Journal of Pharmacy and Pharmacology 5 (2017) 457-466
doi: 10.17265/2328-2150/2017.07.011

Bilirubin Lowering Potential of Aqueous *Carica Papaya* Extract in Induced Jaundice in Rats

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Abstract: Background: The mature unripe fruit of aqueous *Carica papaya* (ACP) is used in Nigerian traditional medicine for the treatment of jaundice. Work done on aqueous extract of the unripe *C. papaya* in an acute oral toxicity study in rats showed the LD_{50} to be 2,520 mg/kg in rats. Current study evaluated the bilirubin-lowering potential of ACP extract in phenylhydrazine (40 mg/kg) induced jaundice in adult rats. Method: Jaundice was assessed by measuring the levels of TB (total bilirubin) and DB (direct bilirubin) in phenylhydrazine-treated animals with or without drug treatment, with CUR (curative), PRO (prophylactic study), and in animals with RLC (reduced liver capacity). Results: Result demonstrated that TB level of 24.0 µmols/L, 22.0 µmols/L and 45.0 µmols/L in the jaundiced group of the CUR, PRO and RLC respectively was significantly lowered ($p < 0.05$) to 10.0, 13.0, and 17.0 respectively by 400 mg/kg of the extract. Total bilirubin level of 24.0 µmols/L, 22.0 µmols/L and 45.0 µmols/L in the jaundiced group of the CUR, PRO and RLC respectively was significantly lowered ($p < 0.05$) to 7.0, 10.0, and 17.0 respectively by 800 mg/kg extract. Conclusion: The study concluded that ACP fruit extract has ability to lower elevated bilirubin level and confer hepatoprotective effect as seen from the liver function test indices thus justifying its ethnomedicinal use.

Key words: Carica papaya, unripe fruit, hepatoprotective, bilirubin, phenylhydrazine, jaundice, rats.

1. Introduction

Jaundice is a yellowish staining of the skin, sclera, and mucous membranes by bilirubin, a yellow-orange bile pigment. Bilirubin is formed usually from metabolized red blood cells [1]. Bilirubin (BR) is the breakdown product of the haem moiety of haemoglobin and other haemoproteins. Because of internal hydrogen bonding, bilirubin is water-insoluble and requires enzyme-mediated glucuronidation in the liver for biliary excretion [2]. In normal circumstances, plasma bilirubin is mostly unconjugated and is tightly bound to circulating albumin. It is taken up through facilitated diffusion and stored in hepatocytes bound to glutathione-S-transferases and conjugated to glucuronides by microsomal uridine-diphosphate glucuronyl transferase (UGT1A1). Bilirubin glucuronides are actively transported into the bile canaliculi by the ATP-utilizing pump MRP2 [2]. Bilirubin is degraded in the intestine by bacteria into urobilinogens, which are partly excreted in the urine. Increased production, reduced uptake and low glucuronidation capacity can increase plasma unconjugated bilirubin levels. In cases of inherited or acquired deficiencies of bilirubin storage or excretion, both conjugated and unconjugated bilirubins accumulate in the plasma. Conjugated bilirubin is less tightly bound to albumin and is excreted in the urine [2]. Bilirubin encephalopathy (kernicterus) is seen in severe cases of exaggerated neonatal jaundice and in patients with very high levels of unconjugated hyperbilirubinaemia owing to inherited disorders of

