

Protective effects of mobola plum seed extracts against paracetamol and carbon tetrachloride-induced liver damage.

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Abstract: *Parinari curatellifolia* has been found implicated in the treatment of diabetics, hypertension and liver disorders. The effects of aqueous and methanolic extracts on paracetamol (PCM) and Carbon tetrachloride (CCl₄)-induced liver damage were investigated, with a view to utilizing the extract of the plant in the management and treatment of liver disorders. *Parinari curatellifolia* seeds were air-dried, soaked in water and methanol, filtered, and rotary evaporator was used to remove the methanol and freeze-dried. The extracts were screened for their phytochemical constituents. The animals were grouped into five (5) groups of four (4) animals each for both aqueous and methanolic extract using PCM and CCl₄ hepatotoxins. Group 1 served as control; group 2 (the hepatotoxin group); group 3 (the extract only group); group 4 (the extract (either aqueous or methanolic) + hepatotoxin group) and Group 5 (the silymarin (standard drug) + the extract group). The extracts were administered for a period of seven days. Animals were sacrificed twenty-four hours after the last administration. Phytochemical screening revealed the presence of saponins, alkaloids, flavonoids, phlobatannins, and tannins in the extracts. The results showed that paracetamol and CCl₄ caused significant ($p < 0.05$) decreases in the activities of liver alkaline phosphatase (ALP), L-aspartate aminotransferase (AST), L-alanine aminotransferase (ALT) and concentration of total protein, total bilirubin, urea and albumin in the liver as compared with their control. Treatment with aqueous extract and silymarin ameliorated the effect of paracetamol and CCl₄. The results of the histopathological study on paracetamol and CCl₄ intoxicated rats revealed that the livers of the control animals were essentially normal while there were severe perinuclear vacuolation of the hepatocytes in the paracetamol and CCl₄ intoxicated rat livers. In animals that received aqueous and methanolic extracts only, livers were essentially normal. Treatment with aqueous and methanolic extracts of the *P. curatellifolia* seed at a dosage of 600 mg/kg (bwt) reversed the effect of paracetamol and CCl₄ in the serum. In conclusion the results of the work therefore suggested that the extracts of *P. curatellifolia* possess hepatoprotective effects against paracetamol and CCl₄-induced liver damage in albino rats. The protection compares well with that of silymarin, (reference drug). The extracts protect the liver damage via antioxidative mechanism

Keywords: Mobola plum, Paracetamol, Carbon tetrachloride, liver damage.

1.0 Introduction

1.1 Medicinal Plants

Medicinal plants are unique type of plants requiring special consideration due to their vital impact on people's health or they are plants, which contain substances that can be used for therapeutic purposes-precursor for the synthesis of useful products (Brussels 2001). The use of medicinal plants was facilitated in the past by several inadvertent or indirect roles and some intentional

management practice. In South Africa (and probably elsewhere) metal machetes and axes were widely available, plants were collected with a pointed len digging stick or small axes, which intended to limit the quality of the barks or roots leered. For example, traditional subsistence harvesting of *Cassine papillosa* causes relatively damage to the tree. Medicinal plants have been found useful in the area of pharmacology among which are;

Atropa belladonna used to relax distended organs, especially the stomach and the tine (Barrette, 1994). *Atropa belladonna* used in conventional anesthetic (Honychurch, \\\Artemitemisia absinthium(composited) provides bitter taste to some well-known beverages liquor (Comerford, 1996}. *Maranta arundinacea (Marantaced)* roots are used as a poultice small pox sores and as an infusion for urinary infection (Comerford, 1996). *Ocimum sancton (labiatae)* has the ability to reduce blood sugar level thereby preventing peptic ulcers and other js related conditions like hypertension, colitis and asthma (Barrett, 1994). *Styrax benzion raceae)* is used externally to fight tissue inflammation and disinfecting of wounds (Comerford, 1996)

1.2 HISTORY OF MEDICINAL PLANTS

In contrast with western medicine, which is technically and analytical based, traditionally African medicines take a holistic approach, good health, diseases success or misfortune are not as chance occurrences but are believed to arise from the actions of individuals and ancestral spirits according to the balance or im balance between the individual and the social environment (Ralfeson *et al*, 2005).

Traditionally rural African communities have relied upon the spiritual and practical skills of the T M P s (Traditional Medicinal Practitioners) whose botanical knowledge of plant species and their ecology and scarcity are invaluable.

In Nigeria, the first man to practice the art of healing through the use of herbal medicine in Yoruba speaking part of Nigeria was Orunmila who was believed to be endowed with the knowledge from God. A record of medicinal plant in earliest period in Nigeria was virtually not available because there was no record for their isolation and preparation. Every fact about potent herbal plants was passed by word of mouth from generation to generation.

However, by watching the effect produced by various plants when eaten by domestic animal (Akpata, 1999) pressure on medicinal plant resources has remained low in remote areas and in countries such as Mozambique and Zambia where the commercial trade in traditional medicines has only developed to a limited extent due to the small size for major urban centres. Taboos, seasonal and social restrictions on gathering equipment all severed to limit medicinal plant harvesting.

In Southern Africa (and probably elsewhere, before metal machetes and axes were widely available; plants were collected with a pointed wooden digging stick or small axe, which tends to limit the quantity of barks or roots gathered. For example, traditional substance harvested of cassia siberana bark causes relatively little damage to the tree (World Health Organization, 1997)

1.3 Justification of The Study

A satisfactory remedy for serious liver diseases is not still available, hence search for effective hepatoprotective plant continues. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional systems of medicine all over fc world (Chopra and Handa, 2006).

parinari curatellifolia leaves, fruits and root has been reported to be useful in the treatment of various diseases such as toothache, snakebite and fractures (Chopra and Handa, 2006). However, there is little or no information as regards the hepatoprotective potential of this plant. Therefore, the present study is designed to investigate hepatoprotective activities of aqueous and methanolic extracts of *Parinari curatellifolia* seed against acetaminophen and carbon tetrachloride-induced liver damage in rat.

1.4 Objectives of The Study

The specific objectives of the study are to:

obtains extracts (aqueous and methanolic) and evaluate the phytochemical constituents of the plant;

evaluate the possible hepatoprotective potential of the extract against acetaminophen and carbon tetrachloride-induced toxicities; and

iii. carry out the histopathological studies on the liver of the animals treated.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 plant materials: collection and identification of plant materials

Fresh seeds of *Parinari curatellifolia* were collected from Akure in Ondo State, Nigeria. The authentication of the plant was done by Dr A. A. Debooye of the Department of Crop Production and Protection, Obafemi Awolowo University of Ile-Ife.

2.1.2 Reagents and Chemicals

Diagnostic kits for assays of ALT, AST, ALP, Total protein, Total bilirubin, Albumin, Urea were purchased from Randox Laboratory Ltd, Diamond Rod, Crumlin, Co Antrim, United Kingdom. Silymarin, Paracetamol and CCl₄ were procured from Jawal Pharmaceutical Company Lagos, Nigeria and other reagents used were of analytical grade.

2.1.3 Experimental Animals

Eighty (80) healthy albino rats of both sexes weighing between (150-200g) were collected from the Department of Biochemistry Federal University of Technology Akure. The rats were fed with guinea feeds for ten days before the experiment commenced.

2.2 Methods

2.2.1 Preparation and Extraction of *P. curatellifolia*

The seeds of *Parinari curatellifolia* were peeled, cleaned, air dried at room temperature for two weeks. The dried seeds were ground to powder (1kg), soaked with methanol and water in ratio 4:1 (i.e. 4L: 1L) for 72 hours (3 days). This suspension was filtered using sieve cloth. The filtrate was concentrated on a rotary evaporator to remove the methanol and the aqueous portion was freeze dried. To prepare the aqueous extract, ground seeds (1kg) was soaked in 5L distilled water overnight, filtered and freeze-dried.

