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Original Research Article

Effect of ethanol leaf extract of pawpaw (carica papaya) on carbon tetrachloride induced-oxidative stress and hepatotoxicity in wistar rats

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Abstract

Introduction: Carbon tetrachloride (CCl4) is a potent hepatotoxin that causes oxidative stress and liver damage. Carica papaya has been traditionally used in traditional medicine for its antioxidant and hepatoprotective properties, suggesting potential benefits in mitigating CCl4-induce liver damage. This study investigates the hepatoprotective effect of ethanol leaf extract of Carica papaya against CCl4-induced oxidative stress and hepatotoxicity in male Wistar rats.

Materials and Methods: Twenty five wistar rats weighing 140 ± 20g were divided into five groups (n=5): normal control, CCl4 control(positive), low-dose treatment (200mg/kg), high-dose treatment (400mg/kg) and Silymarin(140 mg/kg). The rats were induced twice a week with CCl4 and treatment followed immediately after induction per their body weight for 28 days. Biochemical analysis such as Haematological Assays, Hepatic Function Assays, Kidney Function Endogenous, Antioxidant Status and Histopathology were evaluated and also phytochemical property such as Phenols, tannins, alkaloids, arthraquinones, cardiac glycosides, flavonoids and saponins were determined.

Result: The results showed that the extract significantly reduced liver enzyme levels, oxidative stress markers, and histopathological changes induced by CCl4.The Hepatotoxicity effect of carbon tetrachloride (CCL4) and ECPon the activity of wistar rat liver showed that thetreatment with ECP produced a reductionin hepatic enzyme markers (ALT, AST, GGT and ALP) that were high as a result of the induction process by CCL4, this suggest that the phytochemicals in the plant extract have ameliorative potential when compared with the positive control group as shown in table 4.4 For renal enzymatic markers (Blood Urea Nitrogen and The Rate of Creatine, The results showed that the extract significantly reduced liver enzyme levels, oxidative stress markers, and histopathological changes induced by CCl4.

Conclusion: This study revealed the activities of various marker enzymes and levels of metabolites/compounds in the blood taken after the 28 days from the experimental animals in both the control and extract- treated groups, and were used as indicators for renal and hepatic status.This study demonstrates the hepatoprotective and antioxidant effects of ethanol leaf extract of Carica papaya against CCl4-induced oxidative stress and hepatotoxicity in wistar rats.

Keywords: Carica papaya, Carbon tetrachloride, Hepatotoxicity, Oxidative stress, Wistar rats.

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1. Introduction

Carica papaya L., commonly known as papaya, pawpaw, or kates,[1,2] is a perennial horticultural shrub native to Mesoamerica, Central America, and southern Mexico. It belongs to the Caricaceae family and[3] is extensively cultivated in tropical and subtropical regions worldwide, including Brazil, Australia, Malaysia, China, India, Thailand, Myanmar, and the Philippines.

In traditional medicine, papaya leaves are utilized in various ways. A decoction of fresh leaves is used as a tea to treat malaria, while dried leaves are smoked to alleviate respiratory disorders like asthma. In some countries, young leaves are steamed and consumed as a leafy vegetable.

Papaya leaves contain over fifty bioactive components, making them valuable in managing various human ailments.[4,5] They are rich in glycosides, flavonoids, alkaloids, saponins, phenolics, amino acids, lipids, carbohydrates, enzymes, vitamins, and minerals. The crude ethyl acetate isolates of papaya leaves exhibit strong antiplasmodial activity against Plasmodium falciparum, including resistant strains.[6,7]

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The leaves are now recognized as a functional food due to their antiviral and immunity-boosting properties. Papaya leaf tea is also used as a dietary supplement for oxidative stress-related diseases due to its strong antioxidant capacity.[8] Fresh papaya leaves possess antiseptic properties, while dried leaves can act as a blood purifier and digestive tonic.

The juice of papaya leaves has demonstrated various beneficial effects, including anticancer,[9] antioxidative,[1,3] anti-inflammatory,[10] antimicrobial,[11] and antisickling properties.[12] Additionally, it has shown nephroprotective,[13] hepatoprotective,[14] hypoglycemic, and hypolipidemic properties[15] against various toxins.[16] Polar isolates from papaya leaves also exhibit anti-HIV, analgesic, and wound-healing properties.

