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Effect of Methanol Extract of Ananas Comosus Peel on Carbon Tetrachloride (CCl_4)-Induced Hepatotoxicity in Wistar Rats

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Abstract: *This study investigated the ameliorative effect of methanol extract of Ananas comosus peel on carbon tetrachloride (CCl_4)-induced hepatotoxicity in Wistar rats. Twenty-five male albino rats were allocated into five groups: normal control, 200 mg/kg pineapple peel extract (PPE), 500 mg/kg PPE, silymarin-treated positive control, and CCl_4 negative control. At the end of the experimental period, blood and liver tissues were collected for biochemical, antioxidant, and histological evaluation. Quantitative phytochemical analysis showed appreciable amounts of saponins (14.85±0.02 mg/100 g), flavonoids (2.63±0.02 mg/100 g), and alkaloids (2.85±0.02 mg/100 g). The extract contained 5.24±0.02% protein, 5.31±0.02% crude fibre, 1.08±0.01% fat, 3.84±0.00% ash, and 2.36±0.07% carbohydrate. CCl_4 exposure was associated with adverse changes in antioxidant status, lipid profile, hepatic enzymes, renal indices, and liver architecture, whereas PPE treatment showed ameliorative effects comparable in several parameters to silymarin. These findings suggest that methanol extract of A. comosus peel possesses antioxidant and hepatoprotective potential against CCl_4-induced liver injury in Wistar rats*

Keywords: *Ananas comosus, pineapple peel, carbon tetrachloride, hepatotoxicity, antioxidant, Wistar rats*

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1. 0 Introduction

The liver is a vital organ responsible for a wide range of physiological processes, including nutrient metabolism, synthesis of plasma proteins, regulation of lipid and carbohydrate metabolism, xenobiotic biotransformation, and detoxification. Its strategic position in the circulation and its role in metabolizing endogenous and exogenous substances make it particularly susceptible to toxic injury.

Exposure to hepatotoxic chemicals can disrupt hepatocellular integrity and impair normal liver function through mechanisms involving oxidative stress, lipid peroxidation, mitochondrial dysfunction, inflammation, and depletion of endogenous antioxidant defences. Consequently, the identification of natural products capable of protecting the liver against toxic insults remains an important area of experimental research.

Carbon tetrachloride (CCl_4) is a well-established chemical model for inducing experimental hepatotoxicity and oxidative liver injury. Its hepatotoxic effects are associated with metabolic activation and the generation of reactive intermediates that initiate oxidative reactions and lipid peroxidation in hepatic tissues. The resulting oxidative damage can alter cellular membranes and hepatocellular architecture and cause leakage of intracellular enzymes into the circulation. In experimental animals, CCl_4 exposure has been associated with elevated serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), together with increases in bilirubin and other biochemical indicators of hepatic dysfunction. Histological alterations have also been reported following CCl_4 administration, confirming its ability to produce structural liver damage (Mir *et al*., 2010; Munir & Khan, 2023). These characteristics make the CCl_4 model useful for evaluating the potential protective or ameliorative effects of plant-derived substances against chemically induced hepatic injury.

Oxidative stress is an important component of CCl_4-induced hepatotoxicity and provides a mechanistic basis for investigating antioxidant-rich plant materials as potential hepatoprotective agents. Excessive production of reactive oxygen species can overwhelm endogenous antioxidant systems, resulting in oxidative modification of lipids, proteins, and other cellular components. The restoration or enhancement of antioxidant defences may therefore reduce oxidative damage and contribute to the preservation of hepatic structure and function. Evidence from experimental studies supports the potential of plant extracts to attenuate CCl_4-induced liver injury. For example, Hamzah *et al*. (2021) reported hepatoprotective effects of methanol extract of *Senna occidentalis* seeds in CCl_4 treated rats, while Oyeyemi *et al*. (2022) demonstrated that methanol extract of *Luffa cylindrica* fruit reduced serum markers of hepatic injury, enhanced antioxidant enzyme activities, decreased lipid peroxidation, and ameliorated CCl_4-associated liver lesions. Similarly, Mir *et al*. (2010) reported that treatment with *Solanum nigrum* extract attenuated CCl_4-induced alterations in serum transaminases and hepatic histology. These findings indicate that plant-derived bioactive compounds may provide protection against toxicant-induced hepatic damage through antioxidant and related biological mechanisms.

