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# An Investigation of Antioxidant and Anti-inflammatory Activities from Blood Components of Crocodile (*Crocodylus siamensis*)

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**Abstract** Antioxidant and anti-inflammatory activities were found from *Crocodylus siamensis* (*C. siamensis*) blood. The 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging, nitric oxide scavenging, hydroxyl radical scavenging and linoleic peroxidation assays were used to investigate the antioxidant activities of the crocodile blood. Results show that crocodile blood components had antioxidant activity, especially hemoglobin (40.58 % nitric oxide radical inhibition), crude leukocyte extract (78 % linoleic peroxidation inhibition) and plasma (57.27 % hydroxyl radical inhibition). Additionally, the anti-inflammatory activity of the crocodile blood was studied using murine macrophage (RAW 264.7) as a model. The results show that hemoglobin, crude leukocyte extract and plasma were not toxic to RAW 264.7 cells. Also they showed anti-inflammatory activity by reduced nitric oxide (NO) and interleukin 6 (IL-6) productions from lipopolysaccharide (LPS)-stimulated cells. The NO inhibition percentages of hemoglobin, crude leukocyte extract and plasma were 31.9, 48.24 and 44.27 %, respectively. However, only crude leukocyte extract could inhibit IL-6 production. So, the results of this research directly indicate that hemoglobin, crude leukocyte extract and plasma of *C. siamensis* blood provide both antioxidant and anti-inflammatory activities, which could be used as a supplementary agent in pharmaceutical products.

**Keywords** Crocodile blood · Blood components · Antiradical · Inflammation · Nitric oxide

**Abbreviations**

| | |
|---|---|
| SDS-PAGE | Sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| DPPH | 1,1-Diphenyl-2-picrylhydrazyl |
| EDTA | Ethylenediaminetetraacetic acid |
| IL-6 | Interleukin 6 |
| TNF-α | Tumor necrosis factor alpha |
| IL-1β | Interleukin 1 beta |
| ERK | Extracellular signal-regulated kinase |
| JNK | c-Jun N-terminal kinase |
| NF-κB | Nuclear factor-kappa B |

## 1 Introduction

Free radicals are molecules or atoms with unpaired electrons in their outer orbit. The critical types that are of most concern in biological systems are known as reactive oxygen species (ROS). Examples include superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl ($OH^·$) and reactive

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nitrogen species (RNS), specifically nitric oxide (NO) radicals [1]. Free radicals can be generated by many metabolic pathways in cells causing biomolecular damage [2], resulting in various diseases such as atherosclerosis, cancer, Parkinson's disease, heart diseases, diabetes, and other degenerative disorders especially inflammatory disorders [3–6].

During inflammatory processes, high levels of free radicals, such as NO, are generated in macrophage cells. In nature, macrophage plays an important role in response to inflammation via the release of several pro-inflammatory mediators including nitric oxide (NO), prostaglandin$E_2$ ($PGE_2$) and pro-inflammatory cytokines (IL-6, TNF-α, IL-1β). These mediators are also produced in many inflamed tissues, enhancing the inflammation-regulatory transcription factors of their mRNAs via the nuclear factor NF-κB pathway [7, 8]. Based on this evidence, it is expected that if these substances can exhibit anti-inflammatory activity, they might also provide antioxidant activity. Recent studies have been performed observing antioxidant and anti-inflammatory activities on substances extracted from plants [7, 9]; there is limited information on substances extracted from animals.

In biological activity studies in animals, blood components have been reported as being anti-inflammatory or having antioxidant activities. Sivaperumal et al. [10] revealed that the peptides from crab haemolymph of *Ocypoda macrocera* have antioxidant and antimicrobial activity. Likewise, porcine hemoglobin and blood plasma protein hydrolysates also show the strongest antioxidant activities, which are dependent on their peptide or protein compositions [11, 12]. In the Siamese crocodile, Jandaruang et al. [13] studied the antioxidant activity of *Crocodylus siamensis* hemoglobin. They found that hemoglobin exhibited antioxidant activity depending on the pH, and heat between 25 and 65 °C had no effect on its antioxidant activity when tested by DPPH radical scavenging assay. Furthermore, Srihongthong et al. [14] reported that α-chain, β-chain and some enzymatic hydrolysates of *C. siamensis* hemoglobin have both antibacterial and antioxidant activities. Regarding anti-inflammatory activity, Kommanee et al. [15] revealed that *C. siamensis* blood components such as plasma and crude leukocyte extract have anti-inflammatory activity via reduction of the NO levels in a concentration-dependent manner.

