoide were absent.

Effects of Treatment on Haemotological Parameters of Rats Exposed to CCL_4

The effects of treatments on haematological parameters are shown in Table 3 - 4. There was a significant decrease ($P< 0.05$) in the RBC counts, PCV, and Hb concentration of the CCl_4+DW rats compared to the values obtained for control (DW), the *C. papaya* leaves extract (CCl_4+CPE 200, CCl_4+CPE400 and vitamin C (CCl_4+VC) treated groups, respectively. There was no significant difference ($P> 0.05$) between the control group and the treated groups (Table 3).

Furthermore, there was a significant ($P< 0.05$) decrease in the absolute total WBC counts and the differential leucocyte counts of the CCl_4+DW group (Table 4), compared to the values obtained for the control (DW), the *C. papaya* leaves extract (CCl_4+CPE200, CCl_4+CPE400 and vitamin C (CCl_4+VC) treated groups, respectively. There was no significant change ($P> 0.05$) in the total TWBC and differential leucocyte count of the control (DW) compared with the values obtained for the extract (CCl_4+CPE200, CCl_4+CPE400 and vitamin C (CCl_4+VC) treated groups respectively (Table 4). Although the values were comparatively higher in the control (DW) compared to the extract and vitamin C treated groups (Table 3 and 4).

Effects of Treatments on Serum Hepatic Enzymes Activities

A significantly ($P< 0.05$) high activities of alanine aminotransferase (ALT), aspartate aminotransferease (AST) and alkaline phosphatase (ALP) were obtained in the CCl_4+DW group (Table 5) compared to the values obtained for the control (DW), the *Carica papaya*(CCl_4+CPE 200), (CCl_4+CPE400and vitamin C treated groups, respectively. The ALT, AST and ALP activities were comparatively lower in the control (DW) group when compared with the activities in (CCl_4+CPE200, CCl_4+CPE400 and (CCl_4+VC) treated groups, respectively.

Effects of Treatments on Serum Albumin Concentration

The effect of treatments on serum albumin concentration is shown in (Table 5). There was no significant difference ($P> 0.05$) between the treated groups (CCl_4+CPE200, CCl_4+CPE400) CCl_4+VC) and the control (DW) group, However, a comparative increase in the concentration of serum albumin was recorded in the control (DW) groups. There was a significant decrease ($P< 0.05$) in the concentration of serum albumin of the CCl_4+DW group compared to the values obtained for the *C. papaya* extract and vitamin C treated groups respectively (Table 5).

Effects of Treatments on Hepatic Malondialdehyde Concentration

The effect of treatments on hepatic malondialdehyde concentration is shown in Table 6. There was a significant ($P< 0.05$) increase in the concentration of hepatic MDA in the (CCl_4+DW) group compared to the other groups. There was no significant increase ($P> 0.05$) in the concentration of hepatic MDA in between the treated groups and the control (DW) (Table 6).

Discussion

Toxic signs of restlessness, inco-ordination dyspnoea and stupor seen in the rats acutely poisoned with the extract of C. papaya leaves were indicative of respiratory and nervous impairment (Okpara, 2015), and could be attributed to oxidative damage to the cells of these system (Yokomizo and Moriwaki, 2006). Therefore, the observed toxic sign in the acutely poisoned rats could be due to pro-oxidative effect of the high dose of the extract. The pro-oxidative effects occur due mostly to generation of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) (Yokomizo and Moriwaki, 2006, Okpara, 2015; Okpara *et al*., 2017).

Phytochemical screening of the aqueous crude extract of *C. papaya* leaves gave positive results for flavonoids, alkaloids, tannins, glycosides, carbohydrates and reducing sugars. Flavonoids rich extracts have been reported to possess antioxidant properties (Okpara, 2015). A significant decrease in haemoglobin concentration (Hb), red blood cell count (RBC) and packed cell volume (PCV) were seen in rats exposed to CCl_4 only (CCl_4+DW). These effects suggest that CCl_4 causes anaemia perhaps due to intracellular oxidative stress and its attendant fragility (Ebaid *et al*., 2013; Okpara, 2015). The primary metabolites of CCl_4 metabolism, trichloromethyl and trichloromethyl peroxyl free radical are highly reactive. They are capable of initiating lipid peroxidation by attacking polyunsaturated fatty acids (PUFA) in membranes setting off a free-radical-chain sequence (Adewole *et al*., 2013).

Lipid peroxidation is known to cause membrane disruption, resulting in loss of membrane integrity and leakage of microsomal enzymes (Ambali *et al*., 2011; Buloma *et al*., 2017). The anaemia in the CCl_4+DW group affirmed by decrease Hb concentration could also be attributed to the ability of CCl_4 to disrupt homeostasis and haemoposis due to intracellular oxidative stress (Manibusan *et al*., 2007). It may have also resulted from the haemolysis of the erythrocytes in circulation due to increased osmotic fragility (Zaib and Khan, 2014).