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bilirubin glucuronidation [3]. In infants, the transient neonatal jaundice is referred to as "physiologic jaundice" and it results from increased lysis of the erythrocytes red blood cells and impaired hepatic uptake, followed by limited ability of conjugation and excretion [4]. In neonates high elevated levels (> 12mg/dl) of unconjugated bilirubin impairs the antioxidant system of the red blood cells (RBC), enhances oxidative stress causing increases in ROS (reactive oxygen species) formation, morphological alteration and loss of phospholipids symmetry of red blood cell membrane, it also impairs the membrane transport systems of RBC [5] and can cross the blood brain barrier causing kernicterus [6]. Phototherapy is used for therapeutic management of neonatal jaundice, although several side effects of phototherapy have been reported [7]. In adults, phenobarbitone is the drug of choice in jaundice management [8]. In patients with jaundice as a result of Crigler-Najjar syndrome type 1, phenobarbital, ursodeoxycholic acid, calcium (infusions), metalloporphyrins, cholestyramine, chlorpromazine, and clofibrate (no longer on the US market), as well as alkalinization of urine, have all been considered as potential therapies. Problems associated with the use of cholestyramine include taste and concern about bile salt depletion and fat malabsorption. The exact roles and adverse effects of many of these drugs are not yet defined [9-13]. Bilirubin has recently been identified as the first endogenous substrate for cytochrome P450 2A5 (CYP2A5) and it has been suggested that it plays a major role in bilirubin clearance as an alternative mechanism to conjugation by UGT1A1 [14]. The effects of various metalloporphyrins on hepatic hemeoxygenase (EC 1.14.99.3) activity were examined in order to identify compounds that could inhibit heme degradation to bile pigment and might therefore be utilized to suppress the development of hyperbilirubinemia in the newborn [15]. Among nine metal-protoporphyrin IX chelates (i.e., metal-hemes) studied, Sn-heme, Mn-heme, and Zn-heme substantially diminished hemeoxygenase activity *in vivo* in the rat. Metalloporphyrins have been used as a synthetic analogue of heme to inhibit the hemeoxygenase enzyme, the rate-limiting step in heme catabolism to bilirubin. Tin mesoporphyrin (SnMp) is the drug of choice for clinical use because of its increased potency, stability, and photophysical properties. In sub-Saharan Africa, *Carica papaya* has been demonstrated to have healing properties, and it is very safe and cheap compared to conventional methods of management. The mature unripe fruit is used in the management of jaundice in pregnant women and neonates in Nigerian traditional medicine.

2. Method

Seventy five Albino rats of either sex weighing between 150-200 g were obtained from the animal facility of the Department of Pharmacology, Faculty of Pharmacy, Obefemi Awolowo University, Ile-Ife, Nigeria housed in plastic cages for rats (state dimensions of the typical cage), suitably bedded with wood shavings. They were maintained under standard conditions of temperature and humidity and had free access to standard growers mash feed (Vitals Ltd, Nigeria) and normal tap water except an overnight fast on days of experiments. The animals were identified using permanent markers on their tails and back.

2.1 Plant Preparations and Extraction

Matured fresh unripe *Carica papaya Linn.* fruit was obtained in a local garden in the senior staff quarters of the Obafemi Awolowo University, Ile-Ife, and authenticated by the herbarium officer of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The voucher number after deposition was FPI-1885. One kilogram from the mature unripe fruit was peeled and the cream coloured seeds inside the discarded after which it was cut to small pieces and then blended with a domestic blender to a smooth paste using 10 L of distilled water. The ACP extract was sieved into a clean container and concentrated to 100 g of solid mass. (10.00% w/w yield)

using the rotary evaporator at 60 °C.

2.2 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.

2.3 Preparation of Carbon Tetrachloride (CCl_4) Solution

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

2.4 Experimental Design

Five groups of 5 animals each were used for this study. Animals in group 1 served as normal treatment (naïve or negative) control and received sterile distilled water at 1mL/100g body weight twice daily for the entire seven day duration of the experiment. Animals in group 2 served as the Jaundice control receiving either PHZ alone or PHZ and CCl_4. Animals in groups 3 and 4 received 400 mg/kg and 800 mg/kg aqueous *Carica papaya* respectively. Group 5 served as the positive control in which the animals were administered Silymarin 100 mg.

2.5 Curative Studies

For the curative studies, animals in groups 2-5 were treated with PHZ on days 1 and 3, while group 2 served as the Jaundice control receiving PHZ alone. Groups 3, 4 and 5 were treated with 400 mg/kg, 800 mg/kg of ACP and 100 mg/kg Silymarin twice daily from day 2 till day 7. All animals were sacrificed on day 8 after an overnight fast. At sacrifice, blood was obtained from the heart and the weight of the organs of interest (heart, liver, spleen) were taken and recorded after rinsing in normal saline.

2.6 Prophylactic Studies

For the prophylactic studies, animals in groups 2-5 were treated with PHZ on days 2 and 4. Group 2 served as jaundice control and received PHZ alone. Groups 3, 4 and 5 were pretreated with 400 mg/kg, 800 mg/kg ACP and 100 mg/kg Silymarin respectively on day 1 and twice daily till the end of day 7. Groups 3, 4 and 5 received PHZ on days 2 and 4. All the animals were sacrificed on day 8 after an overnight fast. At sacrifice, blood was obtained from the heart and the weight of the essential organs was taken and recorded after rinsing in normal saline.