From previous studies, *C. siamensis* blood had many biological activities. However, combination studies on radical scavenging and inhibition of inflammatory activities of *C. siamensis* blood have never been performed. Hence, the objective of this study was to investigate both antioxidant and anti-inflammatory activities of *C. siamensis* blood, and also confirm the bifunctional mechanisms.

## 2 Materials and Methods

### 2.1 Materials and Reagents

RPMI 1640 medium, antibiotic/antimycotic (penicillin/streptomycin/amphotericin B), trypsin–EDTA and fetal bovine serum (FBS) were purchased from Gibco (USA). Linoleic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carbonic acid (TROLOX), 2,2′- azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2′-azobis-(2- amidinopropane)-dihydrochloride (ABAP), butylated hydroxytoluene (BHT), dimethyl sulfoxide (DMSO) and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (Germany). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Eugene (USA). The other reagents used in this experiment were of the highest quality available.

### 2.2 Crocodile Blood

The Siamese crocodile blood was obtained from Sriracha Moda Farm., Ltd., Chon Buri, Thailand, which was collected from the internal jugular vein of the supravertebral branch using a sterile syringe and kept in 15 mL conical screw cap tubes containing 0.08 g EDTA. The samples were kept on ice until blood cells settled. Leukocytes were presented in the interphase layer between the upper plasma and lower erythrocyte layers. The plasma layer was discarded, and then the leukocyte and erythrocyte layers were individually collected into new conical screw cap tubes.

### 2.3 Hemoglobin Extraction

The extraction of hemoglobin from red blood cells (RBC) was performed by the method of Srihongthong et al. [14]. Shortly, the RBC was separated at a temperature of 4 °C, washed three times with phosphate buffer saline (PBS) pH 7.0 and centrifugation was performed at 3,000×*g* for 5 min at room temperature. The RBC pellet was added to ice-cold distilled water with twofold volume, settled for 10 min, vigorously mixed and then centrifuged at 10,000×*g* for 20 min at 4 °C. Hemoglobin solution was lyophilized and kept at −70 °C until used in the next experiment.

### 2.4 Crude Leukocyte Extraction

Leukocyte was extracted by the freeze–thaw method [16]. Then 10 % (v/v) acetic acid was added, homogenized by an ultrasonicator and the supernatant collected by centrifugation at 12,000×*g* for 20 min at 4 °C. The crude leukocyte extract was lyophilized and stored at −70 °C until used.

### 2.5 Molecular Weight Determination by SDS-PAGE

The protein electrophoretic patterns of the crocodile hemoglobin, crude leukocyte extract and plasma were determined using 13.5 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [17]. Phosphorylase B (97 kDa), bovine serum albumin (66 kDa), chicken ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa) were used as the protein standards (GE Healthcare, Sweden).

### 2.6 ABTS Radical Inhibitory Assay

The ABTS radical cation ($ABTS^{\cdot+}$) was produced after incubation for 12–16 h in the dark at room temperature with the solution of 7 mM ABTS and 2.45 mM potassium persulfate. Then the mixture solution was diluted in 5 mM PBS (pH 7.4) and the absorbance adjusted at 734 nm to 0.7. ABTS radical inhibitory activity was determined by adding 20 µL of each crocodile blood component (hemoglobin, crude leukocyte extract and plasma) to 2 mL of $ABTS^{\cdot+}$ solution and comparing with standard Trolox at the same concentration of blood components (15.62, 31.25, 62.5, 125 and 250 µg/mL).

### 2.7 Nitric Oxide Radical Inhibitory Assay

Nitric oxide (NO) was produced from sodium nitroprusside and detected by Griess reagent according to the method of Yen et al. [18]. The different concentrations (15.62, 31.25, 62.5, 125 and 250 µg/mL) of each *C. siamensis* blood components or positive control, BHT, were mixed with sodium nitroprusside (10 mM) and incubated for 2 h at room temperature. After incubation, the solution (0.1 mL) had 0.1 mL of Griess reagent added. The absorbance was measured at 540 nm.

