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Prophylactic efficacy of aqueous extract of unripe pawpaw (*Carica papaya)* fruit on hematological and biochemical parameters in induced hyperbilirubinemia Wistar rats

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Abstract

This research offers a comprehensive examination of hyperbilirubinaemia, characterized by elevated levels of bilirubin in the bloodstream leading to jaundice. It presents a prevalent challenge during the neonatal phase, as bilirubin can accumulate in various body parts resulting in potential neurotoxicity. The discussion encompasses factors influencing bilirubin production, conjugation, and elimination in the organism, along with an overview of the causes and symptoms of hyperbilirubinaemia. The study delves into the potential advantages of utilizing *Carica papaya*, a tropical fruit, for the prevention of hyperbilirubinaemia. Traditional medical approaches for managing this condition are linked to numerous adverse effects, hence the necessity to develop more affordable, easily accessible, and efficacious drugs with minimal or no side effects. Blood samples were procured and subjected to full blood cell count analysis via an automated hematologic analyzer, while serum samples were utilized for quantifying liver enzymes and bilirubin employing a spectrophotometric technique. Additionally, the weight of visceral organs was determined using a Mettler weighing balance. The outcomes of an experimental study conducted on rodents revealed that pretreatment with *Carica papaya* extract notably decreased bilirubin and liver enzyme levels in the subjects. This highlights the potential role of *Carica papaya* in the preventive management of hyperbilirubinaemia, underscoring the imperative for further investigation in this domain. In essence, this research serves as a valuable repository of knowledge for healthcare practitioners and researchers keen on addressing hyperbilirubinaemia.

Keywords: Jaundice; extract; Liver enzyme; Carbon tetrachloride; Phenyl hydrazine

1. Introduction

Hyperbilirubinaemia, characterized by elevated levels of bilirubin in the blood serum leading to jaundice, is a common issue during the neonatal period. Bilirubin, a byproduct of haem degradation from various sources, accumulates in the skin and mucous membranes, causing visible yellowish discoloration [1]. Physiological jaundice is a transient condition, while pathological jaundice indicates underlying issues with bilirubin production or excretion [2]. During the neonatal period, factors such as G6PD deficiency, hemolysis, and Rhesus factor influence bilirubin metabolism, increasing the risk of complications due to neonatal red blood cell lysis. Serum bilirubin serves as a crucial marker of liver function, as the liver plays a key role in bilirubin metabolism and excretion [3, 4].

Carica papaya, a perennial plant native to tropical regions, is traditionally used in Nigerian medicine to treat jaundice [5, 6]. Its unripe fruit extract, known as Aqueous *Carica papaya* (ACP), has been studied for its medicinal properties, showing a LD50 of 2,520 mg/kg in rats. Papaya contains bioactive enzymes like papain and chymopapain, along with

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various therapeutic compounds with antimicrobial, anti-inflammatory, and wound healing properties [7,8]. This study aims to investigate the prophylactic effects of Aqueous *Carica papaya* extract on hematological and biochemical parameters in a Wistar rat model of hyperbilirubinaemia.

2. Material and methods

2.1. Materials

Animal weighing balance (Apollo GX-AE), Dissecting set and Dissecting board, Cotton wool, Oropharyngeal cannula, pipette (10 mL), Distilled water, Water bath (MO-BC-2D/DE), Wistar rats, Plastic cage, Flat botton flasks (250 mL), Masking tape and permanent markers. Olive oil marked as GOYA by: Andalucia, Spain. Manufactured by GOYA EN ESPANA, SAIL, SAVILIA SPAIN with NAFDAC Reg. NO: A4-0348. Phenyl hydrazine marketed as Elam Pharma by: Laborate pharmaceuticals India LTD. Carbon tetrachloride trade as CARBN TET-104® by laboratories PVT. LTD. C1B, 305/2, 3, 4 & 5, G.I.D.C. Kerala (Bavla), Dist.: Ahmedabad-382 220, Gujarat, India. Paraffin oil marked as DULUX Syrup. Manufactured by SQUARE pharmaceutical. It was purchased from Rovi pharmacy, NO.: 15, Iorkyaa Akor Street, High-Level, Makurdi, Benue State, Nigeria. A brand of Silymarin trade as SILYBON-140® by MICRO LABS LIMITED. HB- 211, Illage Kasho, Kerala (Bavla), Dist.: Ahmedabad-382 220, Gujarat, India. Each film coated tablet contains: Silymarin USP 140mg. It was diluted in distilled water in the ratio of10mg/100ml as stock. Chloroform trade as Liquid Chloroform chemical by Dasaldhan chemical PVT. LTD, Kerala (Bavla), Dist.: Ahmedabad-382 220, Gujarat, India.

