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Bilirubin Lowering Potential of *Annona muricata* (Linn.) in Temporary Jaundiced Rats

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Abstract: Problem statement: *Annona muricata* is used in Ghanaian traditional medicine for the treatment of jaundice. Work done has demonstrated *A. muricata* to be effective in treating carbon tetrachloride-and acetaminophen-induced hepatic damage, hepatic jaundice and toxicologically safe up to 1000 mg kg^{-1} in animals. Current study evaluated the bilirubin-lowering potential of *A. muricata* aqueous extract in phenylhydrazine-(40 mg kg^{-1}) induced jaundice in adult rats. **Approach:** Jaundice was assessed by measuring the levels of total bilirubin and direct bilirubin in phenylhydrazine-treated animals with or without drug treatment with curative, prophylactic study and in animals with reduced liver capacity. **Results:** Phenylhydrazine induced jaundice in animals from 1.50 μmol L^{-1}±0.00 total bilirubin in normal animals up to 29.25 μmol L^{-1}±2.21 in animals with reduced liver capacity. The hyperbilirubinaemia were restored close to normal levels in animals treated with *A. muricata* aqueous extract at 50 and 400 mg kg^{-1}. Total bilirubin level was reduced to 6.22 μmol L^{-1}± 0.27 at 50 mg, 5.68 μmol L^{-1}±0.36 at 400 mg kg^{-1} and 7.94 μmol L^{-1}±0.79 with Silymarin, significantly lower ($p<0.001$) compared with the vehicle group (16.90 μmol L^{-1}±2.21) maintained with distilled water. **Conclusion:** Therefore, *A. muricata* aqueous extract can be used to reduce bilirubin concentration in jaundiced subjects.

Key words: Phenylhydrazine, *Annona muricata*, bilirubin, jaundice, rats

INTRODUCTION

Jaundice is the most common presentation of patients with liver and biliary diseases and other extrahepatobiliary causes (Beckingham and Ryder, 2001). It results from imbalance between bilirubin production and excretion. Jaundice or hyperbilirubinaemia is the appearance of yellow pigmentation in the skin, sclera and mucous membranes (Bishayi *et al*., 2002). Bilirubin (BR), a catabolic product of hemoglobin and other haem-containing compounds in mammals, is transported to the liver by albumin for further metabolism (Zunszain *et al*., 2008). In a healthy adult, approximately 3-4 mg BR per kg of body weight is produced per day. However, in certain metabolic disorders of the liver, congenital disorders that increase the rate of bilirubin production or in newborn infants with genetic deficiency or low levels of albumin, the amount of unconjugated BR in the blood increases and when it exceeds 1 mg dL^{-1}, hyperbilirubinaemia develops (Bittar, 2004). When the BR level reaches a certain concentration (>2.5 mg dL^{-1}), it diffuses into the tissues, causing jaundice in adults and kernicterus in infants.

Four distinct steps of bilirubin metabolism have been proposed (Kamisako *et al*., 2000). Bilirubin is first imported via the sinusoidal surface of the hepatocyte by solute carrier family 21, member 6 (SLC21A6; also known as organic anion transporter 2, OATP2) (Cui *et al*., 2001). Ligandin, a homodimer or heterodimer of Glutathione-S-Transferase (GST) A1 and A2, binds bilirubin with high affinity and thus increases uptake. Bilirubin is then glucuronidated by a specific microsomal bilirubin uridine diphosphate-5′-glucuronosyltransferase (UDP-glucuronosyltransferase 1A1, UGT1A1). The resulting hydrophilic bilirubin diglucuronide is then secreted across the bile-canalicular membrane of the hepatocyte by an active transporter, multidrug resistance-related protein 2 (MRP2) (cMOAT, ABC-C2) (Huang *et al*., 2003). In another mechanism, constitutive androstane receptor (CAR) NR113 has been shown to mediate the response of the liver to phenobarbital and other "phenobarbital-like" compounds (Wei *et al*., 2000). It has been demonstrated that CAR is a key regulator of the bilirubin clearance pathway and that CAR activation increases the rate of bilirubin clearance (Huang *et al*., 2003) by upregulating the synthesis of detoxifying enzymes and transporters.