### 2.8 Hydroxyl Radical Inhibitory Assay

Hydroxyl radical inhibitory activity was assayed by the method of Saumya and Basha [19]. The reaction mixture was added in the following order: 0.01 mL of $FeCl_3$, 0.1 mL of $H_2O_2$, 0.1 mL of EDTA, 0.36 mL of deoxyribose,1.0 mL of crocodile blood components or positive control; BHT (15.62, 31.25, 62.5, 125 and 250 µg/mL), 0.33 mL of phosphate buffer (50 mM, pH 7.4) and 0.1 mL of ascorbic acid. After incubation at 37 °C for 1 h, 1.0 mL of the incubated portion was mixed with 1.0 mL of 0.5 % thiobarbituric acid (TBA), 1.0 mL of 10 % trichloroacetic acid (TCA) then incubated at 90 °C for 15 min. Then the absorbance was measured at 532 nm. The percentage of inhibition was calculated as follows: Inhibition $(\%) = [(A_{control} - A_{test})/A_{control}] \times 100$.

### 2.9 Inhibition of Linoleic Acid Oxidation

According to Hernández-Ledesma et al. [9], the mixture of 50 µL of crocodile blood components or standard Trolox (15.62, 31.25, 62.5, 125 and 250 µg/mL), 50 µL of 50 mM linoleic acid in 80 % ethanol and 10 µL of 0.07 M ABAP were incubated for 10 min at room temperature. After that 150 µL of 20 % (v/v) acetic acid was added and incubated in an oven at 70 °C for 1 h. The incubation solution was used for inhibitory assay of linoleic acid oxidation by the ferric thiocyanate method. All experiments were repeated in triplicate. The inhibition of linoleic acid oxidation was evaluated using the same method as the calculation of hydroxyl radical inhibitory assay.

### 2.10 Cell Culture

A murine macrophage cell line (RAW 264.7) was purchased from the American Type Culture Collection (American Type Culture Collection [ATCC], USA). It was seeded in RPMI 1640 medium supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 100 µg/mL of streptomycin, 100 U/mL of penicillin and 25 µg/mL amphotericin B, then incubated at 37 °C in a 5 % $CO_2$ humidified atmosphere.

### 2.11 Cell Viability

RAW 264.7 cells ($1 \times 10^5$ cells/mL) were seeded on a 48-well plate overnight. Then, cells were divided into two groups; the first group was incubated with *C. siamensis* blood components at different concentrations (7.81, 15.62 and 31.25 µg/mL) and another group was co-incubated with 100 ng/mL of lipopolysaccharides (LPS). After incubation for 24 h, RAW 264.7 cell viability was determined by MTT assay [20]. Cell viability was evaluated by comparing their absorbance with those of the control.

### 2.12 Measurement of Nitric Oxide

RAW 264.7 cells were incubated with LPS (100 ng/mL), and LPS co-incubated with hemoglobin, crude leukocyte extract or plasma (7.81, 15.62 and 31.25 µg/mL) at 37 °C for 24 h. Then 0.1 mL of the culture medium from each sample was lightly mixed with the same volume of Griess reagent and incubated at room temperature for 10 min. Subsequently the absorbance was measured at 540 nm using a microplate reader (BioRad, Model 680, USA). The NO production was calculated as a percentage of control [7].

### 2.13 Measurement of IL-6 and TNF-α

After the anti-inflammation on NO was tested, the same culture media were used for determining IL-6 and TNF-α following the manufacturer's manual ELISA kits (R&D, Minneapolis, MN, USA).

### 2.14 Statistical Analysis

Statistical values of all experimental results were calculated using ANOVA, followed by Dunnett's test. These data are presented as mean ± SD. A value of $P < 0.05$ was accepted as being significant ($*P < 0.05$).

## 3 Results

### 3.1 Determination of Protein Molecular Weight by SDS-PAGE

The molecular weight of proteins contained in crocodile blood components was determined by SDS-PAGE. As shown in Fig. 1, the molecular weight of hemoglobin was composed of three protein bands with 14, 15 and 30 kDa which are identified as α-hemoglobin, β-hemoglobin and the dimer form of hemoglobin, respectively. Also, crude leukocyte extract containing proteins showed a range of 14–80 kDa and plasma showed several protein bands between 27 and 120 kDa and contained two major protein bands including 66-kDa albumin and 120-kDa gamma globulins.