Phytochemicals are natural compounds found in plants, making them valuable for therapeutic applications. Papaya leaves, in particular, are rich in health-promoting phytochemicals as ev idenced by numerous studies.[17] The Studies has identified significant quantities of alkaloids, saponins, glycosides, flavonoids, phenolic compounds, enzymes, amino acids, lipids, carbohydrates, vitamins, and minerals in papaya leaves.[9]

Specific flavonoids and phenolic substances have been identified, including quercetin, kaempferol 3-rutinoside, myricetin 3-rhamnoside, caffeic acid, protocatechuic acid, and chlorogenic acid. Papaya leaves also contain bioactive phytochemicals like carpaine, kaempferol derivatives, luteolin, orientin, and other compounds.[16]

These bioactive components make papaya leaf extracts suitable for use in nutraceutical and herbal medicinal formulations. Traditionally, papaya leaves have been used in various cultures to manage diabetes, malaria, fungal infections, and cancer.[5-7]

The bioactive components in papaya leaves, such as papain, cystatin, chymopapain, tocopherol, phenolic acids, cyanogenic glucosides, glucosinolates, and vitamin C, contribute to their antioxidant potential.[18] The compounds like alkaloids, saponins, glycosides, phenolic compounds, and flavonoids are responsible for the leaves' anti-inflammatory and anticancer properties.[19]

The vitamins, minerals, and amino acids in papaya leaves help enhance hemoglobin levels, protein synthesis, and overall immunity.[20] Carpaine, a significant bioactive compound, possesses properties that help regulate high blood pressure and heart rate, relax the uterus, and exhibit antiplasmodial activity. It is widely used in Ayurvedic formulations for managing physical disorders and viral fevers like dengue and chikungunya.[21]

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals and reagents

Carbon tetrachloride (CCl_4), thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium glutamate, 5',5'-dithiobis-(2-nitrobenzoic acid) (DTNB), adenosine triphosphate (ATP), 6,7-dimethyl-5,6,7,8-tetrahydropterine (DMTHP), sodium pyruvate, benzylamine hydrochloride (BAHC), reduced nicotinamide-dinucleotide (NADH), xanthine, ethylenediaminetetraacetic acid (EDTA), 1-amino-2-naphthol-4-sulfonic acid (ANSA), sodium azide, α-ketoglutarate, N-(1-naphthyl)ethylenediamine, sulfanilamide, ammonium chloride, and ammonium molybdate were sourced from Sigma-Aldrich (St. Louis, MO, USA). Methanol, ethanol, hydrochloric acid, hydrogen peroxide, and formalin were procured from Scharlau (Scharlab S.L, Spain). All other chemicals and reagents were of analytical grade. The assay kits were obtained from Randox Laboratory Ltd. (Antrim, United Kingdom) and Teco Diagnostics (Anaheim, CA, USA).

2.2. Collection of plant material

Carica papaya leaves were collected in Akure, Ondo State. The leaves were identified and authenticated at the Center for Research and Development (CERAD), Federal University of Technology, Akure.

2.3. Animals

Twenty-five (25) Male Wistar rats, weighing between 140 and 250 g, were procured from an animal housing facility in Ogbomoso, Nigeria, and brought to the Animal House in the Department of Biochemistry at the Federal University of Technology, Akure, Nigeria. The animals were allowed to acclimatize for 14 days and they were providedwith free access to feed and water. The handling and use of the animals followed the guidelines of the National Research Council (US) Committee Guide for the Care and Use of Laboratory Animals (2011).

2.4. Methods

2.4.1. Preparation of Carica papaya

Carica papaya leaves were air-dried at room temperature for 14 days and were pulvirised into a fine powder. One thousand grams of the powdered leaves were macerated in 3000 mL of ethanol (the extraction solvent) for 48 hours at 50°C. The filtrates were collected initially through a fine linen cloth and finally through Whatman filter paper. The filtrate was freeze-dried using a freeze-drying machine to give dried residue. The extract was stored in an air-tight container in a desiccator at 4°C for further analysis. The bioactive compounds in the leaf extract were then analyzed using High-Performance Liquid Chromatography (HPLC).

2.5. Experimental design/animal grouping for CCL_4 induction and treatment

Carbon tetrachlorides (CCl_4) were induced to the animals by intravenous injection twice a week. Thereafter, Ethanol leaf extract of carica papaya (ECP) was prepared and administered to animals in each group according to their body weight through oral gauge for twenty-eight days. The animals were divided of similar weight into 5 groups of n=5 animals each:Group 1 (negative control): Administered with vehicle (0.9% normal saline) only,Group 2(CCL_4):Induction with CCL_4 but not treated(positive control or toxicity group), Group 3 (CCL_4 + ECP): Induction with CCL_4 and treatedECP (200 mg/kg), Group 4 (CCL_4+ ECP): Induction with CCl_4 and treatedECP (400 mg/kg) and Group 5 (CCL_4 + Silymarin): Induction withCCL_4 and treated Silymarin (140mg/kg)

2.6. Animal sacrifice and collection of blood and excision of kidney and liver

The rats' body weights were measured before exposure to administrarion and again before sacrifice. Following the treatment period, the animals were fasted for 24 hours and sacrificed by cervical dislocation. Blood samples were collected via cardiac puncture into clean, dry heparinized and non-heparinized centrifuge tubes. The blood in non-heparinized tubes was left to clot for approximately 30 minutes, after which the serum was separated by centrifuging the clotted blood at 4000 rpm for 10 minutes using centrifuge. The clear supernatant was collected and stored in a refrigerator at 4°C. Meanwhile, the blood in heparinized tubes was used immediately for hematological analysis. The kidneys and liver were swiftly excised, rinsed in ice-cold 1.15% KCl, blotted with filter paper, and weighed. Portions of the kidneys and liver were preserved in formalin for histological examination.