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Treatment for neonatal hyperbilirubinaemia includes either phototherapy for mild condition or exchange transfusion under severe conditions (Bittar, 2004). However, certain pharmacological interventions such the use of phenobarbitone and metalloporphyrins have also been used, though these come with severe side effects (Dennery, 2002).

Medicinal plants and herbs contain substances known to the ancient civilizations for their healing properties. People without access to modern medicine rely on these medicinal plants and herbs for treating diseases, as they are similar in terms of active compound. *Annona muricata* is one of such medicinal plants. *Annona muricata* (Linn.) commonly called soursop or "Apre" in the local Ghanaian Twi language, is a small erect evergreen tropical fruit tree plant belonging to the family Annonaceae, growing 5-6 meters in height. It is underutilized (ICUC, 2002) and is grown in Ghana mainly for ornamental purposes and for its fruits. The leaves of *A. muricata* has been reported to contain several groups of substances collectively called annonaceous acetogenins including murihexocin and annocuricin (Kim *et al*., 1998), annopentocin A, B and C, (2,4-cis)-annomuricin-D-one, murihexocin A and B, (2,4-trans)-annomuricin-D-one, 4-acetyl gigantetrocin and cis-gigantrionin (Zeng *et al*., 1996), muricatocin A, B and C (Wu *et al*., 1995) and annohexocin (Zeng *et al*., 1996). The high potency, selectivity, wide chemical and biological diversity and effectiveness of these compounds against microbial resistance could well make them the next class of useful natural antitumor and pesticidal agents (Alali *et al*., 1999) and other pharmacological effects. The basic photochemical screen has revealed *A. muricata* to contain saponins, glycosides, tannins and flavonoids (Arthur *et al*., 2011).

The leaves of *A. muricata* have essential oils with parasiticidal, anti-diarrheal, rheumatological and anti-neuralgic properties (Gleye *et al*., 1998). The boiled water infusion of the leaves have anti-plasmodic, astringent and gastric properties (Khan *et al*., 1997), help treat diabetes and gastric upset (Adewole and Ojewole, 2006), jaundice (Mshana *et al*., 2000) and used in treating kidney aliments (Duke, 1970). The leaves are also hepatoprotective against carbon tetrachloride and acetaminophen-induced liver damage (unpublished data) and in streptozotocin-treated diabetic rats (Adewole and Ojewole, 2008).

Work done has established *A. muricata* to have an LD_{50} of <5000 mg kg^{-1} body weight while low (100 mg) and medium (1000 mg) doses were well tolerated in subchronic studies (Arthur *et al*., 2011). Its hepatoprotective effect against carbon tetrachloride and acetaminophen (paracetamol) has also been established (unpublished data). Further work done has established *A. muricata* to be potent in the treatment of hepatic jaundice (unpublished data). However, its effectiveness in treating pre-hepatic jaundice associated with increase serum bilirubin is yet to be studied.

The general objective of this work was to establish the bilirubin lowering potential of *A. muricata* in Phenylhydrazine (PHZ) -induced haemolysis. Specific objectives were to; evaluate the creative ability of *A. muricata* in temporary jaundiced rats; evaluate the prophylactic ability of *A. muricata* in temporary jaundiced rats; and evaluate the prophylactic ability of *A. muricata* in temporary jaundiced rats with reduced liver capacity.

MATERIALS AND METHODS

Animals: Sprague-Dawley rats of either sex weighing between 150-200 g were used for the study. They were obtained from the animal facility of the Department of Biochemistry and Biotechnology, KNUST. Animals were housed in aluminium cages, suitably bedded with wood shaving. They were maintained under standard conditions of temperature and humidity and had free access to standard feed (GAFCO, Tema, Ghana) and normal tap water except an overnight fast prior to sacrifice. In experimental grouping of the animals, their body weight and sex were taken into consideration to achieve approximately equal conditions among the groups. The animals were identified using permanent markers to mark uniquely on their tails. All animal experiments were conducted in accordance with the guidelines of the committee for the purpose of control and supervision of experiment on animals (CPCSEA, New Delhi, India) and guide for the care and use of laboratory animals (Washington, US).