### 3.2 Determination of ABTS Radical Inhibitory Assay

The generation of ABTS cation radicals was achieved by the oxidation of ABTS by potassium persulfate. All treated concentrations of hemoglobin, crude leukocyte extract and plasma could significantly inhibit ABTS radical ($*P < 0.05$). The inhibition percentages were from 9.48 to 24.88 % for hemoglobin, 10.32 to 40.18 % for crude leukocyte extract and 8.98 to 13.62 % for plasma. At the highest concentration (250 μg/mL), the results showed that crude leukocyte extract was the best antioxidant (40.18 %) which was a little lower than standard Trolox (41.56 %), whereas the plasma was the lowest for ABTS radical inhibitory capacity (13.62 %) (Fig. 2).

### 3.3 Determination of Nitric Oxide Radical Inhibitory Assay

Sodium nitroprusside is well-known to dissolve strongly in aqueous solution at physiological pH, producing NO radicals. Hemoglobin, crude leukocyte extract and BHT (positive control) significantly inhibited the radicals in a concentration dependent manner but plasma had no activity. At the highest concentrations (250 μg/mL), the percentages of inhibition of each sample were 40.58, 33.6 and 37.1 % for hemoglobin,

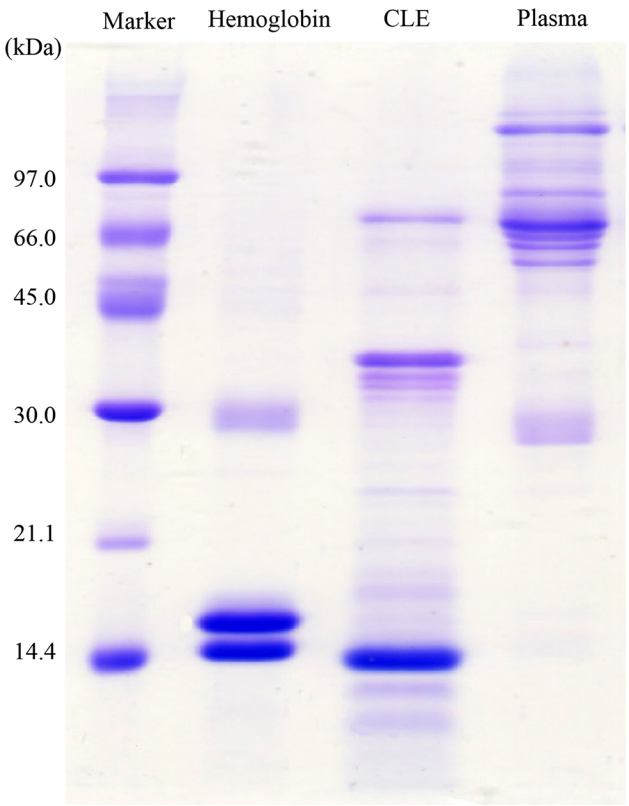


**Fig. 1** SDS-PAGE analysis of crocodile hemoglobin, crude leukocyte extract and plasma on 13.5 % acrylamide gel. *Lane 1* (Marker): low molecular weight protein marker, *Lane 2* (Hemoglobin): hemoglobin (10 μg), *Lane 3* (CLE): crude leukocyte extract (10 μg) and *Lane 4* (Plasma): plasma (10 μg)

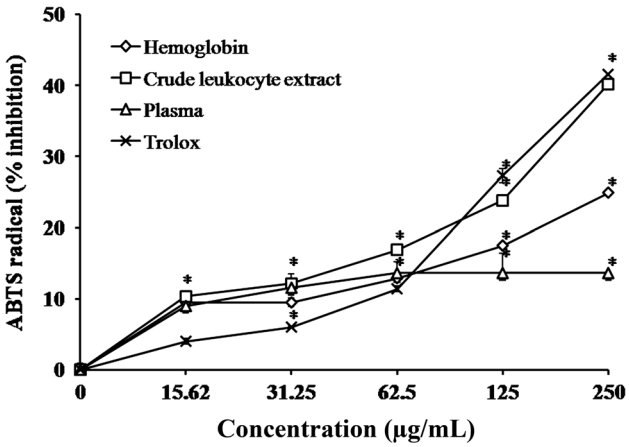


**Fig. 2** Effect of hemoglobin, crude leukocyte extract and plasma on ABTS radicals. Data shown as mean ± SD (n = 8). $*P < 0.05$ versus untreated group