1. Biochemical assays
   a. Evaluation of the in Vivo Antioxidant Activity Ofcaricapapaya
      i. Determination of Malondialdehyde Concentration
      ii. Determination of Glutathione Peroxidase (Gpx) Activity
      iii. Determination of Reduced Glutathione (Gsh) Concentration
      iv. Determination of Glutathione Transferase Activity
      v. Determination of Superoxide Dismutase (Sod) Activity.
   b. Toxicological Assessment of Ethanol Leaf Extract Of Carica Papaya
      i. Serum Parameters
      ii. Evaluation of Hepatic Function
         - Evaluation of Aspartate Amino Transferase (Ast) Activity
         - Evaluation of Alanine Amino Transferase (Alt) Activity
         - Evaluation of Alkaline Phosphatase (Alp) Activity
         - Evaluation of Gamma-Glutamyl Transferase (Ggt) Activity
   c. Evaluation of Kidney Function
      - Determination of Creatinine Clearance Rate
      - Blood Urea Nitrogen Concentration
   d. Evaluation of Inflammation
      i. Determination of Myeloperoxidase Activity
      ii. Determination of Tumor Necrosis Factor-A (Tnf-A) Concentration.
      iii. Determination of Interleukin-1 Beta (Il-1b) Concentration
      iv. Determination of Interleukin-6 (Il-6) Concentration
2. Histological Examination
3. Histopathological Assessment was Carried Out According to De Lillo Et Al, (2010).
4. Statistical Analysis

3. Results

3.1. Phytochemical constituents

Phytochemical screening of ethanol leaf extract of *Carica papaya* confirmed the presence of steroids, phenols, tannins, alkaloids, flavonoids, and terpenoids. (**Table 1**). Spectrophotometric quantification showed an appreciable amount of total tannins, phenols and flavonoids (**Table 2**). **Table 3** showed the phytocompound present in *Carica papaya* leaf extract. The leaf extract has ellagic acid, tannin, as its most abundant phyto-compound with a concentration of 36.42.44mg/kg.

Table 1: Phytochemicals Detected in Extract of *Carica papaya*

Tests	Result
Phenols	+
Tannins	+
Alkaloids	+
Arthraquinones	-
Cardiac glycosides	+
Flavonoids	+
Saponins	++

'+' represent present while '-' represent absent

Table 2: Total Contents of Major Phytochemicals in Extract of *Carica papaya*

Phytochemical	Quantity (µg/g)	Equivalent standard
Phenols	311.03±0.00	Gallic acid
Flavonoids	105.38±0.34	Quercetin
Tannins	45.56±0.79	Tannic acid

Results are expressed as mean ± SD (n=3).

Table 3: High performance liquid chromatography quantification of chemical compounds in extract of *caricapapaya*

Standard compound	Class of compound	Standard retention time	*Caricapapaya*	
			Area (mUA)	Concentration (mg/kg)
Caffeic acid	Phenolic acid	7.625	10160.000	10.82±0.51
Cinnamic acid	Phenolic acid	12.842	19124.406	6.21± 0.99
Ferulic acid	Phenolic acid	16.459	49954.625	12.98±0.77
Quercetin	Flavonoids	39.004	565241.000	5.94±0.38
Gallic acid	Tannins	3.624	03122.215	0.47±0.11

Table 4: Effect of PE on the activities of serum liver enzymes, AST, ALT, ALP, and GGT.

	AST Activity (U/l)	ALT Activity (U/l)	ALP Activity (U/l)	GGT Activity (U/l)
Control	45.24 ± 1.41^{a}	39.63 ± 1.41^{a}	2.95 ± 0.38^{a}	68.29 ± 1.62^{a}
Positive control	87.12 ± 1.30^{e}	82.02 ± 1.42^{b}	9.30 ± 0.06^{d}	121.81 ± 2.36^{d}
200mg/kg (ECP)	58.22 ± 0.84^{d}	53.80 ± 1.75^{c}	4.78 ± 0.08^{c}	91.98 ± 2.17^{c}
400 mg/kg (ECP)	47.63 ± 1.85^{b}	49.49 ± 1.06^{b}	3.26 ± 0.11^{b}	78.80 ± 1.10^{b}
Silymarin 140mg/kg	52.50 ± 0.85^{c}	47.54 ± 0.52^{b}	4.68 ± 0.15^{c}	75.98 ± 1.37^{b}

Effects of ECP against CCL_4 assault on the Activity of AST, ALT, ALP and GGT in the serum. The results are expressed as mean ± standard deviation, with n=5. Values with different superscripts are significantly different ($p<0.05$).