2.7 Prophylactic Studies in Animals with Reduced Liver Capacity

For prophylactic studies in animals with reduced liver capacity, animals in groups 2-5were treated with PHZ on days 2 and 4 and 1 mL CCl_4/kg on day 3. Group 2 served as Jaundice control receiving only PHZ on days 2 and 4 and CCl_4 on day 3. Groups 3, 4 and 5 were pretreated with 400 mg/kg, 800 mg/kg ACP and 100mg/kg Silymarin on day 1 and twice daily till end of day 7 with PHZ on day 2 and 4. Carbon tetrachloride, a hepatotoxin, was administered in addition to PHZ in this group so as to further reduce the bilirubin conjugating capacity of the liver. All the animals were sacrificed on day 8 after an overnight fast. The weights of the animals were recorded before sacrifice. At sacrifice, blood was obtained from the heart and the weight of the essential organs was taken and recorded after rinsing in normal saline.

2.8 Effect of Treatment on Hematological Parameters

Incisions were quickly made in the sacrificed animal's cervical region with the aid of a sterile blade and blood samples collected using a syringe and needle from the heart and dispensed in plain bottles for biochemical assays and EDTA tubes for haematological analysis. Haematological determinations conducted on all the study groups included Hb (haemoglobin) concentration, RBC (red blood cell) count, and HCT (haematocrit)

values.

2.9 Effect of Treatment on Biochemical Parameters

Incisions were quickly made in the sacrificed animal's cervical region with the aid of a sterile blade and blood samples were collected using a syringe and needle from the heart and then dispensed in plain bottles for biochemical assays and EDTA tubes for haematological analysis. The serum obtained from the blood samples 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) and Alb (albumin).

2.10 Preparation of the Vital Organs

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

Relative Organ Weight =

Absolute Organ Weight ÷ Body Weight at Sacrifice × 100%

2.11 Statistical Analyses

Data were analyzed using GraphPad Prism version 5. The experimental results were expressed as the Mean ± SEM (standard error means). Data were assessed by one-way ANOVA followed by Newman-Keuls multiple comparison test. Values for $p < 0.05$ were considered as statistically significant.

3. Results

3.1 Effect of Treatment on Organ Weight

In the curative and prophylactic studies, there were no significant changes in the weights of the liver of the animals; however in the prophylactic with reduced liver capacity study, there were significant increases in the weights of the liver; this demonstrates that both CCl_4 and PHZ adversely affected the size of the liver (Table 1).

In the curative group, prophylactic group and prophylactic with reduced liver capacity group, there was a significant ($p < 0.05$) increase in heart weights of animals in the jaundiced group when compared to the normal group, however (400 mg/kg, 800 mg/kg) ACP

Table 1 Effect of PHZ and ACP on relative organ weights (%) of animals in normal and treated animals.

Treatment group		Liver (g)	Heart(g)	Spleen (g)
Curative study				
	Normal	2.58 ± 0.13	0.28 ± 0.01	0.23 ± 0.01
	Jaundiced	3.42 ± 0.14	0.37 ± 0.01	0.93 ± 0.10 [a]
	400 mg ACP	3.31 ± 0.16	0.36 ± 0.02	0.83 ± 0.02 [a]
	800 mg ACP	3.05 ± 0.09	0.33 ± 0.01	0.84 ± 0.02 [a]
	100 mg Sily	2.91 ± 0.26	0.31 ± 0.03	0.84 ± 0.03 [a]
Prophylactic study				
	Normal	2.71 ± 0.06	0.30 ± 0.01	0.23 ± 0.01
	Jaundiced	3.26 ± 0.17	0.36 ± 0.02	0.99 ± 0.10 [a]
	400 mg ACP	3.39 ± 0.13 [a]	0.37 ± 0.01	0.87 ± 0.03 [a]
	800 mg ACP	3.05 ± 0.16	0.33 ± 0.02	0.86 ± 0.01 [a]
	100 mg Sily	2.77 ± 0.23	0.32 ± 0.01	0.85 ± 0.01 [a]
Prophylactic study with reduced liver capacity				
	Normal	2.51 ± 0.16	0.29 ± 0.02	0.23 ± 0.01
	Jaundiced	3.88 ± 0.03 [a]	0.42 ± 0.01 [a]	1.33 ± 0.16 [a]
	400 mg ACP	3.38 ± 0.13 [a]	0.37 ± 0.01 [a]	1.17 ± 0.14 [a]
	800 mg ACP	3.05 ± 0.09 [a b]	0.33 ± 0.01 [b]	0.88 ± 0.03 [a b]
	100 mg Sily	3.44 ± 0.08 [a]	0.38 ± 0.01 [a]	0.79 ± 0.02 [a b]

[a] Significantly different from Normal at $p < 0.05$; [b] Significantly different from Jaundiced at $p < 0.05$.