Ananas comosus (pineapple) is an economically important tropical fruit that is widely consumed and processed. Although the pulp is the principal edible portion, pineapple processing generates considerable quantities of peel and other residues. Rather than being regarded solely as waste, pineapple peel may represent a useful source of nutrients and biologically active phytochemicals. The presence of compounds such as flavonoids, alkaloids, glycosides, tannins, and other secondary metabolites in pineapple peel suggests that this underutilized material may possess antioxidant and other pharmacological properties. In addition to its phytochemical constituents, pineapple-derived materials have been investigated for their potential biological effects. Johari *et al*. (2023), for instance, reported that administration of pineapple

extract influenced the platelet count of male Wistar rats without significant adverse effects on several other haematological parameters, supporting continued investigation of the biological properties of pineapple-derived preparations.

Of particular relevance to the present study, Unanma *et al*. (2021) investigated the effects of methanol extracts of *A. comosus* and *Citrus sinensis* peels in CCl_4-induced liver injury in Wistar rats. Their preliminary antioxidant screening identified pineapple and orange peels among the fruit peels with relatively high antioxidant activity. The authors subsequently reported that *A. comosus* methanol peel extract at 250 and 500 mg/kg increased serum superoxide dismutase and catalase activities, reduced malondialdehyde levels, and improved alterations in ALT, AST, total protein, and total bilirubin. Improvements in lipid profile and liver histology were also observed, and phytochemical analysis revealed flavonoids, alkaloids, glycosides, tannins, and acidic compounds in the extracts (Unanma *et al*., 2021). This study provides important evidence that pineapple peel possesses hepatoprotective potential and establishes a strong basis for further investigation of *A. comosus* peel as a natural source of hepatoprotective compounds.

Despite these promising findings, further investigation remains warranted because the hepatoprotective effects of plant extracts can vary according to extraction solvent, dose, experimental design, treatment regimen, animal characteristics, and the biochemical and histological endpoints evaluated. Although Unanma *et al*. (2021) demonstrated protective effects of methanol extract of *A. comosus* peel against CCl_4-induced liver injury, additional experimental evidence is needed to further characterize the effects of pineapple peel extract across a broader range of biochemical and physiological indicators. In particular, simultaneous assessment of hepatic enzymes, bilirubin fractions, serum proteins, lipid profile, renal indices, antioxidant-related parameters, and liver histopathology can provide a more comprehensive assessment of the systemic and hepatic effects associated with treatment. Furthermore, establishing the phytochemical and proximate composition of the extract alongside these biological responses may help provide a clearer basis for relating its biological activity to its chemical constituents.

The utilization of pineapple peel for hepatoprotective applications may also have broader environmental and economic significance. Large quantities of fruit peels generated during household consumption and commercial processing are commonly discarded, creating opportunities for their conversion into value-added products. Characterizing the biological properties of pineapple peel could therefore contribute to the development of inexpensive plant-based sources of bioactive compounds while simultaneously promoting the beneficial utilization of agro-industrial residues. Such an approach aligns biomedical investigation with sustainable waste valorization and may provide a basis for further pharmaceutical, nutraceutical, or functional-food applications, subject to appropriate toxicological and clinical validation.

Therefore, this study investigated the ameliorative effect of methanol extract of *Ananas comosus* peel on CCl_4-induced hepatotoxicity in Wistar rats. Specifically, the study evaluated the phytochemical and proximate composition of the pineapple peel extract and determined its effects on selected hepatic and renal biochemical indices, lipid profile, antioxidant status, and liver histoarchitecture following CCl_4-induced injury. The study is significant because it provides additional experimental evidence on the hepatoprotective potential of *A. comosus* peel, evaluates the biological response to

different extract doses, and contributes to the scientific utilization of pineapple peel as a potentially valuable source of natural bioactive compounds.

2.0 Materials and Methods

2.1 Collection and Identification of Plant Material

Fresh *Ananas comosus* (pineapple) fruits were obtained from Ndioru Market, Obowo, in Ikwuano Local Government Area of Abia State, Nigeria. The plant material was authenticated and identified as *Ananas comosus* by Dr. Garuba Omosun, a taxonomist in the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The pineapple fruits were thoroughly washed under running tap water to remove adhering soil, sand, and other surface contaminants. The peels were carefully removed using a clean kitchen knife, collected, and air-dried under shade at room temperature for four days. The dried peels were pulverized using an electric blender to obtain a relatively uniform powdered material.