Key:

Group 1: Negative control

Group 1: Positive control

Group 3: CCL_4 induction and 200mg/kg BWT of ECP

Group 4: CCL_4 induction and 400mg/kg BWT of ECP

Group 5: CCL_4 induction and 140mg/kg BWT of Silymarin

Table 5: The effect of CPM on the blood urea nitrogen and % creatinine clearance of thekidney.

	Blood Urea Nitrogen (mg/dl)	% Creatinine clearance
Control	32.28 ± 0.81^{a}	7.48 ± 0.07^{d}
Positive control	69.52 ± 0.65^{d}	3.82 ± 0.20^{a}
200 mg/kg ECP	42.48 ± 1.80^{c}	5.09 ± 0.17^{b}
400 mg/kg ECP	37.99 ± 0.26^{b}	6.78 ± 0.11^{c}
140mg/kgSilymarin	36.71 ± 0.26^{b}	6.42 ± 0.11^{c}

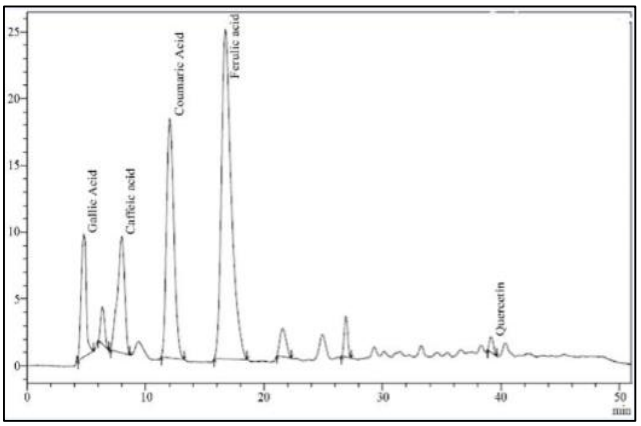

Chart 1: The chromatogram of *Carica papaya*

Effects of ECP against CCL_4 assault on the Concentration of Blood Urea Nitrogen and % Creatine Clearance in the serum.

The results are presented as mean ± standard deviation, with n=5. Values marked with different superscripts indicate significant differences ($p<0.05$).

3.2. The effect of ECP on the activities of serum liver enzymes.

Table 4 shows the effect of ECP on the activities of serum liver enzymes, AST, ALT, ALP, and GGT. A dose-dependent significant decrease ($p<0.05$) was observed in the positive control group in all the liver enzyme markers as revealed in the table below. The treated groups (ECP and Silymarin) shows an ameliorating effect on the activity of the enzyme markers.

3.3. The effect of ECP on the blood urea nitrogen and % creatinine clearance.

Table 5 shows the effect of ECP on the blood urea nitrogen and % creatinine clearance of the kidney. These biochemical parameters reflect the kidney's functioning. In the present study, it was recorded that the serum urea level in treated Group iii-v decreased significantly ($p < 0.05$) with an increase in the dose of ECP.

3.4. Antioxidant potentials of ECP

Carbon tetrachloride IV (CCL_4) induction significantly decreased ($p<0.05$) the concentration of reduced glutathione

(GSH) (**Chart 2**) when compared to the un-intoxicated groups (negative control group), treatment with ECP extract boosted the GSH concentration in the treated groups (groups 3 and 4) and Silymarin (Group 5), thus restoring the depleted defense against free radicals. Superoxide dismutase (SOD) activity (**Chart 3**) was depressed in the testes due to CCL_4 induction, but treatment with dose-dependent ECP significantly ($p<0.05$) elevated the activity of SOD in both the ECP treated group and the Silymarin group. The activity of glutathione transferase (GST) (**Chart 4**) was significantly ($p<0.05$) inhibited by CCL_4-inhibtion when compared to the negative control group ($p<0.05$), administration of dose-dependent ECP and Silymarin to their respective groups significantly ($p<0.05$) elevated the activity of GST.

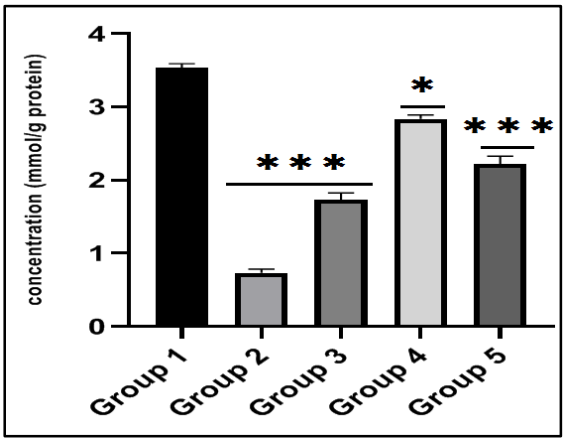

Chart 2: Effects of ECP against CCL_4 assault on the Concentration of reduced glutathione. The results are expressed as mean ± standard deviation, with n=5. Values with different superscripts indicate statistically significant differences ($p<0.05$).