The significant increase in erythrocyte parameters by the various doses of *C. papaya*

leaves extract demonstrated the role of oxidative stress in the toxic mechanism of CCl_4 exposure. Theprotective effects of the *C. papaya* extract are comparable to that of vitamin C although the effect is dose dependant. The antioxidative activity of most flavonoids and flavonoid rich plant extract involves hydrogen atoms donation to peroxyl radical, thus terminating the chain rections (Okpara, 2015). The findings agrees with previous findings of Babalola *et al*. (2000); Anaga *et al*. (2000) and Okpara, (2017) that the hepatoprotection and remarkable improvement in haematological parameters of rats treated with flavonoid fraction, and flavonoid rich extract before exposed to CCl_4 was due to the antioxidant and hepatoprotective effects of flavonoids. This implies that oxidative stress plays a significant role in the damage induced by CCl_4 which is attenuated by the aqueous extract of *C. papaya* leaves.

The exposure of rats to CCl_4 in the present study also resulted in a significant reduction in absolute total white blood cell (WBC) and differential leucocyte counts. These findings also agreed with the result obtained in a similar study by Okpara, 2015). Activated neutrophils have been demonstrated to play an essential role in free radical mediated injury by inducing extracellular release of superoxide and other free radical (Khan and Saddique, 2012), which are toxic to cells, including neutrophils and lymphocyctesresulting in their decrease in the peripheral circulation (Okpara, 2015)

However, treatment with various doses of *C. papaya* leaves extract and vitamin C caused significant restoration of the total and differential leucocyte counts. This could be attributed to their chain breaking antioxidant activities which plays a vital role in the maintenance of cellular membrane integrity, as well as stimulating the immune system (Ebaid *et al*., 2013), especially in the face of oxidative assault by chemical toxicants like CCl_4 (Adewole *et al*., 2013). This may have contributed to the improvement of leucocyte count by stabilizing cellular membranes from the effect of free-radical-mediated toxicity.
In addition, the significantly low serum albumin concentration in the CCl_4+DW rats may have been due to oxidative stress which disrupted hepatic and renal functions (Adewole and Abiodun, 2014). Furthermore, the CCl_4-evoked hypoalbuminemia may be partly due to the fact that during oxidative stress antioxidants such as albumin are used to combat the menace of oxidative stress (Ebaid *et al*, 2013; Okpara, 2017).The various doses of *C. papaya* leave aqueous extract significantly improved the concentration of albumin comparable to the effect of vitamin C – a chain breaking antioxidant.

The result also showed that exposure of rats in CCl_4+DW to CCl_4 caused significant increased the activity of AST, an enzyme found not only in the liver but also in the skeletal muscles and myocardial cells. The significant increase in AST activity may indicate hepatic or muscle damage induced by CCl_4, thereby provoking increased AST liberation into the peripheral circulation. Treatment with *C. papaya* leaves extract and vitamin C caused a decrease in the activity of AST suggesting amelioration of hepatic and muscular damages. The significant increase in ALT activity recorded in the CCl_4+DW group is an indication of hepatic damage since the enzyme is more liver specific than AST (Ambali *et al*., 2011; Adewole *et al*., 2014; Okpara,*et al*., 2016). The ameliorative effect of various doses of *C. papaya* leaves extract is comparable to vitamin C - a chain breaking antioxidant.

A significant elevation of ALP activity was also seen in the CCl_4+DW group exposed to CCl_4. Increased ALP activity is not limited to the liver damage only. It is also associated with pathological changes in the bone, kidneys, bile duct and testes (Adewole and Abiodun, 2011; Ambali *et al*., 2011).

Therefore, the high ALP activity in the CCl_4+DW rats may be due to perturbation in any of these organs. The significant increase in serum MDA concentration observed in the CCl_4+DW group indicated that CCl_4evoked lipoperoxidative damage to the tissue through free radical induction. The finding agreed with results obtained in previous studies (Adewole *et al*., 2014; Okpara, 2015; Bulama *et al*., 2017). Tissue lipid peroxidation is a degenerative phenomenon as a consequence of free radical chain production and propagation which affect mainly PUFA. The significantly low MDA concentration in vitamin C (CCl_4+VC) and the various *C. papaya* leaves extract (CCl_4+CPE200, CCl_4+CPE400) treated groups respectively showed their ability to quench CCl_4-evoked tissue lipoperoxidative damage. This may have been responsible for the amelioration of the CCl_4-provoked clinical, haematological and biochemical change by *C. papaya* leaves crude aqueous extract and vitamin C.

In conclusion, the use of aqueous extract of *C. papaya* leaves in folk medicine has positive correlation with scientific data. *C. papaya* is used by many tribes for treatment of anaemic conditions and liver related disorders. The plant can be relatively safe to use in the field of ethno-medicine considering its high median lethal dose.

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