2.2 Preparation of Methanol Extract

The powdered pineapple peels were extracted by cold maceration using methanol following the procedure described by Sukhdev *et al*. (2008), with appropriate modifications. About 1 g of the powdered peel was soaked in in methanol in a Winchester bottle for 72 h at room temperature. The mixture was intermittently agitated to facilitate extraction of the soluble constituents. After extraction, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary vacuum evaporator maintained at 40 °C. The resulting semi-solid extract was weighed, and the percentage extraction yield was calculated. The extract was stored in a sealed container at 4 °C until required for administration and analysis.

2.3 Experimental Animals and Management

Twenty-five male albino Wistar rats, with an average body weight of approximately 120 g, were obtained from the Breeding Animal House of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The animals were housed in clean aluminium cages, with five rats per cage, and maintained under standard laboratory conditions with a natural light–dark cycle. The animals were fed standard laboratory rat chow (grower's mash) and provided ad libitum access to clean drinking water.

The animals were allowed to acclimatize to the laboratory environment for one week before commencement of the experiment. The rats were handled and used in accordance with internationally accepted guidelines for the care and use of laboratory animals (NIH, 1985).

2.4 Experimental Design and Treatment

The twenty-five rats were randomly assigned into five groups of five animals each (n = 5):

Group 1 (Normal control): received and was not exposed to CCl_4. Group 2 (PPE 200 mg/kg): received pineapple peel methanol extract at 200 mg/kg body weight.

Group 3 (PPE 500 mg/kg): received pineapple peel methanol extract at 500 mg/kg body weight. **Group 4 (Positive control)**: received silymarin at 50 mg/kg body weight.

Group 5 (Negative control): received CCl_4 to induce hepatotoxicity The administration of pineapple peel extract, silymarin, and CCl_4 was carried out according to the experimental protocol. The exact dose, route, frequency, and duration of CCl_4 administration should be reported to ensure reproducibility of the hepatotoxicity model.

At the end of the experimental period, . Blood samples were collected into appropriate sample tubes and processed for serum biochemical analyses. The liver tissues were excised,

washed with ice-cold normal saline to remove adhering blood, and processed for antioxidant and histological analyses.

2.5 Phytochemical and Proximate Analyses

Quantitative phytochemical analysis of the pineapple peel extract was performed using established procedures described (AOAC, 2019; Harborne ,1998).

Proximate composition was also determined using AOAC (2019) method. The parameters evaluated included moisture, crude protein, crude fibre, crude fat, ash, and carbohydrate content. Carbohydrate content was determined by difference where applicable.

2.6 Biochemical Analyses

Serum biochemical parameters were determined using recommended analytical procedures. Serum creatinine was measured according to the method of Fabiny & Ertingshausen (1971). The biochemical parameters evaluated included while liver-function indices included , total protein, albumin, total bilirubin, direct bilirubin, etc., as applicable]. Renal-function indices and lipid-profile parameters were also determined using appropriate standard methods and/or commercially available diagnostic kits according to the manufacturers' instructions. Where commercial diagnostic kits were used, the manufacturer, country of origin, and kit method should be stated to ensure reproducibility.

2.7 Antioxidant Analysis

Antioxidant status was evaluated using the liver tissue homogenates. The antioxidant parameters determined included, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), and malondialdehyde (MDA), as applicable]. Liver tissues were homogenized in an appropriate ice-cold buffer, and the homogenates were centrifuged before analysis. Each antioxidant parameter was determined using the appropriate standard analytical procedure.

2.8 Histological Examination

Following sacrifice, representative liver tissue samples were excised and immediately fixed in such as 10% neutral buffered formalin] for histological examination. The tissues were processed by routine dehydration through graded concentrations of alcohol, cleared in an appropriate clearing agent, embedded in paraffin wax. The sections were mounted on clean glass slides and stained with haematoxylin and eosin (H&E). The stained sections were examined under a light microscope for evidence of hepatocellular degeneration, necrosis, inflammation, vascular alterations, and other pathological changes. Histological processing and examination were conducted at the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.9 Statistical Analysis

Data obtained from the study were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS), version 22.0. Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons. Differences were considered statistically significant at $p < 0.05$.