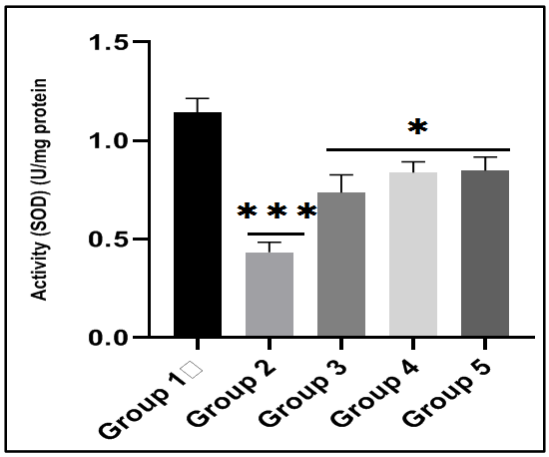

Chart 3: Effects of ECP against CCL_4 assault on the activity of superoxide dismutase. The results are presented as mean ± standard deviation with n=5. Values marked with different superscripts are significantly different ($p<0.05$).

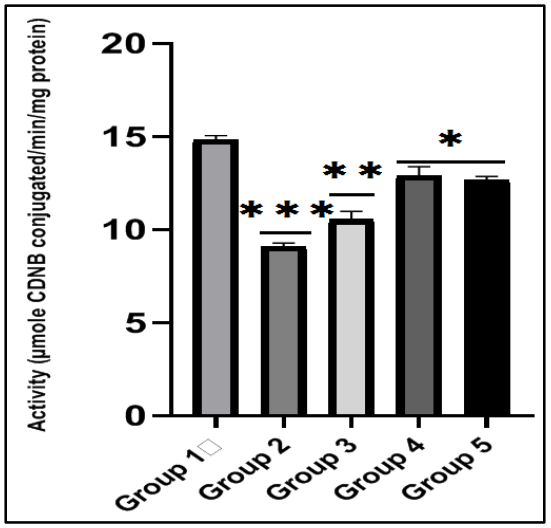

Chart 4: Effects of ECP against CCL_4 assault on the activity glutathione transferase. The results are expressed as mean ± standard deviation (n=5). Values with different superscripts indicate significant differences ($p<0.05$).

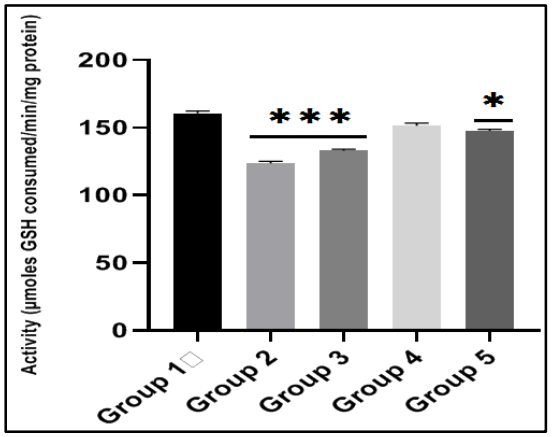

Chart 5: Effects of ECP against CCL_4 assault on the activity glutathione peroxidase. The results are shown as mean ± standard deviation (n=5), with values that have different superscripts being significantly different ($p<0.05$).

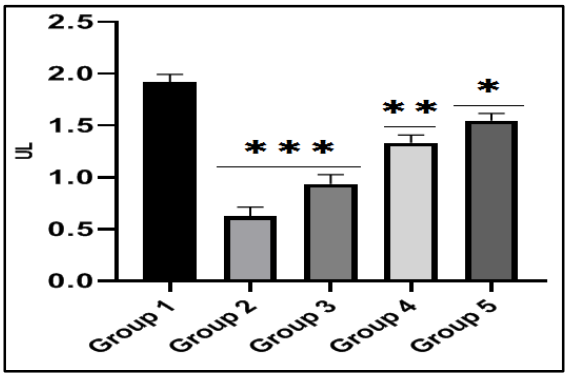

Chart 6: Effects of ECP against CCL_4 assault on the activity MDA. The results are expressed as mean ± standard deviation (n=5), and values with different superscripts are considered significantly different ($p<0.05$).

Glutathione peroxidase (GPx) (**Chart 5**) activity increased after treatment with dose-dependent ECP and Silymarin but

the assaulted group (group 2) were observed to have decreased in their activity of GPx. **Chart 6** MPO, the induced but not treated group showed a significant increase in it concentration, also the treated group (group 3, 4 and 5) was observed to have decreased in their concentration, this is as a result of the effect of the ECP extract reducing the radical effect of MPO.