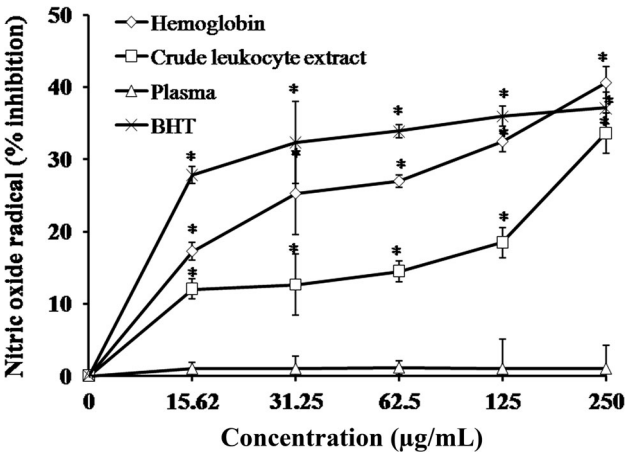


**Fig. 3** Effect of hemoglobin, crude leukocyte extract and plasma on nitric oxide radicals. Data are mean ± SD (n = 8). *$P < 0.05$ versus untreated group

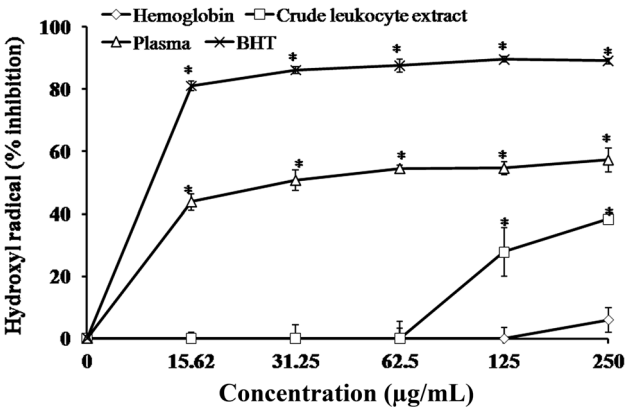


**Fig. 4** Effect of hemoglobin, crude leukocyte extract and plasma on hydroxyl radicals. Data shown as mean ± SD (n = 8). *$P < 0.05$ versus untreated group

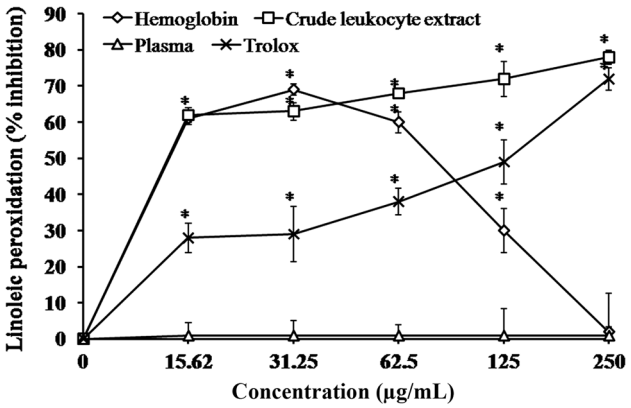


**Fig. 5** Effect of hemoglobin, crude leukocyte extract and plasma on lipid peroxidation. Data shown as mean ± SD (n = 8). *$P < 0.05$ versus untreated group

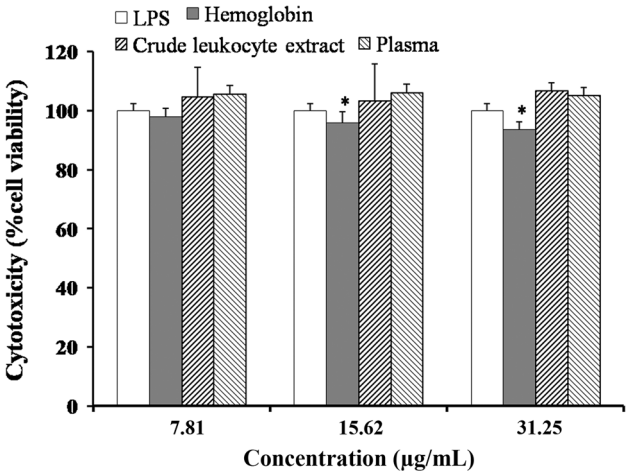


**Fig. 6** Effect of hemoglobin, crude leukocyte extract and plasma on cell viability by MTT assay. Cells were incubated with 100 ng/mL of LPS and co-incubated with 7.81, 15.62 and 31.25 µg/mL of crocodile blood components for 24 h. Each *bar* represents the mean ± SD (n = 8). *Significant at $P < 0.05$ probability levels

crude leukocyte extract and BHT, respectively (Fig. 3). When comparing the NO radical scavenging capacity of each sample, the results show that hemoglobin had the highest capacity at the concentration of 250 µg/mL and was higher than the standard BHT.