Plant preparations and extraction: Leaves of *Annona muricata* were collected in the month of April 2010, from the surrounding fields of the Department of Biochemistry and Biotechnology Annex offices and was authenticated at the Department of Herbal Medicine, KNUST and voucher specimen (KNUST/HM1/2011/L057) deposited at the faculty herbarium. The leaves were washed, shade-dried, milled and decocted (1.41 kg with 10 L water). The aqueous extract was freeze-dried to obtain the *A. muricata* aqueous extract (AMAE) weighing 211 g (14.96% w/w yield) which as used in the study.

Preparation of phenylhydrazine: In all cases, hyperbilirubinaemia was induced with an aqueous solution of 40 mg kg^{-1} PHZ (Sigma-Aldrich Co., Germany) administered orally for two alternate days (Roque *et al*., 2008) with modification.

Experimental design: Five experimental groupings of 5 animals in each group were used for the study. In all cases, group 1 served as normal (naive) control and received sterile distilled water at 1mL 100^{-1} g b. wt twice daily for the entire duration of the experiment. In curative studies, group 2-5 were treated with PHZ on day 1 and 3. In addition, Group 2 served as Jaundice control and was sacrificed 6 h after last PHZ treatment. Group 3 served as Vehicle control and were maintained on sterile distilled water for 6 h after last PHZ treatment till day 7. Group 4 and 5 were treated with 50 and 400 mg kg^{-1} AMAE twice daily from day 2 till day 7. All the animals were sacrificed on day 8 after an overnight fast.

In prophylactic studies, group 2-5 were treated PHZ on day 2 and 4. In addition, Group 2 served as Jaundice control and was sacrificed 6 h after last PHZ treatment. Group 3 served as Vehicle control and were treated with sterile distilled water from day 1-7 with PHZ treatment on day 2 and 4. Group 4 and 5 were pre-treated with 50 and 400 mg kg^{-1} AMAE on day 1 and twice daily till end of day 7 with PHZ on day 2 and 4. All the animals were sacrificed on day 8 after an overnight fast.

For prophylactic studies in animals with reduced liver capacity, group 2-6 were treated with PHZ on day 2 and 4 and 1 ml CCl_4/kg b. wt (1:1 v/v olive oil) on day 3. In addition, Group 2 served as Jaundice control and were sacrificed 6 h after last PHZ treatment. Group 3 served as Vehicle control and were treated with sterile distilled water twice daily from day 1-7 with PHZ and CCl_4 treatment on day 2-4. Group 4, 5 and 6 were pre-treated with 50 mg kg^{-1} AMAE, 400 mg kg^{-1} AMAE and 100 mg kg^{-1} Silymarin on day 1 and twice daily till end of day 7 with PHZ and CCl_4 on day 2-4. All the animals were sacrificed on day 8 after an overnight fast.

Effect of treatment on some hematological and biochemical parameters: Animals were sacrificed by cervical dislocation. Incisions were quickly made in the sacrificed animal's cervical region with the aid of a sterile blade and blood samples collected from the heart and dispensed in plain bottles for biochemical assays and EDTA tubes for hematological analysis using Sysmex hematology system (USA). Hematological determinations conducted on curative and prophylactic study included hemoglobin concentration (Hb), Red Blood Cell (RBC) count, White Blood Cell (WBC) count and Haematocrit (HCT). The serum obtained from the blood samples were used for biochemical assays using the Cobas Integra 400 Clinical Chemistry Analyzer (Roche, USA); Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), bilirubin (total and direct) and Albumin (Alb).

Effect of treatment on Liver, Spleen and Heart Weight: Excised liver, heart and spleen of sacrificed rats were washed in buffered normal saline and weighed to obtain the absolute organ weights. Relative weights were calculated using the formula:

$$\text{Relative Organ Weight} = \frac{\text{Absolute Organ Weight}}{\text{Body Weight at Sacrifice}} \chi 100\%$$

Statistical Analysis: Data was analyzed using GraphPad Prism 5 for Windows. The experimental results were expressed as the Mean±Standard Error Means (SEM). Data were assessed by one-way ANOVA followed by Newman-Keuls multiple comparison test. Values for which $p<0.05$ was considered as statistically significant.

RESULTS

Effect of treatment on relative organ weights: The effect of Phenylhydrazine (PHZ) and AMAE on relative organ weights of animals are as shown in Table 1. There were no significant changes in the weights of the liver and heart of animals in a curative and prophylactic study. However, the administration of the hepatotoxin, CCl_4 resulted in a significant increase in liver weight of jaundiced animals. There were however, significant increases ($p<0.001$) in spleen weight in PHZ treated animals.