2.2. Plant Preparations and Extraction

Fresh unripe *Carica papaya* linn fruit was obtained from a local garden in the University of Agriculture, and authenticated by the herbarium officer of the Department of Crop production, Faculty of Agronomy, University of Agriculture Makurdi, Benue state. Aqueous extraction of unripe *Carica papaya* was carried out at the Biochemistry Department laboratory, Benue State University, Makurdi as follow; the fresh unripe pawpaw fruit was peeled and the cream-coloured seeds inside were discarded after which it was cut to small pieces and washed with distilled water, air dried in the shade at room temperature over a period of two weeks then blended with a domestic blender and weigh to one kilogram. Smooth paste was made by placing it in a Soxhlet extractor with 2L distilled water and was prepared according to the method reported by [9]. The aqueous *Carica papaya* extract was sieved into a clean container and concentrated to solid mass using the water bath and continuously heated at 60 °C for nine days until the paste was formed. The water extracted was concentrated and the solid material residue was weighed on an LS series electronic weighing balance (ORMA, Italy). The extract was stored in a sealed dark glass bottles in a deep freezer. The residue was later constituted in water in the ratio of 10g/100mL before used.

2.3. The Percentage (%) Yield

The percentage (%) yield of aqueous unripe C. papaya was calculated using the formula proposed by Sheneni et al. [10].

$$\% \text{ Yield of the extract} = (\text{Extract weight}) / (\text{Dry weight of pawpaw fruit}) \times 100 \quad \ldots\ldots(1)$$

2.4. Preparation of PHZ (Phenylhydrazine) Solution

In all cases, hyperbilirubinaemia was induced in the rats with an aqueous solution of 40 mg/kg of PHZ administered orally for two alternate days. The aqueous solution of 40 mg/kg of PHZ (100 mL) was made up to 200 mL with inert liquid paraffin before being administered.

2.5. Preparation of Carbon Tetrachloride (CCl_4) Solution

One hundred milliliters of Carbon tetrachloride were measured out into a 200 mL flat bottom flask and made up to 200mL with inert olive oil (Goya Spain).

2.6. Animals

Twenty-five Wistar rats of either sex weighing between 200-300 g were obtained from the animal House of the College of Health Sciences, Benue State University Makurdi and allowed to acclimatize for 3 weeks before the commencement of the experiment. They housed in plastic cages for rats (60cmx40cmx30cm), suitably bedded with iron roof and also, ethical clearance for the uses of animal for experiment was obtained from the Ethical committee in college of Health Sciences, Benue State University, Makurdi (CREC/DIS/003). They were maintained under standard conditions of room temperature (25°C) and 12/12-hour light-dark cycle. They were also adequately fed with standard rat chow and allowed

access to water ad libitum. The animals were identified using permanent markers on their head, neck, right leg, tails and back [11].

Figure 1 A group of Wistar rats use for the experiments

2.7. Experimental design

The animals were randomly assigned to five experimental groups with 5 animals each. Animals in group A served as naïve or negative control and received sterile distilled water at 5mls/Kg body weight twice daily for the entire seven days duration of the experiment. Animals in group B served as the Jaundice control received PHZ 40 mg/kg and CCl_4 1mL/kg. Animals in groups C and D received 400 mg/kg and 800 mg/kg aqueous *Carica papaya* respectively twice daily. Group E served as the positive control in which the animals were administered Silymarin 100 mg/kg twice daily.

2.7.1. Animal Grouping and Treatment

The adults Wistar rats (total n=25) were divided into five groups and each group contained five (n=5) rats.

- Group B to E were treated with PHZ and CCl_4 according to Ayeni et al., 2018
- Group A (Negative control): Wistar Rats were treated with normal saline at 5mL/kg/b.wt for 7 days.
- Group B Wistar Rats were treated with PHZ 40 mg/kg on day 1 and 3, CCl_4 1mL/kg on day 2.
- Group C Wistar Rats were treated with PHZ 40 mg/kg on day 1 and 3, CCl_4 1mL/kg on day 2 and ACP extract 400mg/kg /b.wt on day 2 to 7.
- Group D Wistar Rats were treated with PHZ 40 mg/kg on day 1 and 3, CCl_4 1mL/kg on day 2 and ACP extract 800mg/kg /b.wt on day 2 to 7.
- Group E (Positive control): Wistar Rats were treated PHZ 40 mg/kg on day 1 and 3, CCl_4 1mL/kg on day 2 and Silymarin 100mg/kg /b.wt on day 2 to 7.