2.2.2 Phytochemical Screening extracts (aqueous and methanolic)

(a) Test for Saponins

The extract (0.5g) was shaken with 10ml of distilled water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins.

(b) Test for tannins

The extract (0.5g) was stirred with 10ml of distilled water filtered and a few drops 10% ferric chloride reagent was added to the filtrate. A blue-black, green or black-green precipitate was taken as evidence for the presence of tannins (Trease and Evans, 1989).

(c) Test for Anthraquinones

The extract (0.5g) was shaken with 10ml benzene filtered and 5ml of 10% ammonia solution was added to the filtrate. The mixture was shaken. The presence of a pink, red, or violet colour in the ammoniacal (lower) phase indicate the free anthraquinones (Trease and Evans, 1989).

(d) Test for Phlobatannins

Deposition of a red precipitates when the aqueous extract of the plants was boiled with one percent aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins (Trease and Evans, 1989).

(e) Test for Alkaloid

The extract (0.1g) was stirred in 5ml of 1% aqueous hydrochloric acid in a steam bath, the solution was filtered and 1ml of the filtrate was treated with a few drops of Dragendorff's reagent. Turbidity or precipitation was taken as the preliminary evidence for presence of alkaloids in the extract being evaluated (Trease and Evans, 1989)

(f) *Test for Cardiac glycosides*

(i) *Legal test*

The extract (1.0g) was dissolved in pyridine and a few drops of 20% sodium hydroxide (NaOH) were added. A deep red colour, which faded to brownish yellow, indicates the presence of cardenolides (Trease and Evans, 1989)

(ii) *Lieberman's Test*

The extract (1.0g) was dissolved in 2.0ml of acetic anhydride and cooled well in ice, sulphuric acid was carefully added until there was a colour change from violet to blue green, which indicates the presence of steroidal nucleus that is a glycone portion of the cardiac glycoside (Trease and Evans, 1989).

(iii) *Salkowski Test*

The extract (1.0g) was dissolved in 2.0ml of chloroform, sulphuric acid was then carefully added to form lower layer. A reddish brown colour at the interface indicates the presence of steroidal ring.

(iv) *Keller-Killani Test*

The extract (1.0g) was dissolved in 2.0ml of glacial acetic acid containing few drops of ferric chloride solution, this was underlayered with 1.0ml concentrated sulphuric acid.

A brown ring obtained at interface indicated the presence of deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic layer a greenish ring may form just above the brown grease gradually spread throughout this layer.

(g) *Test for flavonoids*

The extract (1.0g) was dissolved in 5ml of ethanol. This was shaken and filtered, to 1ml of the filtrate few drops of 0.5ml KOH was added and a yellow colouration indicates the presence of flavonoids. (Trease and Evans, 1989).

2.3 BIOASSAY

The extracts were first given to the animals (4 animals per group) in varying doses of 150mg, 300mg, 450mg and 600mg respectively, and it was discovered that the 600mg has the most significant effect on the rats.

2.3.1 Experimental Design

Induction and Administration of PCM/Extracts

Aqueous:

Group I: Served as control (uninduced but giving distilled water, 1ml daily for three days)

Group II: Received 2 g/kg bwt of paracetamol orally for three days) Group III: Received 600 mg/kg bwt of aqueous extract of *Parinari curatelifolia* for three days). Group IV: Aq. + PCM (received 600 mg/kg bwt of aqueous extract *Parinari curatelifolia* orally and PCM 2 g/kg bwt for three days (Co-administration).

Group V: Silymarin + PCM (received 25 mg/kg bwt of Silymarin orally for and PCM 2 g/kg bwt for three days (Co-administration).

Methanolic:

Group I: Served as control (uninduced but giving distilled water, 1ml daily for three days) Group II: Received 2 g/kg bwt of paracetamol orally for three days)

Group III: Received 600 mg/kg bwt of methanolic extract of *Parinari curatelifolia* for 3 days. Group IV: Met + PCM (received 600 mg/kg bwt of methanolic extract of *Parinari curatelifolia* orally and PCM 2 g/kg bwt for three days (Co-administration))

Group V: Silymarin + PCM (received 25 mg/kg bwt of silymarin orally and PCM 2 g/kg bwt for three days (Co-administration)).

Induction and Administration of CCl₄ Extracts Aqueous:

Group I: Served as control (uninduced but giving distilled water, 1ml daily for seven days)

Group II: Received 2 ml/kg bwt of carbon tetrachloride orally for seven days.

Group III: Received 600 mg/kg bwt of aqueous extract of *Parinari curatelifolia* for seven days. Group IV: Aqueous + CCU (Received 600 mg/kg bwt of aqueous extract of *Parinari curatelifolia* orally and CCU 2 ml/kg bwt for seven days (Co-administration))

Group V: Silymarin + CCU (received 25 mg/kg bwt of silymarin orally and CCU 2 ml/kg bwt for seven days (co-administration)) Methanolic:

Group I: Served as control (uninduced but giving distilled water, 1ml daily for seven days) Group II: Received 2 ml/kg bwt of carbon tetrachloride orally for seven days. Group III: Received 600 mg/kg bwt of methanolic extract of *Parinari curatelifolia* for seven days. Group IV: Met + CCU (Received 600 mg/kg bwt of methanolic extract of *Parinari curatelifolia* orally and CCU 2 ml/kg bwt for seven days (Co-administration))

Group V: Silymarin + CCU (received 25 mg/kg bwt of silymarin orally and CCU 2 ml/kg bwt for seven days (co-administration))

All administrations were done orally and the animals were sacrificed twenty-four hours after the last administration, (bwt means body weight)

2.4 Biochemical analyses: Preparation of blood serum

After sacrificing, the animals blood was collected through cardiac puncture from all the animals and serum was separated by centrifugation at 4,800 rpm for 15 minutes, which is then used for the estimation of various biochemical parameters namely Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), bilirubin, Total protein, urea and albumin.

Liver Homogenate preparation

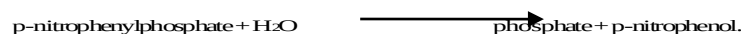
The liver of all the animals were collected, weighed and homogenized using phosphate buffer saline, weight of the liver multiply by 10 was the quantity of phosphate buffer saline used to homogenize the liver with the aid of electric blender. The homogenates were used for the estimation of lipid peroxidation, catalase, glutathione peroxidase, ALT, AST, ALP, Urea, Bilirubin, Albumin, and Total protein. The estimations of ALT, AST, ALP, Urea, Bilirubin, Albumin, and Total protein was done using the same method used for their determination in serum analysis.

2.4.1 Assay of ALP Activity in Serum

This is an optimized standard method according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie. (1960)

Principle

Serum alkaline phosphate hydrolyses a colourless substrate of phenolphthalein monophosphate giving pH values, turns into a pink colour that can be photometrically determined.



Assay procedure

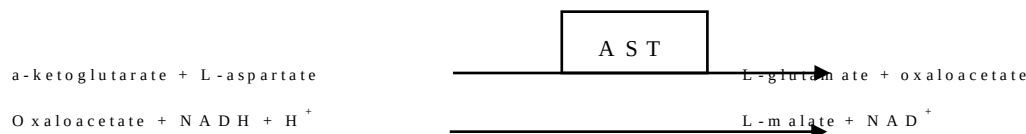
The serum (0.5ml) and 3ml of the reagent (Diethanolamine buffer mol/L, pH 9.8 and MgCl₂ 0.5mmol/L) were pipetted into labelled test tubes and were mixed thoroughly. The initial

absorbance was taken at zero second and later read at 1, 2 and 3 minutes at 405nm. The ALP activity was calculated as: $3300 \times \text{change in absorbance at } 405\text{nm}$.