3.0 Results and Discussion

3.1 Phytochemical and Proximate Composition of Pineapple Peel Extract

The quantitative phytochemical composition of the methanol extract of *Ananas comosus* peel (PPE) is presented in Table 1. The extract contained appreciable amounts of saponins (14.85 ± 0.02 mg/100 g), alkaloids (2.85 ± 0.02 mg/100 g), cyanogenic compounds (2.59 ± 0.01 mg/100 g), flavonoids (2.63 ± 0.02 mg/100 g), phenols (0.92 ± 0.02 mg/100 g),

tannins (0.84 ± 0.02 mg/100 g), and oxalates (0.02 ± 0.00 mg/100 g). Saponins were the most abundant phytochemical detected, followed by cyanogenic compounds, alkaloids, flavonoids, phenols, tannins, and oxalates. The presence of these secondary metabolites indicates that pineapple peel contains a diverse phytochemical profile that may contribute to its biological activity.

Table 1. Quantitative phytochemical composition of pineapple peel extract

Phytochemical	PPE
Flavonoid	2.63 ± 0.02
Alkaloid	2.85 ± 0.02
Tannin	0.84 ± 0.02
Phenol	0.92 ± 0.02
Saponin	14.85 ± 0.02
Cyanide	2.59 ± 0.01
Oxalate	0.02 ± 0.00

The flavonoid content of 2.63 ± 0.02 mg/100 g is particularly relevant to the hepatoprotective potential of the extract because flavonoids are recognized for their free-radical-scavenging and antioxidant properties. Their ability to interact with reactive oxygen species may help limit oxidative damage to cellular lipids and proteins. The presence of phenolic compounds (0.92 ± 0.02 mg/100 g) further supports the antioxidant potential of PPE because phenolic compounds can participate in redox reactions and contribute to the neutralization of reactive species. The relatively high saponin content may also be biologically relevant because saponins have been associated with membrane-interacting, cholesterol-modulating, antioxidant, and anti-inflammatory activities. However, the measured concentration alone cannot establish the contribution of any individual phytochemical to the observed in vivo effects.

The present phytochemical profile is broadly consistent with the findings of Unanma *et al.* (2021), who detected flavonoids, alkaloids, glycosides, tannins, and acidic compounds in methanol extracts of *A. comosus* peel and demonstrated antioxidant and hepatoprotective activity against CCl_4-induced liver injury. Their study provides direct supporting evidence that the phytochemical constituents of pineapple peel may contribute to protection against chemically induced oxidative hepatic injury. The biological effects observed in the present study may therefore reflect the combined activity of several phytochemical classes rather than a single constituent.

The proximate composition of PPE is presented in Table 2. Moisture constituted the largest measured component (82.21 ± 0.04%), followed by crude fibre (5.31 ± 0.02%), crude protein (5.24 ± 0.02%), ash (3.84 ± 0.00%), carbohydrate (2.36 ± 0.07%), and crude fat (1.08 ± 0.01%). The dry matter content was 17.84 ± 0.02%, while the calculated energy value was 40.14 ± 0.07. The high moisture content indicates that fresh pineapple peel contains substantial water, which is expected for a fresh fruit by-product. From a processing perspective, however, the high moisture content of fresh peel can favour microbial deterioration and therefore emphasizes the importance of drying before storage or extraction.

The crude fibre content indicates that pineapple peel contains a measurable structural carbohydrate fraction. Dietary fibre is relevant to nutritional quality and may contribute to gastrointestinal function. The relatively low crude fat content suggests that lipids constitute only a minor component of the peel, while the ash content indicates the presence of mineral constituents. The crude protein content also demonstrates that the peel contains measurable nitrogenous material, although it should not be regarded primarily as a protein-rich food material. Collectively, the proximate composition supports the potential nutritional

and functional value of pineapple peel and provides additional justification for its conversion from an agro-processing residue into a value-added biological material.

Table 2. Proximate composition of pineapple peel extract

Proximate Parameter	PPE
Moisture Content	82.21 ± 0.04
Dry Matter	17.84 ± 0.02
Crude Protein	5.24 ± 0.02
Crude Fibre	5.31 ± 0.02
Fat Content	1.08 ± 0.01
Ash	3.84 ± 0.00
Carbohydrate	2.36 ± 0.07
Energy Value	40.14 ± 0.07

3.2 Antioxidant Status and Lipid Peroxidation

The effects of PPE and silymarin on antioxidant markers are presented in Table 3. The normal control had SOD and catalase activities of 32.18 ± 2.51 and 16.02 ± 0.65, respectively, whereas the CCl_4 negative-control group showed marked reductions to 13.12 ± 0.46 and 10.28 ± 1.51. At the same time, MDA increased from 0.69 ± 0.14 in the normal control to 1.39 ± 0.14 in the CCl_4 group. This pattern is consistent with substantial oxidative stress following CCl_4 exposure.