For the treatment, animals in groups B-E were treated with PHZ on days 1, day 3 and in day 2, they received CCl_4, while group B served as the induced untreated group which received PHZ and CCl_4 but not treated. Groups C, D and E received PHZ and CCl_4 together with 400 mg/kg, 800 mg/kg of ACP and 100 mg/kg Silymarin twice daily respectively [11]. All animals were sacrificed on day 8 after an overnight fast. At sacrifice, blood was obtained through cardiac puncture and the weight of the organs of interest (heart, liver, spleen) were harvested and recorded with Apollo GX-AE weighing balance after rinsing in normal saline.

2.8. Haematological Parameters

After anaesthetizing the rats with chloroform, blood samples were collected using a 5mls syringe and needle from the heart and 2mL of it was dispensed into EDTA tubes for haematological analysis. Haematological determinations conducted on all the study groups included Pack cell volume (PCV), Red Blood Cell (RBC), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin, Concentration (MCHC), White Blood Cell (WBC), Monocyte, Lymphocyte and Granulocyte [1, 12].

2.9. Biochemical Parameters

After anaesthetizing the rats with chloroform, blood samples were collected using a 5mls syringe and needle from the heart and then 3mls remaining in the syringe after dispensing 2mls in EDTA bottle for haematological analysis was dispensed in plain bottles for biochemical assay. The serum obtained from the blood samples was allowed to settle down in the plain bottle and were used for biochemical assays using the Randox test kits for clinical chemistry (Roche, UK); assays performed include Alanine Aminotransferase (ALT), ALP (alkaline phosphatase), LDH (lactate dehydrogenase), bilirubin (total and direct), Total protein and Alb (albumin) [13].

2.10. Preparation of the visceral Organs

Excised liver, heart and spleen of sacrificed rats were washed in normal saline and weighed to obtain the absolute organ weights. Relative organ weights were determined using the formula:

$$\text{Relative Organ Weight} = \text{Absolute Organ Weight} \div \text{Body Weight at Sacrifice} \times 100\%$$

2.11. Statistical Analysis

Data obtained from the study were expressed as mean ±SD. The level of homogeneity among the groups was tested using Analysis of Variance (ANOVA), followed by Tukeys' post hoc test for multiple comparisons using SPSS statistical tool version 22. A value of $p < 0.05$ was considered to indicate a significant difference between groups at alpha level of 5%.

3. Results

The presentation of the results and data analysis, in alignment with the study, is showcased below. It was hypothesized that unripe *Carica papaya* possesses numerous haematological and biochemical advantages. To ascertain these haematological and biochemical parameters, we conducted a research study on hyperbilirubinemia-induced Wistar rats, investigating the effect of the aqueous extract of unripe pawpaw (*Carica papaya*) fruit.

3.1. Effect of Aqueous Extract of Unripe Pawpaw (*Carica papaya*) fruit on Pack Cell Volume (PCV) and Red Blood Cell (RBC) of Wistar rats in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyberbilirubinaemia induced in Wistar rat

Result in table 1 showed that treatment with phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) significantly ($P < 0.05$) reduced the PCV and RBC count relative to control as seen in Group B. Treatment with aqueous extract of unripe *Carica papaya* fruit significantly ($P < 0.05$) elevated the PCV and RBC count. Treatment with the standard drug silymarin (100mg/kg) also significantly ($P < 0.05$) elevated the PCV and RBC count. The increase seen with the standard drug treatment is not significantly different from that caused by the aqueous extract [14].

Table 1 Effect of Aqueous Extract of Unripe Pawpaw (*Carica papaya*) fruit on PCV and RBC in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar rat

PCV and RBC Parameter					
Parameter	**Group A**	**Group B**	**Group C**	**Group D**	**Group E**
PCV (%)	56.04±0.04	41.52±9.96*	49.97±3.87*	52.04±1.56*	48.14±4.32*
RBC($X10^{12}$)/L	9.23±1.4	1.43±1.64*	8.65±8.30*	8.10±3.70*	7.84±3.70*

$P<0.05$; The values in table 1 is expressed as mean ± standard deviations. Values asterisked (*) are statistically significant with $p< 0.05$. N=5

3.2. Effect of Aqueous Extract of Unripe Pawpaw (*Carica papaya*) fruit on White Blood Cell (WBC), Granulocyte (GRA), Monocyte and Lymphocyte of phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar rats

The results indicated that administration of phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) led to a significant ($P < 0.05$) elevation in WBC, Granulocyte, Monocyte, and Lymphocyte counts compared to the control, as observed in Group B. Conversely, treatment with the aqueous extract of unripe *Carica papaya* fruit resulted in a notable decrease ($P < 0.05$) in WBC, Granulocyte, Monocyte, and Lymphocyte counts. Similarly, the standard drug silymarin (100mg/kg) also induced a significant ($P < 0.05$) reduction in these counts. Notably, the decrease observed with silymarin treatment was not significantly different from that caused by the aqueous extract [13, 15].