3.5. Anti-inflammatory potential of ECP

The effects of *C. papaya* extract on the activity of lipid-peroxidase (MDA) shows increased in the induced but not treated group, this is due the effect of the toxicant but for the treated groups of both the extract and the standard drug, the phytochemical component of the extract was able to reverse the effect (**Chart 7**). α-Tumor Necrosis Factor were investigated in the management of oxidative stress and hepatotoxicity in wistar rats as revealed in **Chart 8** the result revealed the effect of ccl_4 induction on the concentration of α-Tumor Necrosis Factor significant increase ($p<0.05$) in the induced but not treated group (positive control group) when compared to the experimental groups. Treatment with ECP significantly decreased the concentration of α-Tumor Necrosis Factor in a dose-dependent manner ($p<0.05$).

Chart 9: Effects of ECP against CCL_4 assault on the activity IL-6. The results are shown as mean ± standard deviation, with n=5. Values marked with different superscripts are significantly different ($p<0.05$). revealed the concentration of Interleukin-6, it was observed that, there was a significant increase ($p<0.05$) in the concentration of Interleukin-6 in the rats due to the induction, but in the experimental groups which the *C. papaya* extract was used for the treatment, a significant decreased on the concentration of Interleukin-6 in a dose-dependent manner ($p<0.05$) was observed.

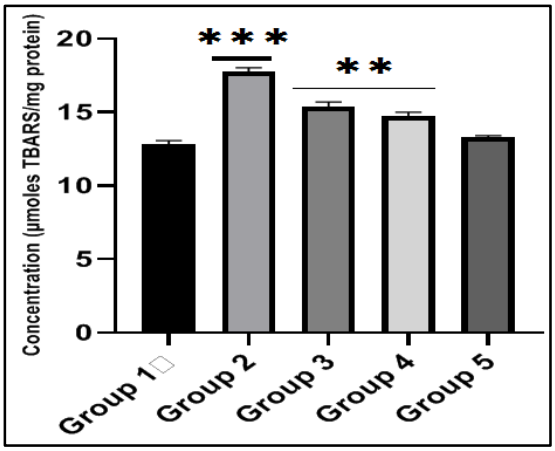

Chart 7: Effects of ECP against CCL_4 assault on the activity MPO. The results are shown as mean ± standard deviation (n=5), with values marked by different superscripts indicating significant differences ($p<0.05$).

Chart 10 interleukin-1β, the concentration of interleukin-1 β increases at the assaulted not treated group, pretreatment with ECP on the treated groups prove that ECP has the ameliorative potentials that elevate the effect of CCL_4toxicant via the decreased in the concentration of interleukin-1β observed.

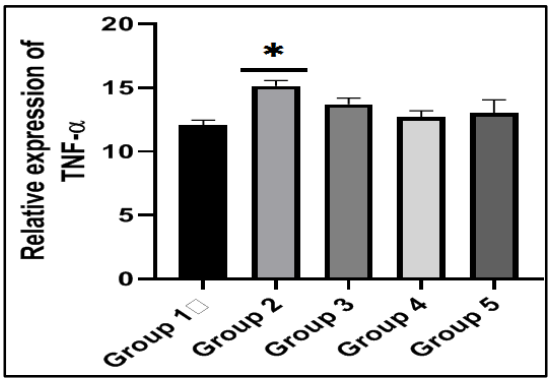

Chart 8: Effects of ECP against CCL_4 assault on the activity α-Tumor Necrosis Factor. The results are expressed as mean ± standard deviation with n=5. Values with different superscripts are considered significantly different ($p<0.05$).

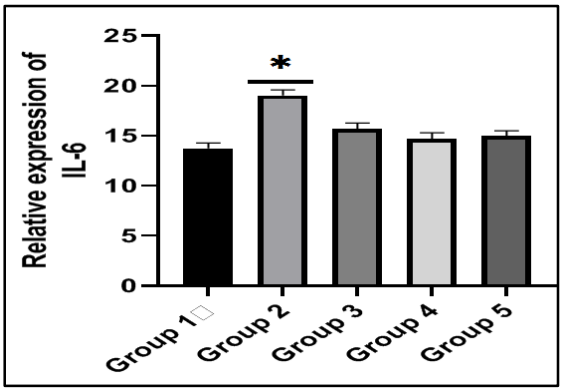

Chart 9: Effects of ECP against CCL_4 assault on the activity IL-6. The results are shown as mean ± standard deviation, with n=5. Values marked with different superscripts are significantly different ($p<0.05$).

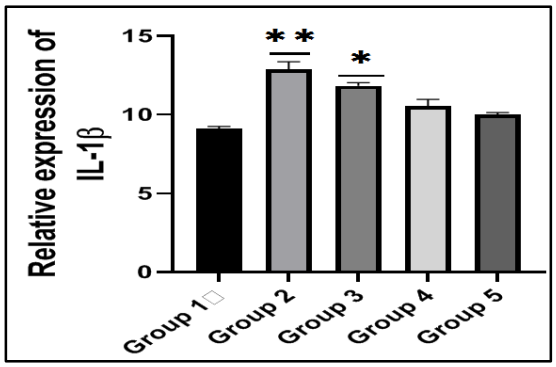

Chart 10: Effects of ECP against CCL_4 assault on the activity IL-β. The results are expressed as mean ± standard deviation, with n=5. Values with different superscripts indicate significant differences ($p<0.05$).