ACP—aqueous *Carica papaya.* Sily—Silymarin.

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doi: 10.17265/2328-2150/2017.07.011

and 100 mg/kg silymarin did not reverse the effect (see Table 1).

In the curative group, prophylactic group and prophylactic with reduced liver capacity group, there was a significant ($P < 0.05$ increase in spleen weights of animals in the jaundiced group when compared with that of the normal group. However, administration of 800 mg/kg ACP and 100 mg/kg silymarin significantly reversed the effect in the jaundiced group (see Table 1).

3.2 Effect of Treatment on Haematological Parameters

In the curative study, prophylactic study and prophylactic with reduced liver capacity study, the normal group had a mean RBC, HCT and HB parameters level that was significantly higher than that of the jaundiced group. The (400 mg/kg, 800 mg/kg) ACP and 100 mg/kg silymarin were significantly different from the normal and the jaundiced group showing that it was able to protect against excessive haemolysis and liver damage (see Table 2)

3.3 Effect of Treatment on Biochemical Parameters of Animals in Control and Treated Groups

In the curative study, prophylactic study and prophylactic with reduced liver capacity study, the normal group had a mean LDH level that was significantly lower than that of the jaundiced group. The (400 mg/kg, 800 mg/kg) ACP and 100 mg/kg silymarin were significantly lower than that of the jaundiced group showing that ACP has hepatoprotective properties. Similar trends were also seen for the ALT, ALP, and ALB. Total direct and indirect bilirubin level values see Figs. 1-3 and Table 3).

4. Discussion

Phenylhydrazine caused significant increase in organ weights. It was observed that PHZ affected the liver weights of the curative, prophylactic and prophylactic with reduced liver capacity groups, especially increasing liver weight in the animals of the prophylactic with reduced liver capacity group (see

Table 2 Effect of treatment on some haematological parameters of animals in control and treated groups.

Treament group		RBC ($\times 10^6$/μl)	HB (g/dl)	HCT (%)
Curative study				
	Normal	5.89 ± 0.22	14.79 ± 0.44	37.68 ± 0.66
	Jaundiced	3.64 ± 0.16 [a]	10.28 ± 0.32 [a]	30.93 ± 0.49 [a]
	400 mg ACP	3.58 ± 0.07 [a]	10.15 ± 0.13 [a]	30.73 ± 0.20 [a]
	800 mg ACP	4.06 ± 0.25 [ab]	11.12 ± 0.50 [a]	32.18 ± 0.75 [a]
	100 mg Sily	3.65 ± 0.29 [a]	10.64 ± 0.42 [a]	31.47 ± 0.54 [a]
Prophylactic study				
	Normal	5.41 ± 0.29	13.83 ± 0.58	36.24 ± 0.87
	Jaundiced	3.45 ± 0.19 [a]	9.91 ± 0.38 [a]	30.36 ± 0.57 [a]
	400 mg ACP	4.35 ± 0.12 [ab]	11.71 ± 0.25 [ab]	33.06 ± 0.37 [ab]
	800 mg ACP	4.18 ± 0.21 [a]	11.37 ± 0.42 [a]	32.55 ± 0.63 [a]
	100 mg Sily	3.61 ± 0.22 [a]	10.49 ± 0.39 [a]	31.65 ± 0.54 [a]
Prophylactic study reduced liver capacity				
	Normal	6.07 ± 0.44	15.13 ± 0.88	38.20 ± 1.33
	Jaundiced	2.89 ± 0.21 [a]	8.79 ± 0.42 [a]	28.68 ± 0.63 [a]
	400 mg ACP	3.50 ± 0.17 [a]	9.99 ± 0.34 [a]	30.49 ± 0.52 [a]
	800 mg ACP	3.82 ± 0.26 [a]	10.64 ± 0.51 [a]	31.46 ± 0.77 [a]
	100 mg Sily	3.38 ± 0.14 [a]	9.76 ± 0.27 [a]	30.13 ± 0.41 [a]

[a] Significantly different from Normal at $p < 0.05$; [b] Significantly different from Jaundiced at $p < 0.05$.
RBC—Red Blood Cell count; HB—Haemoglobin concentration; HCT—Haematocrit.