Table 2 Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on WBC, GRA, Lymphocytes and Monocyte in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wister rat

Parameters	Group A	Group B	Group C	Group D	Group E
WBC($X10^9$)/L	11.00±3.6	15.36±1.3*	12.6±1.0*	10.50±1.4*	10.31±1.2*
GRANULOCYTE (%)	31.30±12.32	36.00±4.70*	35.32±6.21	33.42±9.97*	33.88±6.52*
LYMPHOCYTE (%)	58.82±6.51	66.76±7.04*	59.92±6.61*	54.67±9.42*	56.34±5.25*
MONOCYTE (%)	14.46±3.98	17.00±1.90*	15.86±1.90*	14.67±4.19	14.40±1.86

P<0.05;The values in table 2 are expressed as mean ± standard deviations. Values asterisked (*) are statistically significant with p< 0.05. N=5

3.3. Effect of Aqueous Extract of Unripe Pawpaw (*Carica papaya*) fruit on Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar rat

Table 3 results demonstrate a significant ($p<0.05$) reduction in the levels of Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) in rats (group B) pre-treated with phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) alone, compared to the normal control (group A).

Post-treatment with unripe pawpaw extract (groups C and D) exhibited a statistically significant ($p<0.05$) increase in the levels of Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) compared to groups solely treated with PHZ and CCl_4 (groups B). Similarly, post-treatment with Silymarin 100mg/kg (group E) demonstrated a statistically significant ($p<0.05$) increase in the levels of Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) compared to groups pre-treated solely with PHZ and CCl_4 (groups B) [16].Notably, the increase observed with the standard drug treatment was not significantly different from that caused by the aqueous extract.

Table 3 Effects of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on HB, MCH, MCH and MCHC in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced Wistar rat

Parameter	Group A	Group B	Group C	Group D
HB(g/dl)	16.36±0.72	12.32±1.58*	15.02±1.27*	13.66±0.72*
MCV (fl)	64.4±12.70	61.00±5.29*	61.28±7.79*	65.00±14.23
MCH (pj)	19.84±5.32	18.52±4.46*	18.14±2.60*	21.14±7.08*
MCHC (g/dl)	29.02±1.80	28.18±1.40*	29.62±1.44	30.32±1.94

P<0.05; The values in table 3 are expressed as mean ± standard deviations. Values asterisked (*) are statistically significant with p< 0.05. N=5

3.4. Effect of Aqueous Extract of Unripe Pawpaw (*Carica papaya*) fruit on Platelet count in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl4) Hyperbilirubinaemia induced in Wistar rat

Table 4 showed significant ($p<0.05$) decrease in the level of Platelet in Wistar rats (group B) pretreated with phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) alone when compared to those of the normal control (group A). The groups that were post treated with unripe pawpaw extract (group C and D) had a statistically significant ($p<0.05$)

increase in level of platelet when compared to the groups that were treated alone with PHZ and CCl_4 (groups B). Group E which was post treated with Silymarin 100mg/kg had a statistically significant ($p<0.05$) increase in level of platelet when compared to the groups that were pretreated alone with PHZ and CCl_4 (groups B). The increase seen with the standard drug treatment is not significantly different from that caused by the aqueous extract.

Table 4 Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Platelet in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar rat

Parameter	Group A	Group B	Group C	Group D	Group E
PLATELET COUNT($X10^9$)/L	5.80±1.91	3.02±8.4*	4.77±2.14*	5.90±2.10	4.89±2.90*

P<0.05; The values in table 4 are expressed as mean ± standard deviations. Values asterisked (*) are statistically significant with p< 0.05. N=5

3.5. Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Total Bilirubin and Direct Bilirubin of Wistar rats in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar rat

Table 5 illustrates a significant ($p<0.05$) elevation in the levels of Direct Bilirubin (DB) and Total Bilirubin (TB) in rats (group B) pre-treated with phenyl hydrazine (PHZ) and carbon tetrachloride (CCl4) alone, compared to the normal control (group A). Post-treatment with unripe pawpaw extract (groups C and D) exhibited a statistically significant ($p<0.05$) decrease in the levels of DB and TB compared to groups solely treated with PHZ and CCl_4 (groups B) [17, 18]. Similarly, post-treatment with Silymarin 100mg/kg (group E) demonstrated a statistically significant ($p<0.05$) decrease in the levels of DB and TB compared to groups pre-treated solely with PHZ and CCl_4 (groups B). Notably, the decrease observed with the standard drug treatment was not significantly different from that caused by the aqueous extract.