Table 6: The effect of ECP on the hematological parameter of CCL_4 -Toxified Rats

Groups	WBC (10^9/L)	Lymph# (10^9/L)	Mid# (10^9/L)	Gran# (10^9/L)	RBC (10^12/L)	HGB (g/L)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/L)
Grp 1	6.8	4.9	1.1	0.8	7.96	152	47.6	59.8	19.1	319
Grp 2	10.9	9.9	0.6	0.4	8.91	163	49.7	59.7	19.3	325
Grp 3	7.4	6.9	0.4	0.1	8.06	166	51.6	64	20.6	322
Grp 4	7.2	6.5	0.4	0.3	8.53	169	51.7	60.7	19.8	326
Grp 5	7.3	5.5	0.9	1.9	8.23	158	49.4	59.4	19	320

Table 7: Effect of ECP on Histo-architecture Summary in Rats Liver

Plates	Summary
1: (control)	No lesion seen
2: (Positive control)	There is extensive focus of severe tubular degeneration
3: (ECP 200 mg/ kg bwt)	Mild to moderate congestion occur
4: (ECP 400 mg/ kg bwt)	Almost near normal architecture
5: (Slymarin 140 mg/kg bwt)	Almost near normal architecture

3.6. The effect of ECP on the activities of serum liver enzymes

These are activities of various marker enzymes and levels of metabolites/compounds in the blood taken after the 28 days from the experimental animals in both the control and extract-treated groups, and were used as indicators for renal and hepatic status.

3.7. Effect of ECP on histo-architecture of CCL_4 -Toxified rats in the liver and kidney

Table 7 and **Figure 1-5** represent hematoxylin and eosin stained sections of the hepatotoxic region of rats subjected to carbon tetrachloride-induced oxidative stress and hepatotoxicity and treated with ECP. Atrophic degeneration of cell was observed in the liver and kidney region of carbon tetrachloride administered rats (positive control group) while dissolution of nucleus (Karyolysis) was observed in the liver and kidney region of the carbon tetrachloride-induced rats. These histopathological effects were not present in the control group. The damage to the liver and kidney cells were reversed on treatment with ECP.

Figure 1-5 Effect of ECP on Histo-architecture of CCL_4 -Toxified Rats in the Kidney (mg x40)