Effect of treatment on hematological parameters: The effect of PHZ and AMAE on some hematological indices of animals is as shown in Table 2. The red blood cell count and hemoglobin levels showed significant decreased in all animals treated with PHZ. The decrease in haematocrit is an indication of reduced cell volume following PHZ treatment. These values were however observed to improve with time and AMAE treatment.

Effect of treatment on serum biochemistry: The effect of PHZ and AMAE on some serum biochemistry is as shown in Table 3. PHZ induced significant increases in LDH levels in all animals 6 h after the last PHZ treatment.

Table 1: Effect of PHZ and AMAE on relative organ weight (%) of animals in normal and treated animals

Treatments		Liver	Heart	Spleen
Curative Study				
	Normal	2. 88±0.17	0.34±0.01	0.20±0.00
	Jaundiced	3. 39±0.10	0.43±0.03	0.96±0.05 [a]
	Vehicle	3.18±0.05	0.36±0.04	0.87±0.08 [a]
	50 mg AMAE	3. 36±0.04	0.39±0.03	0.84±0.02 [a]
	400 mg AMAE	2. 77±0.00	0.38±0.03	0.86±0.03 [a]
Prophylactic Study				
	Normal	2. 88±0.17	0.34±0.01	0.20±0.00
	Jaundiced	3. 39±0.10	0.43±0.03	0.96±0.05 [a]
	Vehicle	3.18±0.05	0.36±0.04	0.87±0.08 [a]
	50 mg AMAE	3. 30±0.15	0.39±0.03	1.02±0.03 [a]
	400 mg AMAE	3. 19±0.08	0.37±0.03	1.06±0.02 [a]
Prophylactic Study with reduced liver capacity				
	Normal	3. 09±0.14	0.35±0.00	0.25±0.02
	Jaundiced	4. 41±0.37[a]	0.45±0.02	1.00±0.03 [a]
	Vehicle	3.23±0.14	0.38±0.02	0.90±0.03 [a]
	50 mg AMAE	3. 22±0.10	0.36±0.01	1.17±0.04 [a]
	400 mg AMAE	3. 12±0.03	0.36±0.00	1.02±0.03 [a]
	100 mg Sily	3. 36±0.21	0.38±0.01	0.97±0.01 [a]

[a] Significantly different from normal at p<0.001

Table 2: Effect of treatment on some hematological parameters of animals in control and treated groups

Treatments		RBC	HGB	HCT
Curative Study				
	Normal	6. 44±0.20	12.23±0.49	38.60±1.77
	Jaundiced	3.98±0.08 [a]	8.10±0.11 [a]	21.45±0.31 [a]
	Vehicle	3.36±0.10 [a]	9. 38±0.21 [a]	34. 53±0.39
	50 mg AMAE	3.90±0.14 [a]	10.63±0.49	38.50±1.58
	400 mg AMAE	3.58±0.18 [a]	10.88±0.35	34.25±1.05
Prophylactic Study				
	Normal	7.21±0.34	12.55±0.49	40.63±2.24
	Jaundiced	2.63±0.20 [a]	6.70±0.41 [a]	21.68±1.14 [a]
	Vehicle	3.26±0.36 [a]	8.73±0.56 [a]	28.58±1.28 [a]
	50 mg AMAE	2.74±0.15 [a]	7.25±0.31 [a]	28.45±0.36 [a]
	400 mg AMAE	3.37±0.06 [a]	8.83±0.23 [a]	33.07±0.21 [a]

[a] Significantly different from Normal at p<0.001; RBC-Red Blood Cell count; HGB-Haemoglobin concentration; HCT-Haematocrit

Table 3: Effect of PHZ and AMAE on some biochemical parameters of animals in the control and treated group