2.4.2 Assay of AST Activity in serum

Principle

α -glutarate reacts with L-aspartate in the presence of aspartate aminotransferase (AST) to form L-glutamate and oxaloacetate. The indicator reaction utilizes the oxaloacetate for a kinetic determination of NADH consumption (Reitman and Frankel, 1957)



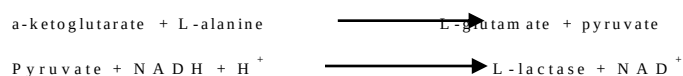
Assay procedure

The serum (0.2ml) and 2ml of the reagent (Tris buffer 80mmol/L, pH 7.5; L-aspartate 240mmol/L; α -Oxoglutarate 12mmol/L; MDH >420U/L; LD >600U/L and NADH 0.18mmol/L) were pipetted into labelled test tubes and were mixed thoroughly. The initial absorbance was taken at 1 min was later read at 2 and 3 minutes at 540nm.

2.4.3 Assay of ALT activity in serum

α -ketoglutarate with L-alanine in the presence of alanine aminotransferase (ALT) to form L-glutamate and pyruvate. The indicator reaction utilizes the pyruvate for a kinetic determination of NADH consumption (Reitman and Frankel, 1957).

Principle

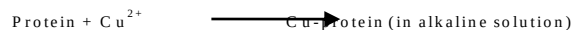


Assay Procedure

The serum (0.2 ml) and 2 ml of the reagent (Tris buffer 100mmol/L, pH 7.5; L-alanine 0.6mol/L α -Oxoglutarate 15mmol/L; LD >1.2U/ml; and NADH 0.18mol/L) were pipetted into labeled test tubes and were mixed thoroughly. The initial absorbance was taken at 1 min after which it was read again at 2 and 3 minutes at 540nm.

2.4.4 Determination of Total Protein Concentration Principle

Cupric ions interact with proteins to give an intense violet-blue complex colour with Cu in alkaline medium. The intensity of the colour was proportional to the amount of proteins present in sample.



Assay Procedure

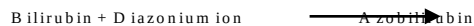
Equal volume (0.02ml) of each of the distilled H₂O, serum and protein standard was pipetted into different test tubes. Then, 1ml of protein reagent was added to all the test tubes and the solutions were thoroughly mixed and incubated at 25°C for 30 min (Weichselbaum, 1974). The absorbance was then read at 546nm against a reagent blank and total proteins were determined using the formula.

$$\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

The protein reagent contained 100mM NaOH, 1mM Na-K-tartrate, 15mM potassium iodide, 6mM CuSO₄ Protein reagent blank contained 100mM NaOH and 16mM Na-K-tartrate. Protein standard solution is 6mg/ml.

2.4.5 Determination of Total Bilirubin Concentration. Principle

Colorimetric method as described by Jendrassik and Grof (1938). Total bilirubin is coupled with diazonium ion in a strong acidic medium in the presence of a suitable solubilizing agent.



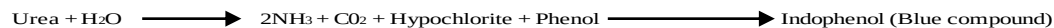
Assay Procedure

Serum (0.2 ml), distilled water and standard were pipette into different labeled tubes after which 1 ml of working reagent (Sulphanilic acid 29 mmol/L; Hydrochloric acid 0.17N; Sodium nitrite 25 mmol/L; Caffeine 0.26 mol/L; Sodium benzoate 0.52 mol/L; Tartrate 0.93 mol/L and Sodium hydroxide 1.9N) was added to all the tubes. The assay mixture was mixed thoroughly and was allowed to stand for 2 min at 25°C. The absorbance of the sample was read against the sample blank (A_{TB}) at 578nm. Total bilirubin was calculated as shown below:

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Concentration of standard}$$

2.4.6 Determination of Urea Principle

The improved Jung (1975) method utilizes a chromogenic reagent that forms a colored complex specifically with urea. The intensity of the colour, measured at 520nm, is directly proportional to the urea concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.



Assay Procedure

Reagents (R1: EDTA Sodium nitroprusside 6 mmol/l; Urease 1g/l, R2: Phenol (diluted) 20 mmol/l; R3: Sodium hypochlorite (diluted) 27 mmol/l and Sodium hydroxide 0.14N) were equilibrated to room temperature. Enough working reagent was prepared by combining equal volumes of Reagent A and Reagent B, shortly prior to assay. Water 5ul (blank), 5ul standard (50 mg/dL) and 5ul samples were, transferred in duplicate into test tubes. Working reagent (200ul) was added and tapped lightly to mix. The tubes were incubated for 30 min at room temperature. Absorbance was then taken at 520nm.

Urea concentration (mg/dl) of the sample is calculated as: $[\text{Urea}] = n \times [\text{STD}]$

Absorbance (sample) - Absorbance (blank)

Absorbance (standard) - Absorbance (blank) (mg/dl) (Jung, 1975)

2.4.7 Estimation of Albumin Concentration Principle

The measurement of albumin is based on its quantitative binding to the indicator 3,3',5,5'-tetrabromocresol sulphonaphthalein (bromocresol green, BCG). The albumin BCG-complex absorbs maximally at 578nm, the absorbance being directly proportional to the albumin in sample.

Assay Procedure

Distilled water (0.01), Albumin standard (0.01 ml) and the sample (0.01 ml) were pipetted into the test tubes labelled Reagent Blank, Standard and samples respectively, 3ml of BCG reagent was added into all the test tubes. The assay mixtures were incubated for five minutes 25°C. the absorbance of sample (A_{sample}) and of the standard (A_{standard}) was measured against the reagent blank at 578nm. The albumin concentration was calculated using the following general formulae;

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}$$

2.4.8. Determination of Concentration of Thiobarbituric Acid Reactive Substance (TBARS).

Principle

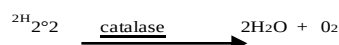
Thiobarbituric acid reacts with malondialdehyde to yield a fluorescent product (Ohkawa *et al*, 1979).

Assay Procedure

This was done according to Ohkawa *et al*, (1979). 100ul of the Liver homogenate was pipette into the test tube , 600ul of acetate buffer (pH 3.4) was added as well as 600ul of 0.8% TEA, the test tube with its content was then incubated at 100°C for 1hr and the absorbance was read at 550nm.

2.4.9. Assay of Catalase Activity (Aebi, 1974) Principle

Catalase is a hemoprotein containing four heme groups. In addition to possessing peroxidase activity, it is able to use one molecule of H_2O_2 as a substrate electron donor and another molecule of $\text{I}^\wedge\text{C}^\wedge$ as oxidant or electron acceptor.



Procedure

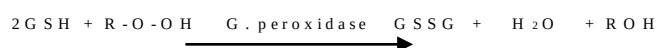
This was done according to Aebi (1974). 70uL of FeCl_3 was pipetted into a test tube and 1950uL phosphate buffer was added as well as 20uL of the homogenate and the absorbance was read at 240nm.

This was done according to Aebi (1974). 70uL of H_2O_2 was pipetted into a test tube and 1950uL phosphate buffer was added as well as 20uL of the homogenate and the absorbance was read at 240nm.

2.4.10 Assay of Glutathione Peroxidase Activity (GPx)

Principle

In the erythrocytes and other tissues, the enzyme glutathione peroxidase, contain Selenium as prosthetic group, catalyzes the destruction of H_2O_2 and lipid hydroperoxides by reduced glutathione, protecting membrane lipids and hemoglobin against oxidation by peroxides.



Procedure

This was done according to (Chance, 1951)

1.0ml of 0.03M phosphate buffer was pipetted into a test tube and .3ml of 10mM GSH was added, then 0.3ml H_2O_2 , 1.37ml of distilled water and 0.03ml of homogenate were also added and the absorbance was read at 340 nm.