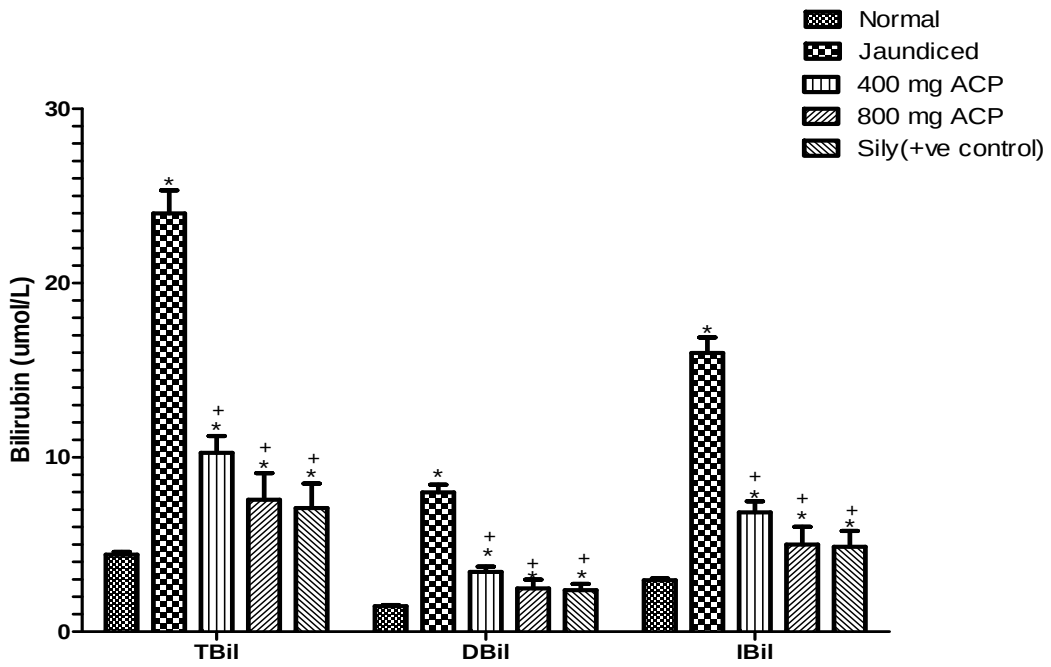

Fig. 1 Showing Total, Direct and Indirect Bilirubin Levels in Curative Group

*Significantly different from Normal at $p<0.05$; +significantly different from Jaundiced at $p < 0.05$. TBil=Total Bilirubin. DBil=Direct Bilirubin. IBil=Indirect Bilirubin

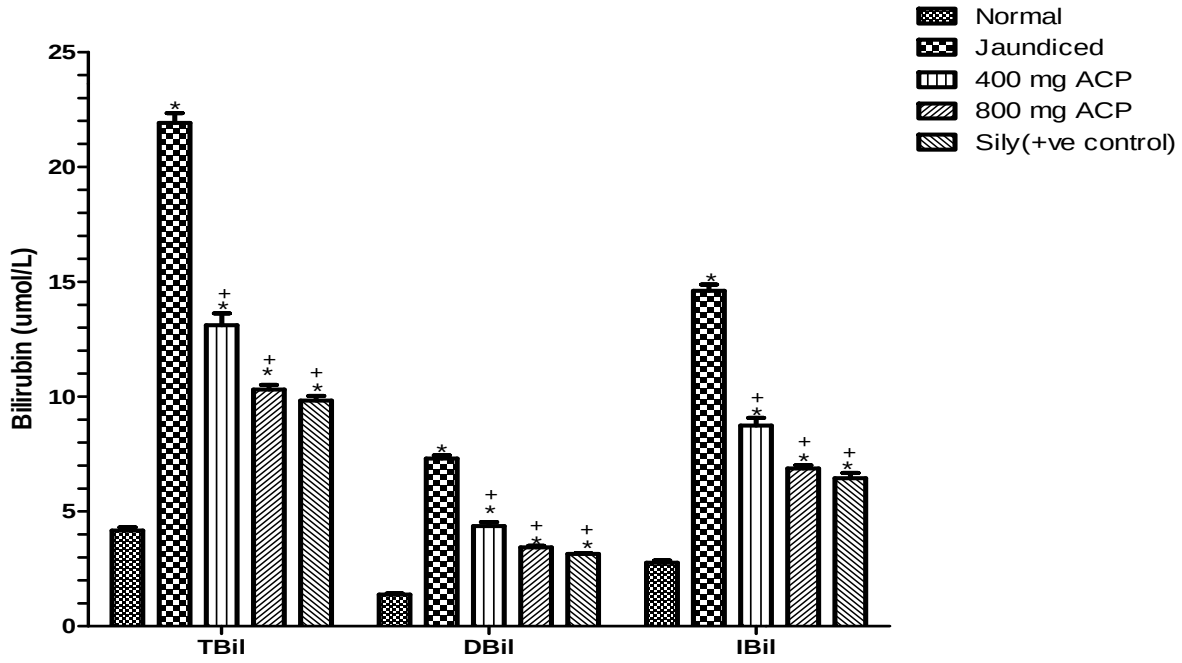

Fig. 2 Showing Total, Direct and Indirect Bilirubin Levels in the Prophylactic Group