CCl_4 is metabolically activated in the liver to reactive intermediates that initiate lipid peroxidation and disrupt cellular antioxidant defences. The observed reduction in SOD and catalase in the negative control therefore indicates impairment or depletion of antioxidant defence during CCl_4-induced injury, while the elevation of MDA indicates increased membrane lipid peroxidation. Similar changes in antioxidant enzymes and lipid peroxidation have been reported in experimental CCl_4 hepatotoxicity, supporting the use of these parameters as indicators of oxidative hepatic injury (Unanma *et al*., 2021).

Table 3. Antioxidant markers

Group	SOD	Catalase	MDA
Normal Control	32.18+2.51	16.02+0.65	0.69+0.14
200mg/kg PPE	20.41+0.91	14.08+2.13	0.65+0.04
500mg/kg PPE	24.61+3.05	13.81+1.69	0.48+0.15
Positive Control (Silymarin Group)	26.33+2.69	13.75+0.53	0.60+0.08
Negative Control (Ccl4 group)	13.12+0.46	10.28+1.51	1.39+0.14

PPE treatment partially improved the antioxidant profile. SOD increased from 13.12 ± 0.46 in the CCl_4 group to 20.41 ± 0.91 and 24.61 ± 3.05 in the 200 and 500 mg/kg PPE groups, respectively. Catalase similarly increased to 14.08 ± 2.13 and 13.81 ± 1.69, compared with 10.28 ± 1.51 in the negative control. The 500 mg/kg group also recorded the lowest MDA concentration (0.48 ± 0.15), compared with 0.65 ± 0.04 at 200 mg/kg and 1.39 ± 0.14 in the CCl_4 group. The overall pattern suggests that PPE attenuated CCl_4-associated oxidative stress, particularly with respect to lipid peroxidation.

Interestingly, the 500 mg/kg PPE group had lower MDA than the normal control, whereas SOD and catalase did not return completely to normal-control values. This suggests that the protective action of the extract may not depend solely on restoration of endogenous antioxidant enzyme activities and may also involve direct scavenging of reactive species or suppression of lipid peroxidation. The antioxidant effect is

biologically plausible given the flavonoid and phenolic constituents detected in the extract. Unanma *et al*. (2021) similarly reported that *A. comosus* peel extract increased antioxidant enzyme activities and reduced MDA in CCl_4-treated rats, providing independent support for the antioxidant response observed in the present investigation.

The implication of these findings is that PPE may help preserve hepatic redox balance during chemical injury. Since oxidative stress is an important component of CCl_4-mediated hepatocellular damage, attenuation of lipid peroxidation and improvement of antioxidant status could contribute to the subsequent improvements observed in hepatic biochemical parameters and tissue integrity.

3.3 Renal Function Indices

The effects of PPE on serum urea and creatinine are shown in Table 4. The normal control had urea and creatinine concentrations of 26.27 ± 1.06 and 1.17 ± 0.03, respectively. These values remained relatively close to normal in the 200 mg/kg PPE group (27.69 ± 0.65 and 1.22 ± 0.02), the 500 mg/kg group (28.51 ± 2.95 and 1.19 ± 0.27), and the silymarin group (26.03 ± 1.64 and 1.21 ± 0.06). In contrast, the CCl_4 negative-control group showed substantially higher urea (40.71 ± 0.71) and creatinine (2.29 ± 0.18).

The marked elevation of both renal indices in the CCl_4 group suggests that exposure to the hepatotoxicant was associated with systemic biochemical disturbance extending beyond the liver. The near-normal values observed following PPE administration indicate attenuation of this disturbance. The 500 mg/kg group, in particular, had a creatinine concentration (1.19 ± 0.27) almost identical to the normal control (1.17 ± 0.03), while its urea concentration was only modestly higher.