2.4.11 Statistical Analysis

Data were presented as mean and standard deviation analysed by SPSS 16. One way analysis variance (ANOVA) and Duncan tests were carried out to test any significant difference between their means significance was accepted at $p < \text{less than } 0.05$.

3.0 RESULTS.

3.1 Phytochemical Constituent of Methanolic and Aqueous Extracts of *Parinari curatelifolia* (seed).

The yield of seed aqueous methanolic and aqueous extracts of *Parinari curatelifolia* were 61.6g and 99.7g respectively, from starting weight of 1kg.

Table 3.1 is the summary of phytochemical constituents of the seed extracts of *P. curatelifolia*. It reveals that alkaloid, flavonoids, phlobatannins, saponins and tannins were all present while cardiolides and anthraquinones were absent in *Parinari curatelifolia* seeds.

3.1 Table 3.1 Phytochemical Constituent for both Aqueous and Methanolic Extracts of *Parinari curatelifolia*(seed)

Metabolites	Methanolic	Aqueous
Alkanoids	+	+
Flavonoids	+	+
Phlobatannins	+	+
Saponins	+	+
Tannins	+	+
Steroidal ring	+	+
Steroidal nucleus	+	+
Cardiolides	-	-
Anthraquinones	-	-

Key

- Means absent

+ Means present

3.2 Effect of aqueous extract of *Parinari curatelifolia* on some serum biochemical indices of paracetamol hepatotoxic albino rats.

Table 3.2 is the summary of the effect of aqueous extract of *P. curatelifolia* seed on some serum biochemical parameters of paracetamol induced liver damage in albino rats. The result revealed that paracetamol caused a significant increase in the activities of ALP, AST and ALT as compared with control. Treatment with aqueous extract significantly reduced the effect of paracetamol on these parameters as compared with silymarin.

Also, administration of paracetamol produced significant increases in the concentration of the total protein, total bilirubin, urea and albumin respectively. While the aqueous extract significantly reduced the activities of these biochemical parameters.

TABLE 3.2 EFFECT OF AQUEOUS EXTRACT OF *PARINARI CURATELIFOLIA* ON SOME SERUM BIOCHEMICAL INDICES OF PARACETAMOL HEPATOTOXIC ALBINO RATS

GROUPS	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	T.Protein (g/dl)	T.Bilirubin (g/dl)	Urea (g/dl)	Albumin, (g/dl)
Control	27.48±0.19a	14.34±0.39a	10.75±0.20C	1.14±0.01b	1.65±0.25a	9.70±0.16"	0.57±0.01L
PCM (2g/kgbw t)	190.50±1.00d	142.00±0.82d	31.93±0.66d	7.84±0.20e	13±0.30d	15.39±0.24d	2.28±0.02e
Aqueous (600mg/kgbw t)	29.05±0.65a	14.88±0.00f1	6.24±0.02"	1.80±0.14d	2.75±0.10b	9.87±0.26b	0.75±0.01d
Aque. (600mg/kgbw t) PCM (2g/kg)	64.30±0.75b+	35.63±0c	8.47±0.28 b	0.69±0.08a	3.33±0.34C	7.68±0.18a	0.37±0.01b

Sil. (25m g/kg bw t) P C M . (2gflkgbw t)	13.05±1.92 +	18.61±0.37b	8.27±0.38b	1.36±0.17C	3.25±0.27C	11.66±0.01C	0.24±0.01E
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ALP - Alkaline phosphatase, AST- aspartate amino transferase, ALT- alanine aminotransferase., T. Protein- Total protein, T. Bil- Total bilirubin, PCM Paracetamol, Sil.-Silymarin, Aque-Aqueous.

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically ($P > 0.05$) different. SD; Standard Deviation.

3.3 Effects of methanolic seed extract of *P. curatellifolia* on some serum biochemical indices of paracetamol treated albino rats.

Table 3.3 presents the effect of methanolic extract of *Parinari curatellifolia* on some serum biochemical indices of paracetamol intoxicated albino rats.

This table revealed that the methanolic extract was able to reverse the increased activities of ALP and AST in the serum caused by paracetamol intoxication. Paracetamol elevated the level of total protein, total bilirubin and urea as compared to the control groups. The protection offered by methanolic extract compared very well with that of the silymarin treated groups.

TABLE 3.3 EFFECT OF METHANOLIC EXTRACT OF PARINARI CURATELIFOLIA ON SOME SERUM BIOCHEMICAL INDICES OF PARACETAMOL TREATED ALBINO RATS

Group	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	T. Protein (g/dl)	T. Bilirubin (g/dl)	Urea (g/dl)	Albumin (g/dl)
Control	27.48±0.19b	14.34±0.39a	10.75*0.20"	1.14±0.01a	1.65±0.25a	9.70±0.16	0.57 0.01c
PCM (2g/kg bw t)	190.50±1.00C	142.00±0.82d	31.93±0.66d	7.84±0.20C	6.13±0.30d	15.39±0.24e	2.28 0.02d
Meth. (600mg/kg bw t)	25.78±0.21a	15.37±0.21b	8.12±0.80a	1.66±0.36b	3.18±0.06b	10.15±0.05b	0.44±0.01b
Meth. (600mg/kg bw t)+PCM (2g/kg bw t)	84.25±0.96C	15.59±0.33b	12.68±0.20c	1.56±0.05b	3.61±0.25C	10.66±0.02C	0.59±0.03c
Sil.(25mg/kg bw t) + PCM .(2g/kg bw t)	113.05±1.92	18.61±0.37c	8.27±0.38a	1.36±0.17a	18.61±0.37c	8.27±0.38a	1.36±0.17a

ALP - Alkaline phosphatase, AST- aspartate amino transferase, ALT- alanine aminotransferase, T. Protein- Total protein, T. Bil- Total bilirubin, PCM -Paracetamol, Sil.-Silymarin, meth-methanolic.

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically ($P > 0.05$) different from each other. SD; Standard Deviation.

3.4 Effect of aqueous extract of *P. curatellifolia* on some liver biochemical indices of paracetamol treated albino rats.

Table 3.4 shows the effect of aqueous extract on some liver biochemical indices of paracetamol intoxicated albino rats.

This shows that paracetamol caused significant decreases in the levels of all the biochemical indices (ALP, AST, ALT, Total protein, Total bilirubin, Urea and Albumin) of the liver while the aqueous extract of *Parinari curatellifolia* seed reversed the action of this hepatotoxin.

Table 3.4: EFFECT OF AQUEOUS EXTRACT OF PARINARI URATELIFOLIA ON SOME LIVER BIOCHEMICAL INDICES OF PARACETAMOL TREATED ALBINO RATS.

GROUPS	ALP (IU/mg prot.)	AST (IU/mg prot.)	ALT (IU/mg prot.)	T. Protein (g/dl)	T. Biliru (mg/dl)	Urea (mg/dl)	Albumin, (g/dl)
Control	266.75±72.4g	180.27±7.26d	64.33±1.46c	10.31±0.24d	6.67±0.24c	39.25±3.62d	3.31±0.35c
PCM (2g/kg bw t)	82.80±0.00a	14.15±1.90a	6.19±0.15a	4.81±0.20a	1.65±0.19a	9.67±0.10c	1.62±0.16a
Aqueous (600mg/kg bw t)	233.38±1.94d	271.60±3.04e	69.18±0.76d	9.76±0.08d	9.54±0.18e	39.65±0.52d	2.71±0.10b
Aque. (600mg/kg bw t+ PCM (2g/kg bw t)	194.40±1.39c	20.96±0.75b	101.75±0.86c	8.52±0.21c	4.29±0.17b	29.75±0.65c	2.47±0.06b
Sil.(25mg/kg bw t)+PCM (2g/kg bw t)	165.48±1.76b	26.54±0.50c	10.29±0.54b	7.46±0.80b	7.29±0.26d	22.81±2.27b	2.47±0.33b

3.5 Effect of methanolic extract of *Parinari curatellifolia* on some liver biochemical indices of paracetamol treated albino rats.