*Significantly different from Normal at $p<0.05$; +significantly different from Jaundiced at $p<0.05$. TBil=Total Bilirubin. DBil=Direct Bilirubin. IBil=Indirect Bilirubin

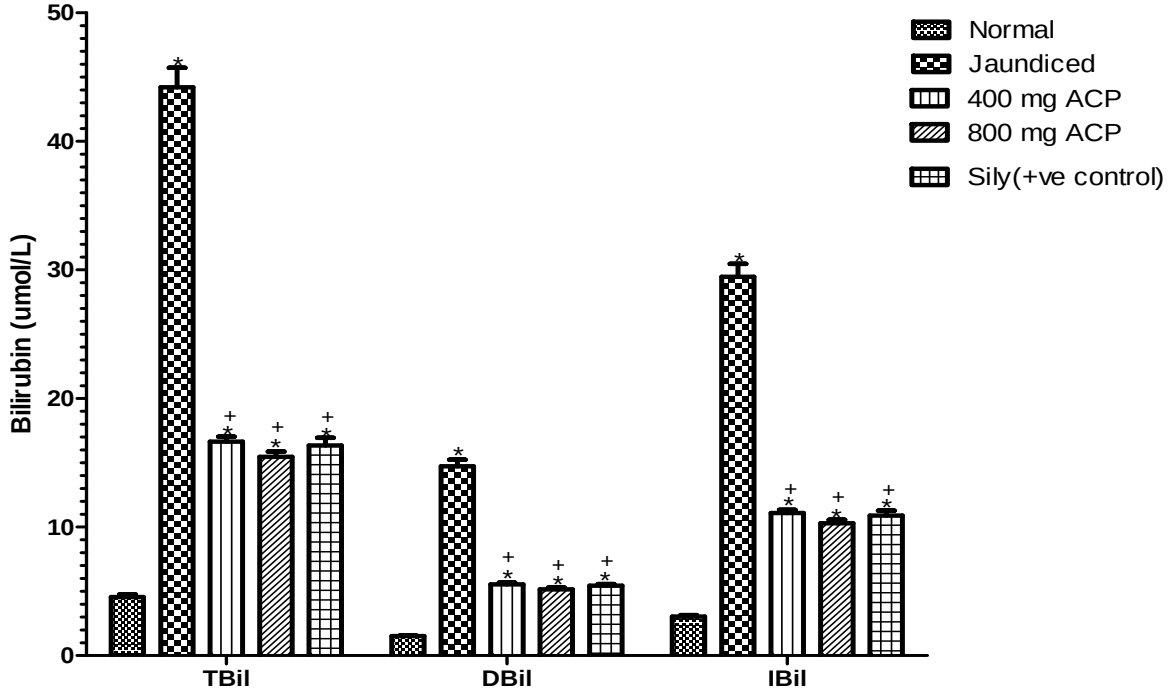

Fig. 3 Showing Total Direct and Indirect Bilirubin Levels in the Prophylactic With Reduced liver Capacity Group
*Significantly different from Normal at p<0.05 +significantly different from Jaundiced at p<0.05. TBil=Total Bilirubin. DBil=Direct Bilirubin. IBil=Indirect Bilirubin

Table 3 Effect of PHZ and ACP on biochemical parameters of animals in the control and treated group.