Table 4. Renal function indices

Group	Urea	Creatinine
Normal Control	26.27+1.06	1.17+0.03
200mg/kg PPE	27.69+0.65	1.22+0.02
500mg/kg PPE	28.51+2.95	1.19+0.27
Positive Control (Silymarin Group)	26.03+1.64	1.21+0.06
Negative Control (Ccl4 group	40.71+0.71	2.29+0.18

These observations suggest that PPE did not produce an obvious adverse effect on the measured renal indices at the doses investigated and may have attenuated CCl_4-associated renal biochemical alterations. Nevertheless, urea and creatinine alone cannot establish complete renal protection; additional markers and histological examination of the kidney would be required to confirm a direct nephroprotective effect. The findings are therefore best interpreted as evidence of preservation of selected renal-function indices rather than definitive proof of nephroprotection.

3.4 Lipid Profile

The lipid-profile results are presented in Table 5. CCl_4 exposure produced an unfavourable lipid-profile pattern relative to the normal control. Total cholesterol increased from 73.46 ± 10.23 in the normal group to 106.11 ± 3.55 in the negative-control group, while triglycerides increased markedly from 48.08 ± 29.13 to

132.52 ± 52.21. Conversely, HDL decreased from 104.37 ± 25.83 in the normal control to 37.00 ± 6.17 in the CCl_4 group, while LDL increased from 34.46 ± 7.23 to 63.71 ± 5.33. These changes indicate disruption of lipid homeostasis following CCl_4 exposure.

Table 5. Lipid profile

Group	CHOL	TAGS	HDL	LDL
Normal Control	73.46+10.23	48.08+29.13	104.37+25.83	34.46+7.23
200mg/kg PPE	78.07+3.41	78.66+45.36	86.05+2.95	34.84+6.58
500mg/kg PPE	71.92+10.00	53.18+17.22	84.95+4.07	37.03+11.15
Positive Control (Silymarin Group)	85.28+6.48	89.17+35.50	100.80+21.63	29.92+15.26
Negative Control (Ccl4 group	106.11+3.55	132.52+52.21	37.00+6.17	63.71+5.33

PPE treatment moderated several of these alterations. At 200 mg/kg, cholesterol was 78.07 ± 3.41 and LDL was 34.84 ± 6.58, both considerably lower than the corresponding values in the negative control. At 500 mg/kg, cholesterol and triglycerides were 71.92 ± 10.00 and 53.18 ± 17.22, respectively, while LDL was 37.03 ± 11.15. The silymarin group showed cholesterol of 85.28 ± 6.48 and LDL of 29.92 ± 15.26. Although HDL in the PPE groups remained lower than the normal control, both PPE groups had considerably higher HDL concentrations than the CCl_4 group.

The improvement in lipid profile following PPE treatment may reflect partial restoration of hepatic lipid metabolism following attenuation of CCl_4-induced hepatic injury. The liver is central to cholesterol, triglyceride, and lipoprotein metabolism; therefore, preservation of hepatocellular function can contribute to normalization of circulating lipids. The findings are consistent with previous observations that pineapple peel extracts can modulate lipid abnormalities associated with CCl_4-induced liver injury (Unanma *et al*., 2021).

However, the original interpretation that these effects were necessarily caused by inhibition or activation of specific cholesterol-metabolizing enzymes is not directly supported by the present data because enzyme activities involved in cholesterol esterification, absorption, or lipoprotein metabolism were not measured. The present findings therefore support an association between PPE administration and improved lipid homeostasis rather than a definitive mechanism.

3.5 Liver Function Indices

The effects of PPE on hepatic biochemical indices are shown in Table 6. The CCl_4 negative-control group demonstrated clear biochemical evidence of hepatic injury. AST increased from 70.25 ± 18.18 in the normal control to 127.75 ± 20.15 in the negative control, while ALT increased from 40.75 ± 29.29 to 71.25 ± 15.10. ALP similarly increased from 45.29 ± 13.02 to 74.97 ± 3.96. These changes indicate disruption of hepatocellular integrity following CCl_4 exposure.