Table 3.5 shows the effect of methanolic extract of *Parinari curatellifolia* on some liver biochemical indices of paracetamol intoxicated albino rats.

It was revealed that paracetamol caused a significant decrease in the levels of paracetamol on the liver biochemical indices (ALP, AST, ALT, Total protein, Total bilirubin, Urea and Albumin) in the liver homogenates. On the other hand the methanolic extract of *Parinari curatellifolia* seed was able to ameliorate the effect of paracetamol on all these parameters.

TABLE 3.5 EFFECT OF METHANOLIC EXTRACT OF PARINARI CURATELIFOLIA ON SOME LIVER BIOCHEMICAL INDICES OF PARACETAMOL HEPATOTOXIC ALBINO RATS

GROUPS	ALP (IU/mg prot.)	AST (IU/mg prot.)	ALT (IU/mg prot.)	T. Protein (g/dl)	T. Biliru (mg/dl)	Urea (mg/dl)	Albumin, (g/dl)
Control	266.75±72.4e	180.27±7.26C	64.33±1.46e	10.31±0.24e	6.67±0.24L	39.25±3.62d	3.31±0.35'
PCM (2g/kg)	82.80±0.00a	14.15±1.901	6.19±0.15a	4.81±0.20a	1.65±0.19a	9.67±0.10f	1.62±0.16f
Meth. (600mg/kg bw t)	218.75±2.50d	232.62±1.89f	62.60±1.70d	9.68±0.15d	8.63±0.04e	39.58±0.38d	3.68±0.19c
Meth.	185.50±2.69C	36.56±2.53C	35.87±1.12C	6.75±0.33b	3.14±0.14b	27.88±2.39C	2.48±0.36

(600 mg/kg bwt) + P C M (2g/kg bwt)							b
Sil. (25 mg/kg bwt) + P C M .(2g/kg bwt)	165.48 ± 1.76 b	26.54 ± 0.50 b	10.29 ± 0.54 b	7.46 ± 0.80 °	7.29 ± 0.26 d	22.81 ± 2.27 b	2.47 ± 0.33 b

ALP - Alkaline phosphatase, AST- aspartate amino transferase, ALT- alanine aminotransferase, T. Protein- Total protein, T. Bil- Total bilirubin, PCM -Paracetamol, Sil-Silymarin, meth-methanolic.

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically (P > 0.05) different from each other. SD; Standard Deviation.

3.6 Effect of aqueous extract of *Parinari curatellifolia* on some serum biochemical indices of CCl₄ treated albino rats,

Table 3.6 presents the effect of aqueous extract of *Parinari curatellifolia* on some serum biochemical indices of CCl₄ intoxicated albino rats.

This shows that the levels of ALP, AST and ALT in CCl₄ group were significantly higher than that of the control group. The aqueous extract was able to reverse the effect of carbontetrachloride on the total protein in the serum. While in total bilirubin, urea and albumin, aqueous extract offered a comparable protection like that of silymarin.

TABLE 3.6 EFFECT OF AQUEOUS EXTRACT OF *PARINARI CURATELIFOLIA* ON SOME SERUM BIOCHEMICAL INDICES OF CCl₄ TREATED ALBINO RATS

GROUPS	ALP (IU/mg prot.)	AST (IU/mg prot.)	ALT (IU/mg prot.)	T. Protein (g/dl)	T. Biliru (mg/dl)	Urea (mg/dl)	Albumin, (g/dl)
Control	27.48 ± 0.19 a	14.34 ± 0.39 d	10.75 ± 0.20 U	U 4 ± 0.01 P	1.65 ± 0.25'	9.70 ± 0.16 a	0.57 ± 0.01 ab
CCl ₄ (2 ml/kg bwt)	194.98 ± 1.196	129.68 ± 0.21 d	128.21 ± 0.83 e	10.70 ± 0.12 e	4.24 ± 0.18 d	17.43 ± 0.15 c	2.54 ± 0.50 c
Aqueous (600 mg/kg bwt)	29.05 ± 0.65 b	14.88 ± 0.00 a	6.24 ± 0.12 a	1.80 ± 0.14 C	2.75 ± 0.10 °	9.87 ± 0.26 a	0.75 ± 0.01 b
Aque. (600 mg/kg bwt + C Cl ₄ (2 ml/kg bwt)	99.36 ± 0.00 C	19.13 ± 0.53 b	10.13 ± 0.16 b	0.77 ± 0.13 a	1.45 ± 0.27 a	10.35 ± 0.10 b	0.67 ± 0.02 b
Sil. (25 mg/kg bwt) + CCl ₄ . (2 ml/kg bwt)	103.68 ± 1.61 d	35.13 ± 0.57 e	17.46 ± 0.23 d	2.44 ± 0.12 d	2.39 ± 0.36 b	10.48 ± 0.28 b	0.26 ± 0.02 a

ALP - Alkaline phosphatase, AST- aspartate amino transferase, ALT- alanine aminotransferase, T. Protein- Total protein, T. Bil- Total bilirubin, ccl₄- carbontetrachloride, Sil.- Silymarin, Aque-Aqueous.

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically (P > 0.05) different from each other. SD; Standard Deviation.

3.7 Effect of methanolic extract of *Parinari curatellifolia* on some serum biochemical indices of CCl₄ treated albino rats

The effect of methanolic extract of *Parinari curatellifolia* on some serum biochemical indices of CCl₄ intoxicated rats is presented in Table 3.7

The table shows that CCl₄ caused significant increases in the levels of ALP, AST and ALT compared to the control while the methanolic extract of *Parinari curatellifolia* seed significantly reverse the effects of CCl₄ on these parameters. The protection offered by methanolic extract on total protein, total bilirubin, urea and albumin compared well with that of the silymarin.

TABLE 3.7 EFFECT OF METHANOLIC EXTRACT OF *PAWNARI CURATELIFOLIA* ON SOME SERUM BIOCHEMICAL INDICES OF CCl₄ TREATED ALBINO RATS

GROUPS	ALP (IU)	AST (IU)	ALT (IU)	T. Protein (g/dl)	T. Biliru	Urea (mg/dl)	Albumin, (g/dl)
Control	27.48±0.19a	14.34±0.39a	10.75±0.20b	1.14±0.01b	1.65±0.25a	9.70±0.16a	0.57±0.01ab
CCl ₄ (2ml/kg bwt)	194.98±1.19d	129.68±0.21d	128.21±0.83d	10.70±0.12e	4.24±0.18d	17.43±0.15C	2.54±0.50C
Meth. (600mg/kg bwt)	25.78±0.21a	15.37±0.21b	8.12±0.80a	1.66±0.36C	3.18±0.06C	0.44±0.01ab	10.15±0.05b
Meth.(600mg/kg bwt)+ CCl ₄ (2ml/kg)	94.47±2.47b	34.79±0.53C	11.04±0.26b	0.75±0.02a	1.56±0.39a	11.45±0.34C	0.67±0.01b
Sil.	103.68±1.6ic	35.13±0.57°	17.46±0.23C	2.44±0.12d	2.39±0.36b	10.48±0.28b	0.26±0.02a

ALP - Alkaline phosphatase, AST - aspartate amino transferase, ALT - alanine aminotransferase, T. Protein - Total protein, T. Bil - Total bilirubin, CCl₄ - carbontetrachloride, Sil. - Silymarin, meth - methanolic.

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically (P > 0.05) different from each other. SD; Standard Deviation.

3.8 Effect of aqueous extract of *Parinari curatellifolia* on some liver biochemical indices of CCl₄ treated albino rats.