Table 5 Effect of Aqueous Extract of Unripe Pawpaw (*Carica papaya*) fruit on Total Bilirubin and Direct Bilirubin in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar rat

Parameter	Group A	Group B	Group C	Group D	Group E
Total Bilirubin (3.4-20.0) Nmol/L	4.68±1.11	27.22±1.94*	21.52±1.16*	15.68±2.46*	20.12±2.63*
Direct Bilirubin (1.7-5.1) Nmol/L	2.92±0.76	17.82±2.01*	8.84±1.47*	4.72±0.68*	3.80±0.94*

P<0.05;The values in table 3.5 are expressed as mean ± standard deviations. Parameters asterisked (*) are statistically significant with p< 0.05, the parameters not indicated with asterisk (*) not significant (P>0.05). N=5.

3.6. Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl4) Hyperbilirubinaemia induced in Wistar rat

Table 6 Effect of Aqueous Extract of Unripe Pawpaw (*Carica papaya*) fruit on Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar rat

Parameter	Group A	Group B	Group C	Group D	Group E
AST (SGOT) (10-50)IU	42.02±4.06	167.96±182.45*	48.84±15.73*	41.16±7.19	51.14±4.63*
ALT (SGTPT) (10-50)IU	36.38±10.08	104.16±58.43*	62.58±5.67*	44.20±4.35*	41.36±4.16*
ALK.PHO (100±250)IU	107.98±10.58	280.00±53.51*	221.02±40.77*	171.72±29.76*	142.10±31.18*
AST (SGOT) (10-50)IU	42.02±4.06	167.96±182.45*	48.84±15.73*	41.16±7.19	51.14±4.63*

P<0.05;The values in table 6 are expressed as mean ± standard deviations. Parameters asterisked (*) are statistically significant with p< 0.05, the parameters not indicated with asterisk (*) not significant (P>0.05). N=5.

Significant ($p<0.05$) increase in the level of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) in rats (group B) pretreated with phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) alone when compared to those of the normal control (group A) is presented in table 6 below. The groups that were post treated with unripe pawpaw extract (group C and D) had a statistically significant ($p<0.05$) decrease in level of Alanine aminotransferase (ALT), Aspartate Aminotransferase (AST) and alkaline Phosphatase (ALP) when compared to the

groups that were treated alone with PHZ and CCl_4 (groups B). Group E which was post treated with Silymarin 100mg/kg had a statistically significant ($p<0.05$) decrease in level of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) when compared to the groups that were pretreated alone with PHZ and CCl_4 (groups B). The decrease seen with the standard drug treatment is not significantly different from that caused by the aqueous extract [19].

3.7. Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Total Protein and Albumin in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar rat

Table 7 illustrates a notable ($p<0.05$) decline in the concentrations of Total protein (TP) and Albumin (Alb) in rats (group B) pre-exposed to phenyl hydrazine (PHZ) and carbon tetrachloride (CCl4) individually in comparison to those in the control group (group A).

The groups subjected to post-treatment with unripe pawpaw extract (group C and D) exhibited a statistically significant ($p<0.05$) elevation in the levels of Total protein (TP) and Albumin (Alb) when contrasted with the groups treated solely with PHZ and CCl_4 (groups B). In contrast, Group E, receiving post-treatment with Silymarin at a dosage of 100mg/kg, demonstrated a statistically significant ($p<0.05$) rise in the levels of Total protein (TP) and Albumin (Alb) when compared to the groups that were solely pretreated with PHZ and CCl_4 (groups B). The increment observed with the standard pharmaceutical intervention does not differ significantly from that induced by the aqueous extract.

Table 7 Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Total Protein and Albumin in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar rat

Parameter	Group A	Group B	Group C	Group D	Group E
Total Protein (65-80) g/l	69.00±4.47	54.00±3.16*	60.60±5.18*	62.80±3.35*	59.00±1.58*
Albumin(39-51) g/L	42.00±2.15	25.42±3.87*	33.44±2.52*	39.14±1.09*	33.90±5.12*

P<0.05;The values in table 3.7 are expressed as mean ± standard deviations. Parameters asterisked (*) are statistically significant with p< 0.05, the parameters not indicated with asterisk (*) not significant (P>0.05). N=5.