Treatment group		LDH (U/L)	ALT (U/L)	ALP (U/L)	ALB (g/dl)
Curative study					
	Normal	1315.0 ± 21.78	34.99 ± 2.44	51.08 ± 0.97	4.24 ± 0.09
	Jaundiced	2698.0 ± 60.45[a]	68.65 ± 0.48[a]	75.16 ± 0.74 [a]	6.99 ± 0.66 [a]
	400 mg ACP	1902.0 ± 35.69[a b]	42.46 ± 0.52[a b]	61.12 ± 0.71[a b]	5.29 ± 0.11[b]
	800 mg ACP	1705.0 ± 16.23[a b]	41.69 ± 0.64[a b]	54.74 ± 0.68[a b]	5.03 ± 0.17[b]
	100 mg Sily	1680.0 ± 22.18[a b]	40.58 ± 0.91[a b]	54.53 ± 0.38[a b]	4.84 ± 0.14[b]
Prophylactic study					
	Normal	1294.0 ± 20.82	32.41 ± 2.67	51.63 ± 0.31	4.83 ± 0.31
	Jaundiced	3157.0 ± 43.59[a]	62.77 ± 0.85[a]	72.53 ± 0.66 [a]	7.87 ± 0.40[a]
	400 mg ACP	2179.0 ± 52.40[a b]	48.70 ± 0.19[a b]	65.64 ± 0.65[a b]	7.27 ± 0.84[a]
	800 mg ACP	2029.0 ± 49.51[a b]	38.34 ± 0.69[a b]	62.42 ± 0.51[a b]	7.18 ± 0.18[a]
	100 mg Sily	1990.0 ± 52.16[a b]	40.54 ± 0.65[a b]	61.21 ± 0.56[a b]	6.48 ± 0.18[a]
Prophylactic with reduced liver capacity study					
	Normal	1395.0 ± 39.08	35.89 ± 1.41	53.29 ± 0.67	5.61 ± 0.19
	Jaundiced	4173.0 ± 36.99[a]	93.88 ± 1.20[a]	74.90 ± 0.78 [a]	7.99 ± 0.51 [a]
	400 mg ACP	2410.0 ± 27.02[a b]	53.76 ± 1.01[a b]	64.85 ± 0.31[a b]	6.14 ± 0.26[b]
	800 mg ACP	2212.0 ± 18.01[a b]	51.89 ± 0.47[a b]	64.19 ± 1.02[a b]	5.64 ± 0.37[b]
	100 mg Sily	1496.0 ± 67.86[a b]	57.28 ± 0.55[a b]	54.46 ± 0.37[b]	5.75 ± 0.09[b]

[a] Significantly different from Normal at $p < 0.001$; [b] Significantly different from Jaundiced at $p < 0.05$.
LDHL—actic dehydrogenase. ALT—Alanine tranferase. ALP—Alkaline phosphatase.

Table 1). However, ACP (400 mg/kg and 800 mg/kg) was able to reverse that effect. Phenylhydrazine also caused a significant ($p < 0.05$) increase in the spleen weight (splenomegaly) of the curative, prophylactic and prophylactic with reduced liver capacity group especially increasing those of the animals in the prophylactic with reduced liver capacity group (see Table 1), however, the splenomegaly was significantly ($p < 0.05$) reversed by ACP (400 mg/kg and 800 mg/kg). This significant weight increase observed must have been caused by phenylhydrazine breaking down more red blood cells which probably had affected the spleen because the spleen is an essential organ in the destruction of ruptured or expired red blood cells, the spleen is also a major organ involved in erythropoiesis and where there is excessive destruction of RBC, the burden of making RBC and replacing or removing senescent ones becomes too much for the spleen to cope with hence splenomegaly results. The splenomegaly observed is supported by the earlier work of Sharma and Haldar [16] wherein the study reported that PHZ administration caused a significant weight increase in the spleen of *Funambulus pennant* (the Indian squirrel). In the prophylactic with reduced liver capacity group (see Table 1), phenylhydrazine effect was seen mainly in the liver (hepatomegaly) and the spleen (splenomegaly). The significant weight increase seen in the liver and spleen must have been caused by the lethal combination of the haematotoxin, PHZ and the hepatotoxin, carbon tetrachloride (CCl_4). Studies have shown that PHZ can also be a hepatotoxin as demonstrated in the study by Toth [17], where it was noticed that PHZ caused tumours, active metastases and general organomegaly in rats. Looking at the blood indices of the curative, prophylactic and prophylactic with reduced liver capacity groups (see Table 2), it was demonstrated that PHZ caused a significant decrease in the RBC, HGB and HCT levels. The PHZ induced toxicity is attributed to the lipid peroxidation which occurs in the membrane of RBC. Formation of Methaemoglobin and Heinz body formation are the other effects of PHZ toxicity [18]. PHZ is known for its ability to produce haemolysis in rats and humans [19-21]. PHZ is known to decrease haemoglobin levels, RBC count and PCV [18, 22], however ACP (400 mg/kg and 800 mg/kg) especially the 800 mg/kg extract was able to significantly ($p < 0.05$) reverse the effect suggesting that ACP has the ability to boost red blood cell, haemoglobin and haematocrit levels as earlier demonstrated by a study conducted by Dharmarathna et al. [23] on ACP, where it was observed that administration of ACP leaf extract caused a significant increase in the level of platelets and RBC in mice. In the prophylactic with reduced capacity group (see Table 2), RBC, HB and HCT level restorations were not as high as that of the curative and prophylactic group probably because of the addition of the hepatotoxin CCl_4 to PHZ. CCl_4 is a chemical that causes hepatotoxicity and release free radicals such as trichloromethyl (CCl_3) or trichloroperoxy radical (CCl_2O_2) into the blood stream. These free radicals cause lipid peroxidation which produces hepatocellular damage to red blood cells and enhances production of fibrotic tissue in the liver as shown in group two of the prophylactic with reduced capacity group. CCl_4 is also suggested to cause liver injury and damage to the red blood cells, which agrees with the study of Recknagel et al. [24] and study of Brent and Rumack [25], where it was found that CCl_4 was able to cause damage to hepatocytes and destroy RBC. However, the ACP (400 mg/kg and 800 mg/kg) extract was able to significantly ($p < 0.05$) reverse the effect of CCl_4 and PHZ combination. Looking at the effect of phenylhydrazine on biochemical parameters as seen in the curative, prophylactic and prophylactic with reduced liver capacity group (see Table 3), it was demonstrated that PHZ induced significant ($p < 0.05$) increase in LDH levels in all animals. These levels were however restored to near normal with drug treatment showing that ACP extract (400 mg/kg and 800 mg/kg), especially the 800 mg/kg ACP extract was able to lower significantly ($p < 0.05$) LDH levels (see Table 3)