The effect of aqueous extract of *Parinari curatellifolia* seed on some biochemical indices of the liver homogenate of CCl₄ intoxicated albino rats is presented in table 3.8.

This shows that CCl₄ significantly lowered the levels of all the biochemical indices in the liver of the albino rats as compared to the control groups. While the aqueous extract of *Parinari curatellifolia* significantly reversed the effects of this hepatotoxin (CCl₄)

TABLE 3.8 EFFECT OF AQUEOUS EXTRACT OF *PARINARI CURATELIFOLIA* ON SOME LIVER BIOCHEMICAL INDICES OF CCl₄ TREATED ALBINO RATS

GROUPS	ALP (IU/mg prot.)	AST (IU/mg prot.)	ALT (IU/mg prot.)	T. Protein (g/dl)	T. Biliru	Urea (mg/dl)	Albumin, (g/dl)
Control		180.27±7.25	64.33±1.46C	10.31±0.2	6.67±0.2	39.25±3.62	3.31±0.35

	266.75±7.24 a	D		4e	4		
CC14 (2ml/kg bw t)	121.60±7.70 a	64.64±2.27a	22.30±3.30a	3.82±0.25 a	1.05±0.1 1a	0.92±0.30a	0.92±0.30a
Aqueous (600mg/kg bw t)	233.38±1.94 C	271.60±3.04 d	69.18±0.76d	9.78±0.08 d	9.54±0.1 8d	39.65 ±0.52d	2.71 ±0.10c
Aque. (600mg/kg bw t)+	222.00±1.39 b	202.23±0.83 C	63.97±3.45C	7.58±0.03 C	4.24±0.1 1b	25.75±0.29 C (mg/dl)	2.47±0.06C (g/ dl)
Sil. (25mg/kg) + CC14.(2ml/k g)	222.45±1.95 b	182.77±3.89 b	56.50±0.58b	6.85±0.14 b	4.29±0.1 7b	21.88±1.25	2.23±0.06 b

ALP - Alkaline phosphatase, AST - aspartate amino transferase, ALT - alanine aminotransferase, T. Protein- Total protein, T. Bil- Total bilirubin, CCU - carbontetrachloride, Sil.-Silymarin, Aque.-Aqueous.

Values are mean ± SD; n = 4; Values with the same superscript and Idler(s) down a column are not statistically (P > 0.05) different from each other. SD; Standard Deviation.

3.9 Effect of methanolic extract of *Parinari curatellifolia* on some liver biochemical indices of CC14treated albino rats.

The effect of methanolic extract of *Parinari curatellifolia* seed on some liver biochemical indices of CC14 intoxicated rats is presented in table 3.9.

It was seen here that CC14 significantly reduced the level of these biochemical indices as compared with the control groups. While the methanolic extract of *Parinari curatellifolia* seed significantly increased the level of these parameters as compared with CC14 groups.

TABLE 3.9 EFFECT OF METHANOLIC EXTRACT OF PARINARI CURATELIFOLIA ON SOME LIVER BIOCHEMICAL INDICES OF CC14TREATED ALBINO RATS

GROUPS	ALP (IU/mgprot.)	AST (IU/mgprot.)	ALT (IU/mgprot.)	T.Protein (g/dl)	T.Biliru (mg/dl)	Urea (mg/dl)	Albumin, (g/dl)
Control	266.75±7.24U	180.27±7.25D	64.33±1.46fc	10.31±0.24e	6.67±0.24°	39.25±3.62C	3.31±0.35l
CC142ml/kg bw t)	121.60±7.70a	64.64±2.27a	22.30±3.30a	3.82±0.25a	1.05±0.11a	17.88±2.02	0.92±0.30f
Meth. (600mg/kg bw t)+ CC14(2ml/kgbw t)	218.75±2.50C	232.62±1.89d	62.6Q±1.70C	9.68±0.15d	8.63±0.046	39.58±0.38C	3.68±0.19C
Meth. (600mg/kg bw t)+ CC14(2ml/kgbw t)	176.90±3.05b	191.53±1.94c	56.97±2.27b	6.07±0.27b	3.13±0.13b	24.75±0.29b	2.39±0.14t
Sil (25mg/kgbw t)+ CCU.(2ml/kg bw t)	222.45il.95"	182.77±3.89"	56.50±0.58D	6.85±0.14C	4.29±0.17C	21.88±1.25D	2.23±0.06b

A L P - Alkaline phosphatase, A S T - aspartate amino transferase, A L T - alanine aminotransferase, T. Protein - Total protein, T. Bil - Total bilirubin, carbontetrachloride, Sil.-Silymarin, meth-methanolic.

3.10 Effects of aqueous and methanolic extracts on TBARS concentration in the liver of paracetamol treated rats.

The effects of aqueous and methanolic seed extracts of *Parinari curatellifolia* on malonaldehyde concentration in the liver of paracetamol treated rats is presented in table 3.10.

Paracetamol caused a significant increase in TBARS formation as compared with control. Treatment with extract ameliorated the effect of paracetamol on the malonaldehyde concentration in the liver homogenate.

TABLE 3.10 LIVER TEARS (THIOBARBITRIC ACID)

Effect of Aqueous and Methanolic Extracts of *Parinari curatellifolia* on TBARS Concentration in the liver of Paracetamol treated rat.

Group	MDA (nM /g tissue)
Control	0.047±0.0126b
PCM (2mg/kg bwt)	0.136±0.13f
Aqueous (600mg/kg bwt)	0.044±0.01a
Methanolic (600mg/kg bwt)	0.049±0.005b
PCM (2g/kg bwt) + Aque. (600mg/kg bwt)	0.078±0.01c
PCM (2g/kg bwt) + Meth. (600mg/kg bwt)	0.093±0.013d
PCM (2mg/kg bwt) + Sil. (25mg/kg bwt)	0.113±0.01e

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically (P > 0.05) different from each other. SD; Standard Deviation.

3.11 Effects of Aqueous and Methanolic Extracts of *P. curatellifolia* on MDA Concentration in the Liver of CCl₄ Treated Rats.

The effects of aqueous and methanolic seed extracts of *Parinari curatellifolia* on TBARS concentration in the liver of CCl₄ treated rats is presented in table 3.11. CCl₄ caused a significant increase in TBARS formation as compared with control. Treatment with the extracts (aqueous and methanolic) extracts ameliorated the effect of CCl₄ on the TBARS level.

3.12 Effects of Aqueous and Methanolic Extracts of *P. curatellifolia* on Catalase Activity in the Liver of Paracetamol Treated Rats.

The effects of aqueous and methanolic seed extracts of *Parinari curatellifolia* on catalase activity in the liver of paracetamol treated rats is presented in Table 3.12. Paracetamol intoxication caused significant decrease in the activity of catalase as compared with control. Treatment with the extract nullified the effects of paracetamol on catalase activity.

TABEL 3.11 LIVER TBARS

Effect of Aqueous and Methanolic Extracts of *Pannari curatettifolia* on TBARS Concentration in the Liver of CCl₄ treated rat.

GROUP	TBARS (uM /g tissue)
Control	0.047±0.0126a
CCl ₄ (20ml/kg bwt)	0.141±0.335f
Aqueous (600mg/kg bwt)	0.044±0.01a
Methanolic (600mg/kg bwt)	0.049±0.005b

CC14 (20ml/kg) + Aque. (600mg/kg bwt)	0.081±0.005c
CC14 (20ml/kg bwt) + Meth. (600mg/kg bwt)	0.096±0.005d
CC14 (20ml/kg bwt) + Sil. (25mg/kg bwt)	0.105±0.006e

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically (P > 0.05) different from each other. SD; Standard Deviation.