3.8. Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Lactate Dehydrogenase (LDH) of Wistar rats in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar Rat

Table 8 demonstrates a significant ($p < 0.05$) elevation in lactate dehydrogenase (LDH) levels in rats pretreated with phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) alone (group B) compared to the normal control (group A). Moreover, groups C and D, which received post-treatment with unripe pawpaw extract, exhibited a statistically significant ($p < 0.05$) increase in LDH levels compared to groups treated solely with PHZ and CCl4 (groups B). Interestingly, group E, which received post-treatment with Silymarin 100mg/kg, also demonstrated a significant ($p < 0.05$) increase in LDH levels compared to groups pretreated solely with PHZ and CCl_4 (groups B). Notably, the LDH elevation observed with standard drug treatment did not significantly differ from that caused by the aqueous extract.

Table 8 Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on LDH of Wistar rats in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced in Wistar Rat

Parameter	Group A	Group B	Group C	Group D	Group E
LDH (IU/L)	203.08±14.60	325.76±45.95*	378.34±50.70*	272.20±37.60*	431.1±28.55*

P<0.05;The values in table 8 are expressed as mean ± standard deviations. Parameters asterisked (*) are statistically significant with p< 0.05, the parameters not indicated with asterisk (*) not significant (P>0.05). N=5.

3.9. Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Relative Organ Weight (ROW) of Liver in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced Wistar rat

Figure 2 illustrates that there was no statistically significant ($P > 0.05$) increase in the relative organ weight of the liver in rats pretreated with phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) alone (group B) compared to the normal control (group A). Additionally, groups C and D, which received post-treatment with unripe pawpaw extract, showed no statistically significant ($P > 0.05$) decrease in the relative organ weight of the liver compared to groups treated solely with PHZ and CCl_4 (groups B). Similarly, group E, which received post-treatment with Silymarin 100mg/kg, did not exhibit a statistically significant ($P > 0.05$) decrease in the relative organ weight of the liver compared to groups

pretreated solely with PHZ and CCl_4 (groups B). Notably, the decrease observed with standard drug treatment was not significantly different from that caused by the aqueous extract.

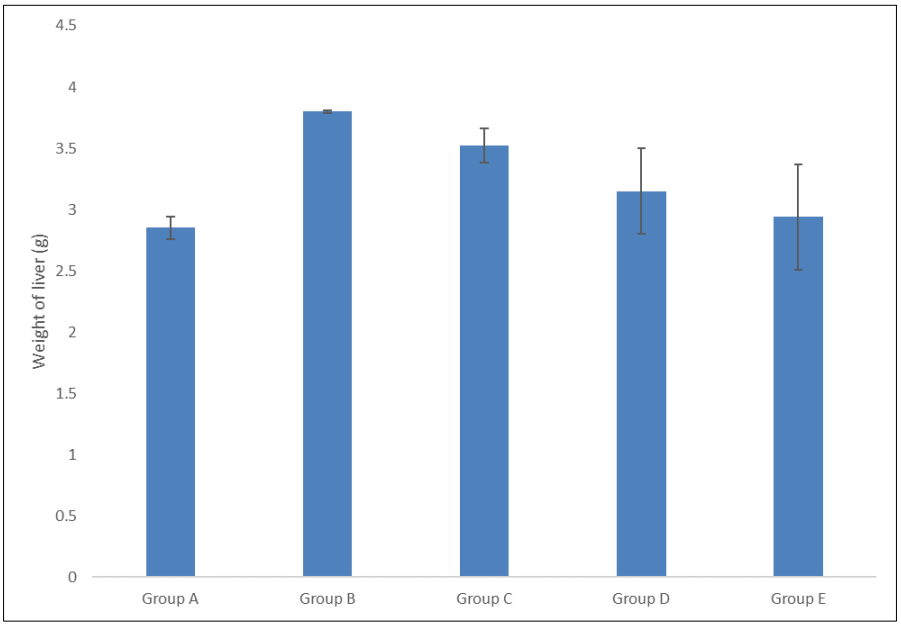

Figure 2 Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Liver Weight in PHZ and CCl4 induced Hyperbilirubinaemia

3.10. Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Heart Weight in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) induced Hyperbilirubinaemia