and preserve the synthetic ability of the liver as shown in the values. ALT, ALP and ALB levels also rose beyond the normal values but the levels were restored to near normal by ACP (400 mg/kg and 800 mg/kg) extract, this is in agreement with an earlier study performed by Rajkapoor et al. [26] where it was demonstrated that the dried fruit extract of ACP was able to reduce elevated LDH, ALP, ALT, ALB levels in animals that were treated with CCl_4. Furthermore, another study conducted by Adeneye et al. [27], also demonstrated that the seed extract of ACP prevents CCl_4 induced hepatotoxicity in rats. In the prophylactic with reduced liver capacity group biochemical parameters showed (see Table 3) that PHZ induced significant increases in LDH, ALT, ALP and ALB levels much greater than that seen in the curative and prophylactic group mainly because the hepatotoxin CCl_4 was administered in addition to PHZ in all animals of this group. These levels were however restored to near normal levels with drug treatment showing that ACP (400 mg/kg and 800 mg/kg) extract was able to lower LDH, ALT, ALP and ALB and the synthetic ability of the liver was also preserved as shown in the values. 800 mg/kg ACP demonstrated lower levels of the liver function markers than the 400 mg/kg ACP extract. This is in agreement with a study performed by Rajkapoor et al. [26] where it was demonstrated that the dried fruit extract of ACP was able to reduce elevated LDH, ALP, ALT, ALB levels in animals that were treated with CCl_4. PHZ administration also adversely increased serum bilirubin levels in the curative and prophylactic and prophylactic with reduced liver capacity group. In the curative and prophylactic group, total, direct and indirect bilirubin levels were significantly elevated in the jaundiced group, however, ACP (400 mg/kg and 800 mg/kg) extract was able to significantly ($p < 0.05$) lower bilirubin levels as seen in Figs. 1 and 2, and this is in tandem with the study carried out by Oduola et al. [28] where the bilirubin lowering potential was observed when the aqueous leaf extract of *Carica papaya* was administered to sickle cell patients. In the prophylactic with reduced liver capacity group (see Fig. 3), total, direct and indirect bilirubin levels were high in the jaundiced group and much higher than that seen in the curative and prophylactic groups because the hepatoxin CCl_4 was added to PHZ to further cause liver compromise and lower conjugating capacity of the liver, however ACP (400 mg/kg and 800 mg/kg) extract was able to significantly ($p < 0.05$) lower total, direct and indirect bilirubin levels as demonstrated in Fig. 3.

5. Conclusions

The study concluded that ACP fruit extract has ability to lower elevated total, direct and indirect bilirubin levels, confer prophylactic effect as well as reverse the effect of liver toxicants. The degree of hepatoprotective effects are seen on the liver of laboratory rats especially when compared to silymarine, thus justify its ethno medicinal use.

Acknowledgements

The researchers will like to acknowledge and thank Dr. Christopher Larbie of the Department of Biochemistry and Biotechnology, Faculty of Biosciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. He provided vital information regarding the methodology without which this research could not have been done.

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