### 3.4 Determination of Hydroxyl Radical Inhibitory Assay

The results show that at the highest concentrations (250 µg/mL), plasma had the strongest ability to inhibit hydroxyl radicals. The hydroxyl radicals inhibition showed 38.35, 57.27 and 89.02 % for crude leukocyte extract, plasma and standard BHT, respectively (*$P < 0.05$). While no inhibitory activity for hydroxyl radical was observed for hemoglobin (Fig. 4).

### 3.5 Inhibition of Linoleic Acid Peroxidation

The results show that crude leukocyte extract at the concentration of 15.62–250 µg/mL was similar to Trolox (positive control). Across the experiment, the inhibitory percentage of leukocyte extract was between 62 and 78 %, whereas the inhibitory percentage of Trolox was between 28 and 72 %. For hemoglobin the highest capacity for inhibiting linoleic acid oxidation was observed at 31.25 µg/mL (68.7 %). When the concentrations of hemoglobin were increased, it was likely that the percentage of inhibition gradually reduced until no activity was seen at 250 µg/mL. This result indicates that the inhibitory ability

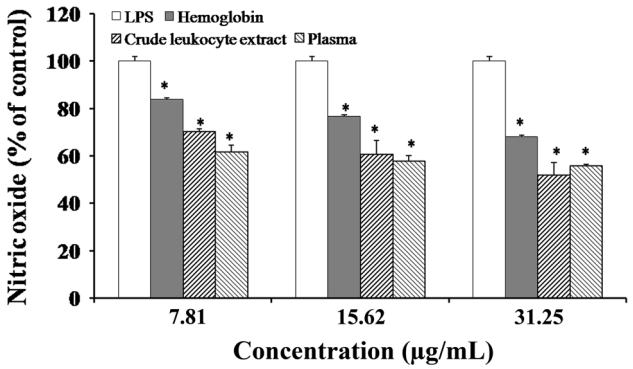


**Fig. 7** Effect of hemoglobin, crude leukocyte extract and plasma on NO generation in LPS-activated RAW264.7. Cells were incubated with 100 ng/mL of LPS and co-incubated with 7.81, 15.62 and 31.25 µg/mL of crocodile blood components for 24 h. Each condition media was used to measure nitrite level. Each *bar* represents the mean ± SD (n = 8). *Significant at $P < 0.05$ probability levels compared with LPS treatment alone

of hemoglobin on linoleic acid oxidation does not occur in a dose-dependent manner. Moreover, no effects of plasma on the inhibition of linoleic acid oxidation have been observed (Fig. 5).

### 3.6 Effect of Crocodile Blood on Cell Viability

In order to evaluate the anti-inflammatory activity of crocodile blood components, the cytotoxicity of each component to RAW 264.7 cells was determined. The percentage of cell viability treated with LPS was taken as 100 % viability. The results indicate that crude leukocyte extract and plasma did not affect cell viability whereas co-incubating hemoglobin with LPS leads to slightly decreased percentage viability ($*P < 0.05$) (Fig. 6). The different concentrations were tested to guarantee that the crocodile blood had no or less cytotoxicity. Therefore, these percentages of crocodile blood were chosen for further experiments.

### 3.7 Effect of Crocodile Blood on NO Production

After inflammation of RAW 264.7 was induced by LPS for 24 h, hemoglobin, crude leukocyte extract and plasma could reduce the production of NO in a concentration-dependent manner. The reductions were 16.26–31.9 % for hemoglobin, 29.78–48.24 % for crude leukocyte extract and 38.3–44.27 % for plasma. The data showed that the ability to reduce NO production was at the highest level in crude leukocyte extract with 48.24 % at the highest concentration (31.25 µg/mL), whereas hemoglobin had the lowest ability to reduce NO production. At the highest concentration of hemoglobin, the reduction of NO was 31.9 % (Fig. 7).