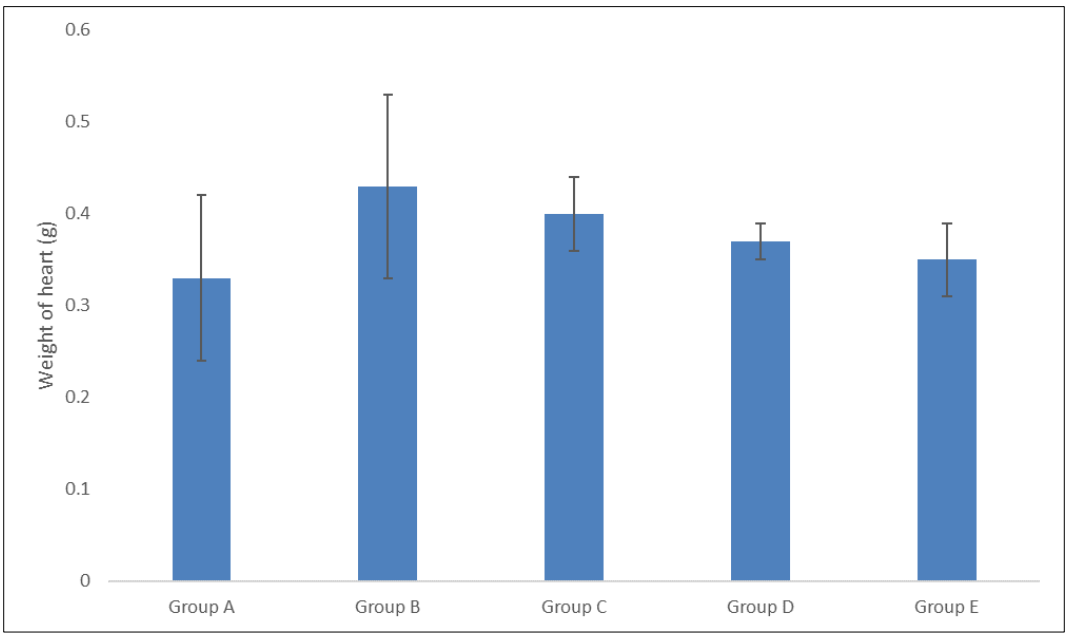

Figure 3 Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Heart Weight in PHZ andCCl_4 induced Hyperbilirubinaemia

Figure 3 illustrated a lack of statistically significant (P>0.05) elevation in the Relative Organ Weight of the Heart in rats (group B) pre-exposed to phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) individually, in comparison to the control group (group A). The groups subjected to post-treatment with unripe pawpaw extract (groups C and D) exhibited no statistically significant (p>0.05) reduction in the Relative Organ Weight of the Heart when compared to the groups treated solely with PHZ and CCl_4 (groups B). Group E, which underwent post-treatment with Silymarin at a

dosage of 100mg/kg, displayed no statistically significant ($p>0.05$) decline in the Relative Organ Weight of the Heart in comparison to the groups solely pretreated with PHZ and CCl_4 (groups B). The reduction observed with the standard medication intervention does not differ significantly from that induced by the aqueous extract.

3.11. Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Relative Organ Weight of Spleen of Wistar rats in phenyl hydrazine (PHZ) and carbon tetrachloride (CCl_4) Hyperbilirubinaemia induced Wistar Rat

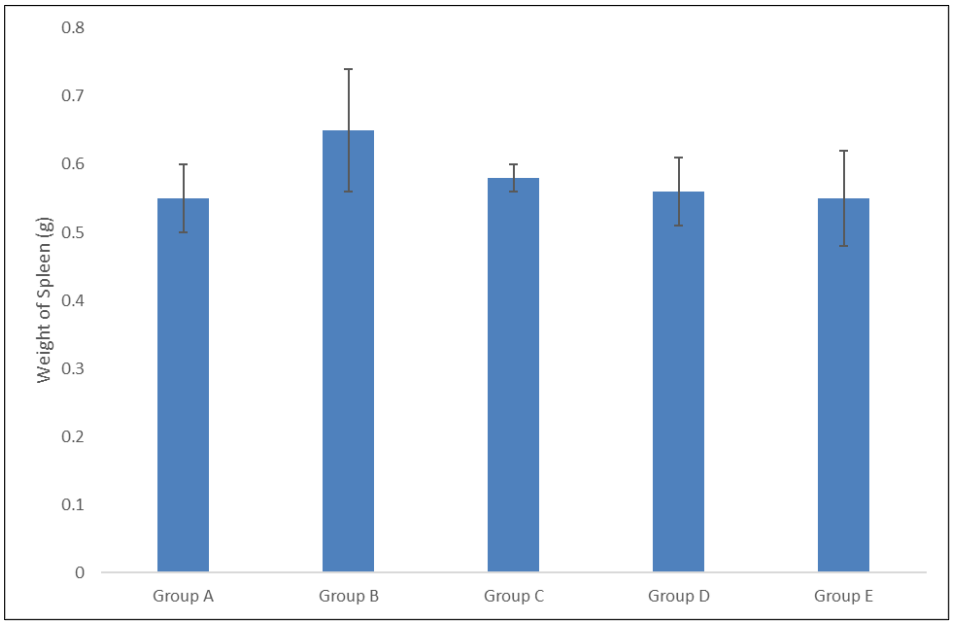

Figure 4 Effect of Aqueous Extract of Unripe Pawpaw *(Carica papaya)* fruit on Spleen Weight in PHZ andCCl_4 induced Hyperbilirubinaemia