Treatments		LDH	ALT	ALP	Albumin
Curative Study					
	Normal	1497. 0±281.70	56.5±3.15	124.4±2.42	44.9±1.22
	Jaundiced	4081. 2±432.20[a]	71.6±4.40	148.6±5.72[a]	43.0±1.04
	Vehicle	2374.8±174.17	57.5±3.43	106.2±2.34	44.8±0.77
	50 mg AMAE	1939. 8±281.75	52.0±4.92	107.6±6.11	46.1±0.54
	400mgAMAE	1806.2±184.01	55.3±4.65	93.5±1.74[a]	43.5±1.41
Prophylactic Study					
	Normal	2141. 8±179.18	64.1±9.19	148.7±10.64	37.2±0.70
	Jaundiced	4471. 5±508.99[a]	66.4±6.14	123.6±10.53	37.6±0.95
	Vehicle	1990.5±458.67	50.8±3.78	123.4±11.57	38.1±1.16
	50 mg AMAE	2082. 8±379.49	57.0±2.39	170.0±10.91	36.4±1.13
	400 mg AMAE	2118. 0±174.03	44.1±4.08[a]	128.2±9.69	33.8±1.27
Prophylactic Study with reduced liver capacity					
	Normal	2787.8±136.67	57.0±7.43	187.2±16.95	50.7±1. 49
	Jaundiced	4359. 5±128.81[a]	612.5±28.71[a]	186.9±15.34	47.6±1.27
	Vehicle	2685.2±211.15	142.1±4.93[a]	116.1±14.79 [a]	50. 8±1.10
	50 mg AMAE	2769. 2±254.35	39.3±2.30[b]	110.0±8.83 [a]	53. 7±0.99
	400 mg AMAE	2357. 4±240.16	38.8±3.05[b]	151.1±7.82	52.5±0.32
	100 mg Sily	1996. 4±346.51	57.9±3.15[b]	189.3±27.24	48.9±0.91

[a] Significantly different from Normal at p<0.001; [b] Significantly different from Vehicle at p<0.001; LDH-Lactate dehydrogenase; ALT-Alanine aminotransferase; ALP-Alkaline phosphatase

These levels were however restored to normal levels with or without drug treatment. Also, no significant difference was observed in ALT, ALP and albumin levels. However, CCl_4 treatment resulted in significant increases in ALT ($p<0.001$) levels. AMAE and Silymarin restored these increases to normal levels.

Effect of treatment on bilirubin concentration: The effect of PHZ and AMAE on bilirubin concentration is as shown in Fig. 1-3. Total bilirubin level increased in jaundiced groups 6 h after the last PHZ treatment. These values were observed to decrease in vehicle group, in animals with no AMAE treatment and with normal liver capacity. However, administration of AMAE improved the conjugating capacity of the liver by decreasing the total bilirubin level. Similar trends were observed in direct and indirect bilirubin levels. In all studies, 400 mg AMAE significantly decreased bilirubin levels ($p<0.001$) compared with vehicle group.

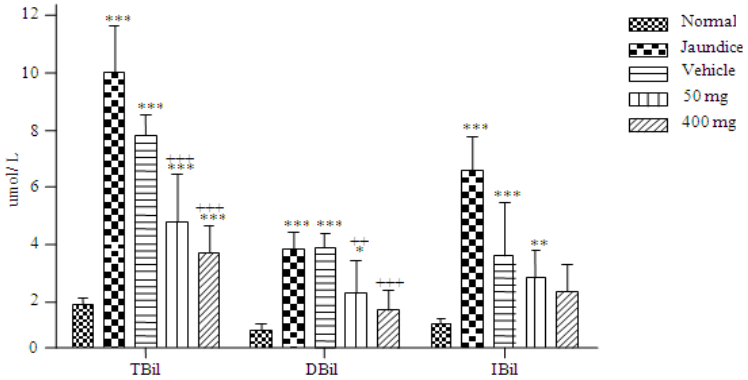

Fig. 1: Effect of PHZ and AMAE on bilirubin levels of normal and treated groups in curative study. Each column represents a mean with SEM of 5 rats. Significantly different from normal, * p<0.05, ** p<0.01, *** p<0.001; from vehicle, ++ p<0.01, +++ p<0.001 TBil – Total bilirubin; DBil - Direct bilirubin; IBil - Indirect bilirubin