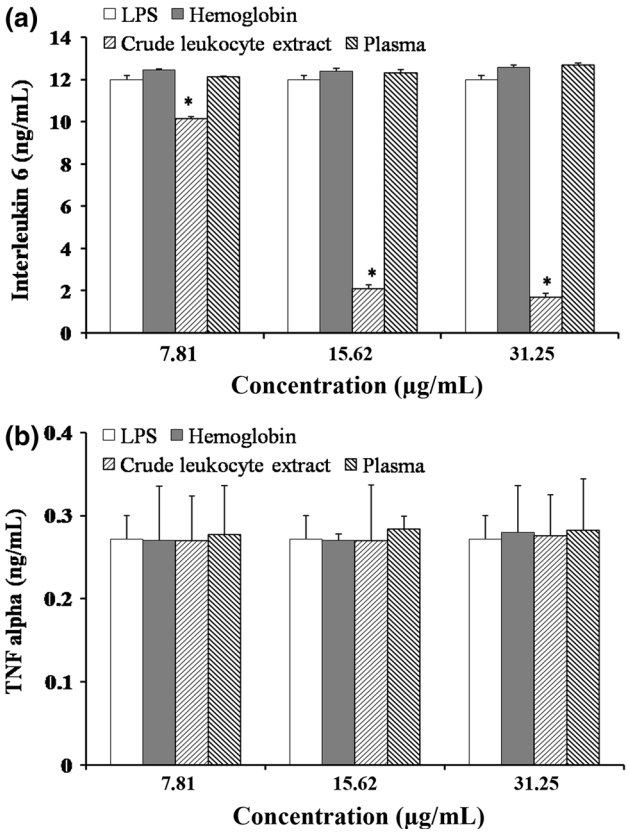


**Fig. 8** Effect of hemoglobin, crude leukocyte extract and plasma on LPS-stimulated IL-6 (**a**) and TNF-α (**b**) production. Cells were incubated with 100 ng/mL of LPS and co-incubated with different concentrations of crocodile blood components for 24 h. Media of each condition were used to measure IL-6 and TNF-α level. Each *bar* represents the mean ± SD (n = 8). *Significant at $P < 0.05$ probability levels compared with LPS treatment alone

### 3.8 Effect of Crocodile Blood on IL-6 and TNF-α Production

The inflammatory marker, IL-6 and TNF-α, level in the RAW264.7 were produced after activation with LPS. Among the crocodile blood samples, crude leukocyte extract was the only one that could decrease the IL-6 production significantly in a concentration-dependent manner as follows: 7.81, 15.62 and 31.25 µg/mL could inhibit IL-6 production by 15.66, 82.5 and 85.3 % ($*P < 0.05$), respectively. In contrast hemoglobin and plasma did not affect IL-6 production (Fig. 8a). Additionally, no effects on TNF-α production were found when hemoglobin, crude leukocyte extract and plasma were added to LPS-stimulated RAW 264.7 cells (Fig. 8b).

## 4 Discussion

Oxidative stress and inflammation play major roles in various diseases, including rheumatoid arthritis, chronic

asthma, multiple sclerosis, diabetes, cancer, cardiovascular problems and other degenerative disorders [3, 4], so the inhibitions of oxidative stress and inflammation can reduce many diseases. Crocodile blood has several biological properties such as antimicrobial activities [14, 16, 21, 22], antioxidant activities [14] and anti-inflammatory activities [15]. These properties might be used for development of food supplements and novel drugs. Therefore, antioxidant and anti-inflammatory activities from crocodile blood components including hemoglobin, crude leukocyte extracts and plasma were investigated.

Our results have demonstrated that crocodile (*C. siamensis*) hemoglobin could scavenge ABTS, NO radicals and inhibit linoleic peroxidation on antioxidant activity. These antioxidant methods were related to electron donors to free radicals. Hemoglobin exhibited antioxidant activity that might involve the thiol groups. Crocodylidae hemoglobin has the highest number of thiols which can intercept ROS or RNS [23]. Similar results have been reported in biothiols for example glutathione (GSH), N-acetylcysteine (NAC), captopril (CAP), homocysteine (HCYS) and cysteine (CYS) that have strong antioxidant effects. This function allows them to protect cells from oxidative damage [24]. The other presumed antioxidant property of *C. siamensis* hemoglobin involves some amino acid in their primary structure such as Val, Leu, Pro, His, Tyr, Trp, Phe, Cys, Glu, Asp, Lys and Arg [14]. The antioxidant activity of peptides containing His and Tyr may show characteristics of chelates and the lipid peroxide radical catching capacity from its imidazole ring. Moreover, Tyr could enhance the antioxidant activity, which is an effective hydrogen donor [14]. The importance of some amino acids in *C. siamensis* hemoglobin to antioxidant action is similar to other reports such as in humans it has been found that hemoglobin β93Cys plays an important role within the red blood cell for antioxidant activity [25]. Therefore, the antioxidant activity of peptides from *C. siamensis* hemoglobin may result from elements of antioxidant amino acid residues and thiol groups presented in their primary structure.