Table 6. Liver function indices

Parameter	Normal	200 mg/kg	500 mg/kg	Positive	Negative
TP	4.90 ± 0.26	5.11 ± 0.37	51.31 ± 0.68	5.31 ± 0.14	2.37 ± 0.35
ALB	2.95 ± 0.12	3.03 ± 0.10	3.11 ± 0.13	2.95 ± 0.15	1.63 ± 0.28
GLB	1.97 ± 0.27	2.08 ± 0.31	2.20 ± 0.58	2.35 ± 0.17	0.71 ± 0.14

AST	70.25 ± 18.18	92.50 ± 5.50	94.72 ± 4.00	87.87 ± 5.26	127.75 ± 20.15
ALT	40.75 ± 29.29	22.75 ± 11.00	27.25 ± 8.95	27.00 ± 6.00	71.25 ± 15.10
ALP	45.29 ± 13.02	41.66 ± 7.87	34.58 ± 5.20	37.20 ± 10.41	74.97 ± 3.96
TB	0.22 ± 0.05	0.22 ± 0.02	0.19 ± 0.02	0.21 ± 0.01	0.25 ± 0.04
DB	0.10 ± 0.02	0.10 ± 0.01	0.11 ± 0.00	0.10 ± 0.00	0.14 ± 0.00
CB	0.12 ± 0.02	0.14 ± 0.04	0.08 ± 0.03	0.11 ± 0.01	0.11 ± 0.04

Note: Values are presented as mean ± standard deviation.

The PPE-treated groups showed lower ALT and ALP activities than the negative control. ALT decreased to 22.75 ± 11.00 and 27.25 ± 8.95 in the 200 and 500 mg/kg groups, respectively, compared with 71.25 ± 15.10 in the negative control. ALP also decreased progressively to 41.66 ± 7.87 at 200 mg/kg and 34.58 ± 5.20 at 500 mg/kg, compared with 74.97 ± 3.96 in the CCl_4 group. The silymarin group showed ALT of 27.00 ± 6.00 and ALP of 37.20 ± 10.41, demonstrating a broadly comparable response to PPE.

AST showed a somewhat different pattern. Although the negative-control group had the highest value (127.75 ± 20.15), PPE-treated groups recorded 92.50 ± 5.50 and 94.72 ± 4.00 at 200 and 500 mg/kg, respectively, while silymarin produced 87.87 ± 5.26. Thus, PPE reduced the CCl_4-associated elevation in AST but did not return it completely to the normal-control value. This partial normalization nevertheless indicates attenuation of hepatocellular injury.

ALT and AST are widely used indicators of hepatocellular injury because disruption of hepatocyte membranes can facilitate leakage of these intracellular enzymes into the circulation. The lower activities observed following PPE treatment therefore suggest preservation of hepatocellular integrity. The response is consistent with the earlier report by Unanma *et al*. (2021), in which *A. comosus* peel extract attenuated CCl_4-associated alterations in ALT and AST. Similar hepatoprotective responses have also been reported for other plant extracts in CCl_4-induced experimental liver injury, demonstrating the usefulness of this model for evaluating natural hepatoprotective agents.

The reduction in ALP following PPE administration is also noteworthy. Since ALP can increase with hepatobiliary injury and cholestatic processes, the lower values in the PPE groups suggest reduced disturbance of hepatobiliary function compared with the untreated CCl_4 group. However, ALP alone cannot establish the absence of cholestasis, and interpretation should therefore be made together with bilirubin and histopathological findings.

The serum protein results also suggest preservation of hepatic synthetic function. Albumin increased from 1.63 ± 0.28 in the negative-control group to 3.03 ± 0.10 and 3.11 ± 0.13 in the 200 and 500 mg/kg PPE groups, respectively. Globulin similarly increased from 0.71 ± 0.14 in the negative control to 2.08 ± 0.31 and 2.20 ± 0.58 in the PPE groups. These values were comparable to, or in some cases higher than, those observed in the normal and silymarin groups. The findings indicate that PPE administration was associated with preservation of serum protein concentrations during CCl_4-induced injury.

The total protein results require particular attention. The normal control recorded 4.90 ± 0.26, while the 200 mg/kg and positive-control groups recorded 5.11 ± 0.37 and 5.31 ± 0.14, respectively. The negative-control group showed a marked reduction to 2.37 ± 0.35, which is consistent with impaired hepatic function. However, the reported value of 51.31 ± 0.68 for the 500 mg/kg group is inconsistent with all other groups and with the

corresponding albumin and globulin values. Because albumin + globulin would be expected to approximate total protein, the reported value of 51.31 appears likely to be a transcription or decimal error. This value should be verified against the original laboratory records before publication. If the intended value was approximately 5.31, the interpretation would be consistent with the other biochemical findings.