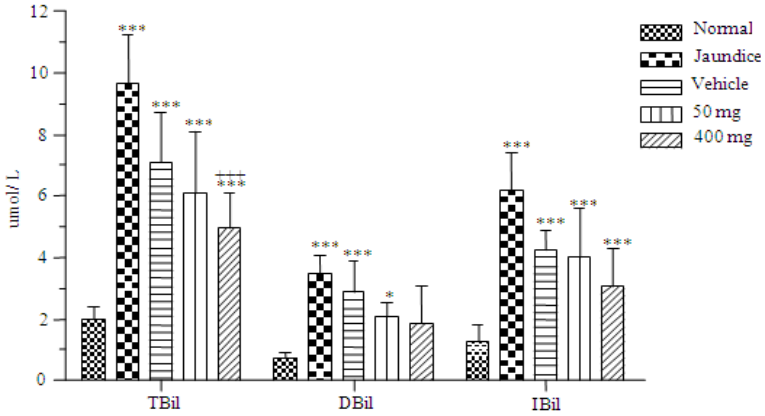

Fig. 2: Effect of PHZ and AMAE on bilirubin levels of normal and treated groups in prophylactic study. Each column represents a mean with SEM of 5 rats. Significantly different from normal, * p<0.05, *** p<0.001; from vehicle group, +++ p<0.001 TBil-Total bilirubin; DBil - Direct bilirubin; IBil - Indirect bilirubin

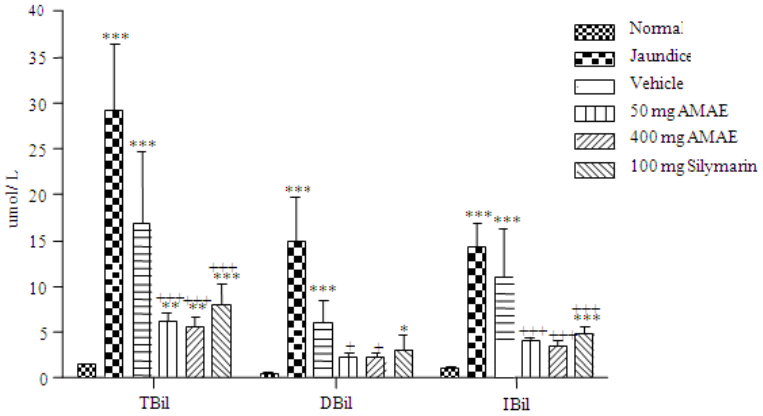

Fig. 3: Effect of PHZ, CCl_4 and AMAE on bilirubin levels of normal and treated groups of animals with reduced liver capacity. Each column represents a mean with SEM of 5 rats. Significantly different from normal, * p<0.05, ** p<0.01, *** p<0.001; from vehicle group + p<0.05, ++ p<0.01, +++ p<0.001

DISCUSSION

In vertebrates, anemia are a common hematological disorder associated with several conditions such as drug toxicity, parasites (e.g., malaria), genetic (e.g., sickle cell diseases and G6PD deficiency) or acquired defects and blood loss (Criswell *et al*., 2000; Jollow and McMillan, 2001). The haemolytic activity of arylhydrazines, such as Phenylhydrazine (PHZ), Dapsone, hydroxylamine and divicine may lead to acute haemolytic anemia in vertebrates (Jollow and McMillan, 2001). The main action of the classical haemotoxicant, PHZ has long been associated with drug-induced oxidative stress occurring within erythrocytes (Kinuta *et al*., 1995). This process produces an increase in the oxidation of oxyhaemoglobin, thus leading to the formation of methaemoglobin which is subsequently converted into irreversible haemachromes which, in turn, lead to the denaturation and precipitation of hemoglobin in the form of Heinz bodies (Rifkind and Danon, 1965). Skeletal protein damage and lipid peroxidation as well as glutathione and ATP depletion, cation imbalances and reduced membrane deformability have been proposed to be involved in the haemolytic response that is induced by oxidant drugs (McMillan *et al*., 1998). PHZ affects the red blood cells, inducing haemolysis with the release of cell content into circulation without affecting other organs such as liver and heart. This lends support to the observed decreases in Red Blood Cell (RBC) count, Hemoglobin Concentration (HGB) and Haematocrit (HCT) of PHZ-treated groups (Table 2). These values were restored to normal level in vehicle and AMAE-treated groups suggesting that in a normal animal with normal liver capacity and erythroid system, anemia is prevented mainly by compensatory erythrocytes spleen hyperplasy (Roque *et al*., 2008). The spleen plays important roles in processing other than blood storage and immune competence and in PHZ-induced haemolytic anemia in rodents and rabbits it acts as the main erythrophagocytic organ (Latunde-Dada *et al*., 2006) resulting in the observed spleenomegaly in animals (Table 1). This implies that damaged cells are removed intact by the spleen which accompanies intravascular lysis (Sinxadi and Martens, 2007).