The effect associated with the anti-inflammation of *C. siamensis* hemoglobin might be related to the inhibition of nitric oxide synthase (NOS), which leaded to the inhibition of the NO secretion. Dreier et al. [26] reported that hemolysis caused the release of hemoglobin into the extracellular space, the regulatory fine-tuning was lost, and hemoglobin played an important role in the NO scavenging. Hence, hemoglobin not only exhibited antioxidant activity that could inhibit ABTS, NO and linoleic peroxidation, but it also had anti-inflammatory activity by inhibiting NO production. Both antioxidant (nitric oxide radicals scavenging) and anti-inflammation also have been reported in humans [27], rat [26] and sheep [28] hemoglobin.

Crude leukocyte extracts have inhibited ABTS, NO, hydroxyl radical and linoleic peroxidation which might involve elements in crude leukocyte extracts such as proteins or peptides. This is consistent with previous reports that reported that the activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) were increased in the human lymphocytes when induced by oxidative stress [29]. Another indication about the peptides in crude leukocyte extracts from *C. siamensis* as described by Theansungnoen et al. [30], who reported the antioxidant activity was observed in two peptides i.e. TDVLGLPAK (912.5 Da) and DPNAALPAGPR (1,148.6 Da).

We also found that crude leukocyte extracts exhibit anti-inflammatory effects by reducing NO and IL-6, but cannot inhibit TNF-α production. This anti-inflammatory effect was likely related to heme oxygenase-1 (HO-1), an antioxidant enzyme found in many cells, especially in leukocyte [31, 32]. HO-1 plays an essential role in the conversion of heme into carbon monoxide and biliverdin, which will be changed to bilirubin that is known to be a potent antioxidant. Both carbon monoxide and bilirubin also have potent anti-inflammatory activity and can decrease the release of inflammatory media such as NO and IL-6, as well as reducing the LPS-stimulated nuclear transcription factor (NF-κB) [33, 34]. Another report has suggested the involvement of ubiquitin. In the American alligator (*Alligator mississippiensis*), ubiquitin in leukocytes has been reported to have several functions in cellular processes such as the control of apoptosis, immune response, antigen processing and especially inflammation [35]. Therefore our studies confirmed that both antioxidant and anti-inflammatory activities were observed in crude leukocyte extracts.

Plasma suppressed ABTS and hydroxyl radicals and also inhibited the production of $NO^{\cdot}$ indicating that plasma had anti-inflammatory activity. The effect of plasma inhibiting free radicals is likely related to albumin, which is the most abundant protein in plasma. It is evident that the cysteine thiol group (Cys34) in their structures play an important role in antioxidant properties [36]. In addition, the reduced form of Cys34 in albumin can scavenge hydroxyl radicals via the Fenton reaction [37]. During oxidative stress, these protein thiols get oxidized, mainly by conversion to disulfide bonds in plasma. Thus protein thiols play a major role in free radical defenses [38, 39].

The anti-inflammatory mechanism of plasma involved the modulation of inhibitory NO mediator, which was secreted during inflammation. Hypothetically, this function is related to activated protein C (APC). This agrees with the results of Esmon [40], who reported the anti-inflammatory functions of APC can decrease the synthesis of NF-

κB components and decrease inflammatory cytokine production through NF-κB.

Our study suggests that antioxidant and anti-inflammatory properties of each crocodile blood component depend on the different kind of protein or peptide composition.

## 5 Conclusion

This is the first report about antioxidant and anti-inflammatory activities of hemoglobins, crude leukocyte extract and plasma from *C. siamensis* blood. We believe that crocodile blood and its components could be developed to be a supplementary product in food and pharmaceutical industries.

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