The bilirubin results showed relatively small differences among the groups. Total bilirubin ranged from 0.19 ± 0.02 to 0.25 ± 0.04, while direct bilirubin ranged from 0.10 ± 0.00 to 0.14 ± 0.00. Conjugated/indirect bilirubin values were also relatively comparable among groups. The relatively limited alteration in bilirubin suggests that the CCl_4 challenge under the present experimental conditions produced more pronounced changes in hepatic enzymes and protein status than in bilirubin metabolism. This observation is important because hepatotoxicity does not necessarily produce equivalent changes in all biochemical markers. Overall, the liver-function results indicate that PPE attenuated several of the biochemical consequences of CCl_4 exposure. The reductions in ALT, AST and ALP, together with restoration of albumin and globulin concentrations, were generally comparable to the response produced by silymarin. The 500 mg/kg dose produced particularly favourable responses in ALP and several other parameters, although a clear dose-dependent effect was not observed for every marker. This suggests that PPE has hepatoprotective potential, but the response is parameter-dependent rather than uniformly dose-dependent.

3.6 Integrated Implication of the Findings

Taken together, the findings across Tables 1–6 provide convergent evidence that methanol extract of *A. comosus* peel can attenuate several biochemical disturbances associated with CCl_4-induced toxicity. The extract contained flavonoids, phenols, tannins, alkaloids, and abundant saponins, providing a plausible phytochemical basis for antioxidant activity. This was reflected in improved SOD and catalase activities and reduced MDA concentration in PPE-treated animals. The extract also moderated CCl_4-associated increases in urea and creatinine, improved several lipid-profile parameters, and reduced the elevations in ALT, AST, and ALP associated with hepatic injury.

The overall pattern is consistent with the proposed role of oxidative stress in CCl_4 toxicity and with previous experimental evidence that *A. comosus* peel possesses antioxidant and hepatoprotective properties. Unanma *et al*. (2021) similarly demonstrated that methanol extracts of pineapple peel improved antioxidant enzymes, MDA, hepatic enzymes, bilirubin, lipid profile, and liver histology in CCl_4-treated Wistar rats. The agreement between the present biochemical findings and that earlier study strengthens the evidence that pineapple peel contains biologically active constituents capable of mitigating chemically induced oxidative and hepatic injury.

The findings have two important implications. First, they suggest that pineapple peel, which is frequently discarded as a fruit-processing residue, may represent a potentially useful source of natural antioxidant and hepatoprotective compounds. Second, the results support the concept of converting an underutilized agricultural by-product into a value-added biological material. However, the present findings are from an animal model and should not be interpreted as evidence of clinical efficacy in humans. Further work involving characterization of the active constituents, dose-response studies, mechanistic investigations, longer-term toxicity assessment, and ultimately appropriately

designed clinical studies would be required before therapeutic applications can be proposed.

Overall, the biochemical evidence indicates that methanol extract of *A. comosus* peel exerted an ameliorative effect against CCl_4-associated oxidative, hepatic, renal, and lipid disturbances in Wistar rats. The response observed with PPE, particularly at 500 mg/kg for several parameters, was broadly comparable with that of silymarin, although the effects were not uniformly dose-dependent across all measured indices. These findings support further investigation of pineapple peel as a source of natural bioactive compounds with potential hepatoprotective activity.

4.0 Conclusion

The findings of this study demonstrate that methanol extract of *Ananas comosus* peel contains appreciable levels of bioactive phytochemical constituents and exhibits protective effects against CCl_4-induced biochemical and histopathological alterations in Wistar rats. Administration of the extract was associated with improvement in the evaluated biochemical indices and attenuation of hepatic tissue damage, indicating a reduction in the deleterious effects of CCl_4-induced hepatotoxicity. The observed hepatoprotective activity may be associated with the presence of phenolics, flavonoids, tannins, saponins, alkaloids, and other phytoconstituents capable of modulating oxidative and lipid-peroxidative processes. These findings support the potential of *A. comosus* peel, an otherwise underutilized agro-industrial by-product, as a promising source of naturally occurring bioactive compounds with hepatoprotective properties. However, further studies involving isolation and characterization of the active constituents, mechanistic investigations, dose optimization, and long-term toxicity assessment are necessary to establish its therapeutic relevance and safety.

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