PHZ haemolysis is accompanied by hyperbilirubinaemia (Maines and Veltman, 1984). PHZ has been shown to exhibit a potent ability to increase haem oxygenase activity in a time-dependent manner in the liver and kidney and this suggest that the induction of hyperbilirubinaemia by PHZ may be related to the enhanced rate of enzymatic conversion of haemoglobin haem to bilirubin. These findings have been supported by the observation that zinc protopophyrin, a haem oxygenase inhibitor, markedly reduced serum total bilirubin levels (Maines and Veltman, 1984).

The curative and prophylactic effect of AMAE in treating jaundice was assessed. On both levels, AMAE exhibited a dose dependent effect, where 400 mg showed a potent effect than 50 mg. Curatively, AMAE reduced bilirubin levels from 9.68 μmol L^{-1}±0.49 in the jaundice group to 4.43 μmol L^{-1}±0.53 at 50 mg and 3.33 μmol L^{-1}±0.31, significantly different ($p<0.001$) from the vehicle group (7.43 μmol L^{-1}±0.23) maintained on distilled water (Fig. 1). This indicates that AMAE possesses a bilirubin-lowering potential and could help clear serum bilirubin faster than in the untreated group. During prophylactic studies, serum bilirubin levels were reduced from 9.68 μmol L^{-1}±0.49 to 6.08 μmol L^{-1}±0.64 at 50 mg and 4.93 μmol L^{-1}±0.36 at 400 mg significantly lower ($p<0.001$) from vehicle group (7.08 μmol L^{-1}±0.50) (Fig. 2). This indicates that AMAE at 400 mg protected animals against jaundice induced by a haemotoxicant.

Finally, the bilirubin-lowering potential was assessed in animals with reduced liver capacity following the administration of carbon tetrachloride. The hepatotoxic agent was expected to injure the liver, reducing its liver conjugation property and further exacerbating the jaundice. This was observed when the combined effect of PHZ and CCl_4 resulted in increased serum bilirubin level (29.25 μmol L^{-1}±2.21, normal 1.50 μmol L^{-1}±0.00) (Fig. 3), accompanied by increased ALT and LDH levels (Table 3) characteristic of CCl_4 toxicity. The administration of AMAE, with its established hepatoprotective effect reduced the effect of PHZ and CCl_4. Bilirubin levels were reduced to 6.22 μmol L^{-1}±0.27 at 50 mg and 5.68 μmol L^{-1}±0.36 at 400 mg which compared well with Silymarin, 7.94 μmol L^{-1}±0.78. These values were significantly lower compared with animals that were maintained on distilled water, 16.90 μmol L^{-1}±2.43 ($p<0.001$). AMAE could help treat jaundice by offering protection on the liver, improving its bilirubin conjugating property and helping clear bilirubin from circulation. This was justified by the lack of significant difference between unconjugated bilirubin levels of normal group and AMAE treated groups.

The following mechanisms could be suggested for the bilirubin-lowering potential of *Annona muricata*. Firstly, the presence of glucosides in the extract might be converted to glucorunic acid for conjugating with bilirubin for excretion. This is evidenced by the fact that high dose of *A. muricata* (400 mg) offered a

significant reduction in bilirubin levels in the study. It could also be suggested that AMAE activated the Constitutive Andostane Receptor (CAR), a key regulator in the bilirubin clearance pathway (Huang *et al*, 2003), increasing the activity of glucuronyl transferases (Ostrow *et al*., 2003), synthesis of ligandin, a transporter of bilirubin, increasing its transport to the liver for conjugation (Greige-Gerges *et al*., 2007). Also, AMAE could inhibit the activity of haem oxygenase, the rate limiting enzyme of the bilirubin pathway, reducing serum total bilirubin in treated animals.

CONCLUSION

Annona muricata aqueous extract possesses bilirubin lowering potential especially at higher doses and can be used in the effective management of hyperbilirubinaemia or jaundice. Therefore, *Annona muricata* aqueous extract can be used successfully to develop a future drug for the management of hyperbilirubinaemia/jaundice.

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