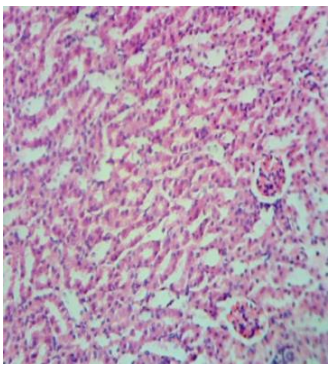

Figure 1: Negative control

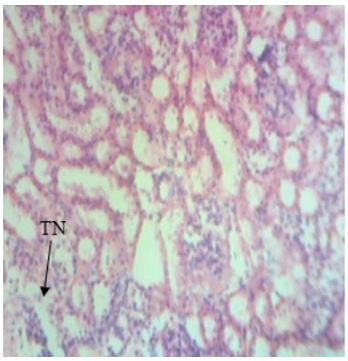

Figure 2: Positive control

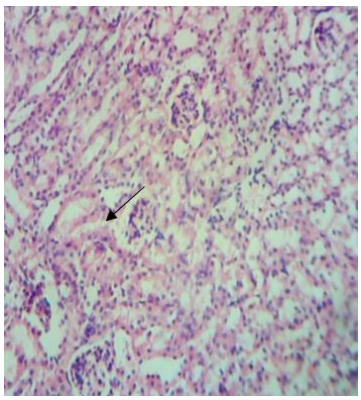

Figure 3: 200mg/ kg bwt

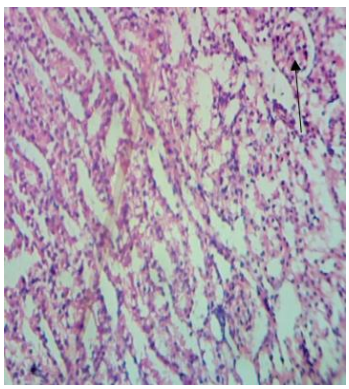

Figure 4: 400mg/ kg bwt

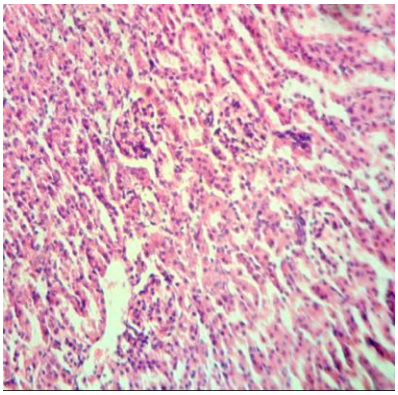

Figure 5: 140mg/ kg bwt

4. Discussion

Carbon tetrachloride (CCl4) is a toxic environmental substance found in both outdoor and indoor air, used in various industrial applications. Exposure to CCl4 can cause acute and chronic effects on the liver, kidneys, and central nervous system (CNS) in humans. Acute inhalation and oral exposure can lead to liver damage (swelling, tenderness, and jaundice) and kidney damage (nephritis, nephrosis, and proteinuria). CNS depression has also been reported[22] and was also observed in this research through various biochemical assays.

The Environmental Protection Agency (EPA) has classified carbon tetrachloride (CCl_4) as a probable human carcinogen (Group B_2). Exposure to CCl_4 has been linked to hepatotoxicity, affecting various hematological parameters, including packed cell volume (PCV), erythrocyte count, white blood cell count (WBC), and others.

However, treatment with Carica papaya leaf extract (ECP) reversed the hematological effects of CCl_4, demonstrating significant positive effects on WBC, RBC, and PCV. ECP's protective role in cellular proliferation is consistent with previous research.[23]

The mechanism involves the potential for iron deposition in Kupffer cells, leading to the generation of reactive oxygen species (ROS) through Fenton and Haber-Weiss reactions. These ROS may contribute to lipid peroxidation, DNA oxidative damage, and potentially carcinogenesis. Despite this, ECP's antioxidant properties appear to mitigate these effects, highlighting its potential benefits in protecting against CCl4-induced toxicity. ECP's role in protecting cellular proliferation aligns with the mechanism described by.[23]

The study also investigated the hepatotoxicity effects of CCl4 and the protective role of ECP. Results in table 3.4 showed that ECP treatment reduced hepatic enzyme markers (ALT, AST, GGT, and ALP) that were elevated due to CCl4 induction. Additionally, ECP treatment improved renal enzymatic markers, including Blood Urea Nitrogen and Creatinine Clearance.

The findings suggest that the phytochemicals in ECP have ameliorative potential, protecting against CCl_4-induced toxicity. The study's results are consistent with previous research, highlighting the potential benefits of ECP in mitigating the adverse effects of CCl_4 exposure.[22,23]

The study investigated the effects of carbon tetrachloride (CCl_4) and Carica papaya leaf extract (ECP) on oxidative stress markers. This result is similar to a report of a research carried out by,[24] it showed an increase in cellular carcinomas in mice exposed to CCl4, consistent with previous research. However, ECP treatment mitigated the hepatotoxic effects by inhibiting CCl_4's toxic activity and increasing antioxidant enzyme activity.

Antioxidant assays (G-S-T, GPx, Reduced Glutathione, SOD, and Malondialdehyde) revealed that ECP-treated groups had significant increases in enzyme concentration/activity due to the presence of bioactive components. In contrast, the CCl_4-induced group showed decreased enzyme activity.

The study also examined pro-inflammatory markers and found that CCl_4 stimulation led to excessive production of TNF-α, IL-1β, and IL-6, causing oxidative stress and tissue injury. However, pre-treatment with ECP reduced NF-κB, a critical factor for pro-inflammatory cytokine expression, thereby decreasing the concentration of pro-inflammatory cytokines. The results shown in figure 3.6 – 3.9, suggest that ECP exhibits antioxidative and anti-inflammatory effects in animal models of oxidative stress and hepatotoxicity. The phytochemical screening of ECP revealed the presence of tannins, phenols, alkaloids, terpenoids, flavonoids, and steroids, which displayed significant antioxidant activities and potential to scavenge radicals and reactive oxygen species.

Overall, the study demonstrated the ameliorating effect of ECP on CCl_4-induced oxidative stress and hepatotoxicity, likely due to the presence of phytochemical constituents with antioxidant properties.[24,25]

5. Conclusion

This research highlights the potential of Carica papaya leaf extract (ECP) in counteracting the harmful effects of carbon tetrachloride (CCl_4) on the liver and kidney. CCl4 induction led to decreased antioxidant activity, oxidative stress, hepatotoxicity, and inflammation, impairing liver and kidney function and histology. ECP mitigated these effects by replenishing phytochemical components, reducing free radicals, and restoring antioxidant balance. By modulating liver and kidney enzyme concentrations, ECP sustained the functions of these vital organs.

The findings suggest that ECP is a valuable plant that can be used to support individuals with hepatocellular carcinoma or other liver-related diseases, helping to maintain endogenous immunity. Encouraging the use of ECP may provide a beneficial adjunct to conventional treatments.

6. Source of Funding

None.

7. Conflict of Interest

None.

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