TABLE 3.12 PCM CATALASE

Effect of Aqueous and Methanolic Extracts of *Parmaricuratellifolia* on Catalase Activity in the Liver of Paracetamol Treated Rat.

GROUP	Catalase (umole/mg protein')
Control PCM	1642.2±0C
PCM	293.05±1.17a
Aqueous Methanolic	1290.3±1.35b
Aqueous + PCM	1759.5±1.35C
Methanolic + PCM	1173±0b
Silymarin + PCM	1231.65±2.24b
	469.20±0a

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically (P > 0.05) different from each other. SD; Standard Deviation.

3.13 Effects of aqueous and methanolic extracts of *Parinari curatellifolia* on catalase activity in the liver of CC14 treated rats,

The effects of aqueous and methanolic seed extracts of *Parinari curatellifolia* on catalase activity in the liver of CC14 treated albino rats are presented in table 3.13. CC14 hepatotoxicity caused significant decrease in the activity of catalase as compared with control. Treatment with the extract ameliorated the effect of CC14 on catalase activity in the liver homogenates.

3.14 Effects of aqueous and methanolic extracts of *Parinari curatellifolia* on glutathione peroxidase activity in the liver of paracetamol treated rats.

The effects of aqueous and methanolic seed extracts of *Parinari* on glutathione peroxidase activity in the liver of paracetamol treated rats is presented in table 3.14. The result indicated that paracetamol hepatotoxicity caused significant decrease in the activity of GPx as compared with control. Treatment with the extract nullified the effects of paracetamol on the activity of the enzyme.

TABLE 3.13 Effect of Aqueous and Methanolic Extracts of *Parinari curatellifolia* on Catalase Activity in the Liver of CCU Treated Rat.

GROUP	<u>catalase (umole/ms protein</u>
Control	1642.2±0e
Ecu	586.5±1.35a -
Aqueous Methanolic	-1290.3±1.35cd

Aqueous + CCl ₄	1759.5 ± 1.35e
Methanolic + CCl ₄	1173.0 ± 0C
Silymarin + CCl ₄	1407.60 ± 0d
	938.40 ± 0b

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically (P > 0.05) different from each other. SD; Standard Deviation

TABLE 3.14 Effect of Aqueous and Methanolic Extracts of *Parinari curatellifolia* on Glutathione peroxidase

Activity in the Liver of Paracetamol Treated Rat.

GROUP	GPx (μmole/GSSG/min)
Control	1.012 ± 0.078e
PCM	0.067 ± 0.012a
Aqueous	0.537 ± (X 018C
Methanolic	0.607 ± 0.005d
Aqueous + PCM	0.511 ± 0.009C
Methanolic + PCM	0.648 ± 0.028d
Silymarin - PCM	0.17 ± 0.029b

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically (P > 0.05) different from each other. SD; Standard Deviation.

3.15 Effects of aqueous and methanolic extracts of *Parinari curatellifolia* on glutathione peroxidase activity in the liver of CCl₄ treated rats.

The effects of aqueous and methanolic seed extracts of *Parinari curatellifolia* on glutathione peroxidase activity in the liver of CCl₄ treated rats is presented in table H[^]. The result indicated that CCl₄ hepatotoxicity caused significant decrease in the activity of GPx as compared with control. Treatment with the extract nullified the effects of CCl₄ on the activity of the enzyme.

3.16 Histopathological Study

The results of the histopathological study of the livers of rats treated with aqueous and methanolic seed extracts of *Parinari curatellifolia* as compared with paracetamol and CCl₄ treated rats are shown in figures 3 and 4. The results revealed that administration of paracetamol and CCl₄ caused perinuclear vacuolation of the hepatocytes as compared with the control. Administration of the extracts alongside with paracetamol or CCl₄ protected against the damage caused by paracetamol or CCl₄. Rats administered with the extracts alone had normal liver morphology like that of the control, the protection offered by the extracts compared well with silymarin (the standard drug).

TABLE 3.15 Effect of Aqueous and Methanolic Extracts of *Parinari curatellifolia* on Glutathione peroxidase Activity in the Liver of CCl₄ Treated Rat.

<u>GROUP</u>	<u>GPx fumole/GSSG/minl</u>
Control	1.012±0.078 ^f
CCl ₄	0.082±0.002 ^a
Aqueous	0.537±0.018 ^d
Methanolic	0.607±0.005 ^e
Aqueous + CCl ₄	0.404±0.003 ^c
Methanolic + CCl ₄	0.327±0.011 ^b
Silymarin + CCl ₄	0.204±0.0015 ^b

Values are mean ± SD; n = 4; Values with the same superscript and letter(s) down a column are not statistically (P > 0.05) different from each other, SD; Standard Deviation.

4.0 DISCUSSION

In the assessment of liver damage by paracetamol and carbon tetrachloride, the determination of enzymes levels such as ALT and AST is largely used. Necrosis of membrane damage releases the enzyme in to circulation; therefore, it can be measured in serum (Bergmeyer and Wahlefeld, 2006). A high level of AST indicates liver damage such as that due to viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver and is thus a better parameter for detecting liver injury (Moss and Butterworth, 2001). Elevated levels of the serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotum and Lawhorn, 1998). Serum ALP and bilirubin level on the other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of biliary enzymes.

Tables 2 and 3 present the serum biochemical indices of paracetamol Hepatotoxic albino rats treated with aqueous and methanolic extracts of *Parinari curatellifolia* as compared with silymarin and controls. There were a significant decrease in the level of ALP, AST, and ALT in the animal treated with the aqueous extracts and silymarin. It is known from the literature that the activities of enzymes are important in the diagnosis of liver damage. (Fransworth, 1997). The predominant source of ALT is the liver but AST has the highest concentration in the heart followed by liver skeletal muscles, pancreas, erythrocytes and spleen. Hence for all practical purposes, it is reasonable to consider that raised serum ALT has its origin in the liver (Kimberly, "2003). The reduced levels of enzyme activities after administration of the extracts may be due to the presence of phytochemicals such as flavonoids, tannin, alkaloid in them.

Elevation in the levels of the marker enzymes is a known effect of toxicity which specifically affects the liver (Fairhurst *et al*, 1997). The elevation of the serum levels of ALT, AST and ALP could be due to destruction of the membrane of the hepatocytes by paracetamol which leads to the leakages of the enzymes from the liver tissues to the extracellular environment hence the increased activities of these enzymes in the blood.

Results also showed a significant elevation in the total bilirubin level in the serum. Also, there were significant increases in the total protein, urea and albumin levels. It has been reported that a high serum total protein could be due to infection or dehydration, while low total protein level might be due to chronic liver damage (Kimberly, 2003). This experiment suggests a chronic liver diseases or damage as there was evidence of serious changes in the enzyme activities in the serum. Jaundice occurs in toxic or infectious diseases of the liver e.g hepatitis B or obstruction of the bile duct and rhesus incompatible babies. High level of conjugated bilirubin indicate that bile is not being properly excreted; therefore, an obstruction may be present in the bile duct or gall bladder. These could also be due to oxidative damage (Halliwell and Chirico, 2003). In Table 2 & 3 the serum ALT activities of the group fed with aqueous and methanolic extracts only (6.24 ± 0.12 and 8.12 ± 0.80) respectively

significantly lower than that of the paracetamol group (31.93 ± 0.66) and were almost similar to the control group (10.75 ± 0.20). These findings indicate that the aqueous and methanolic extracts are not toxic, the serum ALP activities of paracetamol group fed with 600mg/kg aqueous and methanolic extracts (84.25 ± 0.75 and 64.30 ± 0.75) respectively were significantly reduced as compared with paracetamol induced group. This indicates that the extracts are able to protect the liver from PCM toxicity.