The findings depicted in figure 4 revealed a lack of statistical significance ($p>0.05$) in the elevation of Relative Organ Weight of the spleen in rats (group B) pre-exposed to phenylhydrazine (PHZ) and carbon tetrachloride (CCl_4) independently compared to those in the standard control (group A). There was no statistically significant ($p>0.05$) reduction in the Relative Organ Weight of the Spleen in groups C and D, which received post-treatment with unripe pawpaw extract, in comparison to groups B treated solely with PHZ and CCl_4. In contrast, Group E, which underwent post-treatment with Silymarin 100mg/kg, exhibited no statistically significant ($p>0.05$) decrease in the Relative Organ Weight of the Spleen compared to groups B that were only pre-treated with PHZ and CCl_4. The increment observed with the conventional drug therapy does not differ significantly from that induced by the aqueous extract.

4. Discussion

This investigation sought to examine the preventive efficacy of unripe *Carica papaya* on hematological and biochemical parameters in experimental Wistar rats. The findings illustrated a notable positive influence on hematological and biochemical parameters in Wistar rats with hyperbilirubinemia when exposed to the aqueous extract of unripe *Carica papaya* (ACP). Specifically, the ACP extract significantly mitigated the impacts of phenyl hydrazine and carbon tetrachloride by averting the degradation of red blood cells and other hematological, as well as biochemical parameters.

Previous studies [20, 21, 22,23, 24] have underscored the diagnostic significance of hematological parameters in evaluating health indicators, and this investigation substantiated that unripe pawpaw substantially hindered the breakdown of red blood cells, along with modifications in various hematological parameters. The reduction noted in parameters like packed cell volume, hemoglobin, and red blood cell count in untreated rats with ACP extract was consistent with existing literature. Moreover, the research evidenced that ACP extract lowered white blood cell count, suggesting its capacity to regulate immune responses [13, 25, 26, 27].

In terms of biochemical parameters, the administration of phenyl hydrazine led to a detrimental rise in serum bilirubin levels across all groups [28]. Nevertheless, the ACP extract notably diminished bilirubin levels, implying its efficacy in alleviating jaundice by conjugating unconjugated bilirubin. Correspondingly, the ACP extract exhibited protective

effects on the liver by reducing levels of hepatic function markers such as alanine aminotransferase, alkaline phosphatase, and lactate dehydrogenase. The synthetic function of the liver was maintained, particularly with higher dosages of ACP extract [29, 30, 31].

The examination also noted no substantial decline in the weight of the lung and heart in groups treated with ACP extract compared to the control group. Nonetheless, there was no significant increase in spleen weight (splenomegaly) induced by phenyl hydrazine. Overall, the aqueous *Carica papaya* extract displayed significant impacts on hematological parameters, biochemical parameters, and organ weights, indicating its potential therapeutic advantages [32, 33, 34]. The hepatoprotective properties of ACP extract mirrored those of silymarin, a conventional hepatoprotective medication, implying its potential in enhancing liver cell regeneration and averting liver injury.

5. Conclusion

The findings of the current investigation suggest that ACP demonstrates potential hepato-protective and hepato-curative properties against phenyl hydrazine-induced hemolysis and CCl_4-induced hepatotoxicity. These effects may be attributed to bioactive constituents such as papain, chymopapain, ascorbic acid, cyanogenic glucosides, cystatin, and glucosinolates present in the extract from *Carica papaya*. The aqueous extract of *Carica papaya* has shown to mitigate induced hemolysis in Wistar rats, indicating its possible therapeutic utility. However, further studies are warranted to elucidate the underlying mechanisms of action and investigate its clinical implications.

The bilirubin-lowering efficacy of ACP extract was examined in rats with phenylhydrazine-induced jaundice in the present research. Evaluation of hematological and biochemical parameters revealed notable elevations in levels of DB, TB, ALT, AST, ALP, LDH, TP, and Alb in all experimental groups compared to untreated rats. Pretreatment with phenylhydrazine and carbon tetrachloride, followed by post-treatment with either 400mg/kg or 800mg/kg of aqueous *Carica papaya* extract or 100mg of silymarin, exhibited a significant mitigating impact on bilirubin levels and liver function test parameters, thereby substantiating the hepatoprotective properties of ACP fruit extract.

Compliance with ethical standards

Disclosure of conflict of interest

There are no competing interests to declare.

Statement of ethical approval

All experimental protocols were in compliance with the laid down ethical guidelines for the use of animals in research, given by the college of health sciences, Benue State University research and ethics committee.

Author's Contributions

All authors of this study have a complete contribution to data collection, data analyses, and manuscript writing.

Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

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