Tables 4 and 5 present the liver biochemical indices of paracetamol of hepatotoxic albino rats treated with aqueous and methanolic extracts of *Parinari curatellifolia* compared with controls. There was a significant reduction in the activities of all the enzymes (ALT, AST, & ALP) in the liver of the paracetamol induced group. Pretreatment with either aqueous or methanolic extracts produce a significant increase in the activities of these enzymes. The protection offered by the extract compared well with that of silymarin the standard drug. The protection by the extract may be due to the presence of phytochemicals such as flavonoids tannis, alkaloids etc in them (Das and Pereira, 1990). Reduction in the level of the marker enzymes is a known effect of paracetamol toxicity which specifically affects the liver. (Fairhurst *et al.*, 1997). The decrement in the liver level of ALT, AST and ALP could be due to destruction of the membrane of the hepatocytes by paracetamol which leads to the leakages of the enzymes from the liver tissues to the extracellular environment, hence the increment in the activities of these enzymes in the blood. This is in agreement with the finding of Wasen *et al.*, 2001 and Crook, 2006, who reported that enzyme activities lost from some tissues like liver and heart are found in the blood.

There were also significant decreases in the total bilirubin, total protein, urea and albumin levels. It has been reported that low liver total protein might be due to chronic liver damage or disease (Kimberly, 2003). This experiment could suggest a chronic liver disease or damage.

A decrement in bilirubin concentration in the liver tissues and elevation of this marker in the serum is called jaundice. Jaundice occurs in toxic or infectious diseases of the liver e.g. hepatitis B or obstruction of the bile duct and rhesus incompatible babies. (Halliwell and Chirico, 2003).

CCl₄ is known to cause hepatic damage manifested in marked elevation in serum levels of aminotransferase enzymes (AST and ALT), especially ALT, which is considered the primary and specific marker of liver injury. Table 6-7 showed a significant increase in the activities of AST, ALT and ALP thereby confirming the hepatocellular damage in CCl₄ treated rats. After pretreatment with aqueous or methanolic extract of *Parinari curatellifolia* of these enzymes were decreased to values similar to those of controls. Also there was a marked increase in the levels of total protein, total bilirubin, urea and Albumin, in the CCl₄ group while the extracts were able to reduce the levels of these parameters.

Table 8-9 present the liver biochemical indices of PCM and CCl₄ hepatotoxic albino rats treated of *Parinari curatellifolia* as compared with silymarin and control. There was a significant reduction in the values of AST, ALT and ALP in the CCl₄ group but the extracts were able to reverse the effect of CCl₄ on these biomarks this may be due to the presence of flavonoid in the extracts. Table 10 & 11 present the liver TBARS of paracetamol and CCl₄ - intoxicated albino rat as compound with that of silymarin and control groups

Aqueous and methanolic extract of *Parinari curatellifolia* could also prevent the paracetamol and CCl₄ -induced increase in hepatic TBARS levels suggesting that extracts inhibit lipid peroxidation and its propagation reactions. CCl₄ and paracetamol caused marked toxicity by increasing the liver lipid peroxidation as detected by enhanced levels of hepatic TBARS (Poli, 1993). CCl₄ hepatotoxicity depends on the reductive dehalogenation of CCl₄ catalyzed by P450 in the hepatic endoplasmic reticulum leading to the generation of an unstable complex of trichloromethyl peroxy radical (CCl₃) which is reported to be a highly reactive species (Bravo, 1998). This free radical attacks membrane lipids causing their peroxidation, and may also covalently bind to lipids and proteins thereby initiating deleterious processes that ultimately leads to cell damage. Aqueous and methanolic extract of *Parinari curatellifolia* may afford cell protection by impairing CCl₄ mediated LPO, thus preventing the

generation of free radical's derivatives.

Besides the finding that CCl₄ and paracetamol increased lipid peroxidation, they also depleted intracellular GSH levels, indicating that GSH loss might result from the detoxification of CCl₄ and paracetamol by GSH conjugation. In addition, the previous enhancement of carbonyl protein contents was significantly prevented in rats treated with the extracts and silymarin, suggesting a further antioxidant protection against protein oxidation. Therefore, this result strongly suggests that both extracts act as antioxidant (Halim *et al.*, 2007).

The levels of catalase and GPx activities were reduced in paracetamol and CCl₄ groups when compare with the control groups (tables 12-15). Treatment of rats with paracetamol or CCl₄ induces processes in acute injury and also in regeneration, in which injury events are predominantly expressed at early stages, although this regeneration process is latent (Bhave *et al.*, 2008). A possible reason why catalase and GPx were augmented in rats pre-treated with extract or silymarin and then treated with paracetamol or CCl₄ is that these extracts might be able to counteract PCM and CCl₄ - induced massive reactive oxygen species production (Halliwell and Gutteridge, 1999).

It could therefore be speculated *Parinari curatellifolia* might be playing a role during the early stages in CCl₄ and paracetamol-induced liver injury, decreasing lipid peroxidation and protein carbonylation and consequences improving cellular antioxidant status thereby preventing ALT, AST and ALP leakage from liver.

The activity of GPx, an important antioxidant enzyme that takes part in the enzymatic neutralization of free radicals, was significantly decreased by paracetamol and CCl₄ treatment. However the effect of PCM and CCl₄ on GPx was ameliorated by pre-treated with aqueous and methanolic extract of *Parinari curatellifolia*. This result strongly suggests that paracetamol and CCl₄, probably through their conversion to their active metabolite produced in the liver after oral administration, inhibits the activity of GPx. In accordance with the response found in GPx, catalase activity also showed decreased values after CCl₄ and paracetamol treatment and again the pre-treatment with extracts was able to normalize its activity. Taking into account the diminished GSH content together with the persistent enhancement of TBARS levels found in livers of rats treated with paracetamol and CCl₄. These results suggest that this organ was facing a severe oxidative insult. It was strongly suggesting that this process was reversed after the treatment with extracts because the extracts possess some antioxidant properties that can guarantee GSH maintenance probably through reactive oxygen species neutralization.

The histopathology analysis provided complementary evidence that treatment with aqueous and methanolic extract of *Parinari curatellifolia* as well as silymarin attenuated the cytoplasmic changes in rat liver induced by paracetamol and CCl₄ administration. The hepatocytes plate of paracetamol and CCl₄-treated rats resulted in cyto-physiological changes shown by Canson's trichrome staining, revealing different colours in the cytoplasm (fig. 3 and 4). This might have been due to the formation of highly reactive radicals associated with the oxidative threat induced by paracetamol and CCl₄ cellular accumulation of lipid hydroperoxides can cause cytotoxicity associated with peroxidation of membrane phospholipids by lipid hydroperoxides, the basis for a related cellular damage (Halliwell and Gutteridge, 1999). The cytophysiological changes coincide with increased levels of lipid peroxidation and protein oxidation. While the administration of extracts and silymarin prevented such biochemical and histopathological induced by PCM & CCl₄. This effect could be attributable to the antioxidant activity of these two compounds, which significantly attenuated the oxidative threat and led to restoration of normal physiological functions.

Conclusion

Both extracts of *Parinari curatellifolia* seed demonstrated hepatoprotective activities against paracetamol- and carbon tetrachloride-induced liver damage. The protection offered by the extracts appeared to be related to antioxidative mechanism. Therefore, the extracts of *Parinari curatellifolia* can be used in management and treatment of liver diseases.

Recommendation

More research should be done on *Parinari curatellifolia* seed in order to elucidate how this seed can be processed to a synthetic drug for treating liver diseases.

CONTRIBUTION OF THE STUDY TO KNOWLEDGE

The findings from this study are expected to:

1. provide information on the phytochemical constituent of aqueous and methanolic extracts of Mobola plum seeds,
2. Reveal the effect of the extracts on some biochemical indices of paracetamol and carbon tetrachloride hepatotoxic rats.

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