Xanthones protects lead-induced chronic kidney disease (CKD) via activating Nrf-2 and modulating NF-kB, MAPK pathway

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\textbf{ABSTRACT}

Xanthones from a tropical fruit of \textit{Garcinia mangostana} L. is known to possess a wide spectrum of pharmacologic properties, including antioxidant, anti-bacterial, anti-inflammatory, and antidiabetic activities. The current study aimed to assess the possible protective effects of xanthones against lead acetate (PbAc)-induced chronic kidney disease (CKD). To accomplish, \textit{in vitro} antioxidant assays of xanthones, \textit{in vivo} oxidative stress parameters, histopathology, inflammatory markers were evaluated using PbAc-induced IRC male mice. The study was supported by \textit{in silico} molecular docking of respective organ receptor-ligand interaction. Results revealed that xanthones potentially scavenged the DPPH, superoxide, hydroxyl, and nitric oxide radicals. Oxidative stress, kidney dysfunction, inflammatory markers, and kidney apoptosis increased by PbAc were attenuated with the co-treatment of xanthones. The treatment remarkably improved the tissue architecture. Of note, \textit{in silico} prediction of activity study showed that protective role of xanthones could be due to its efficacy to activate the Nrf-2, regulate the intracellular [Ca\textsuperscript{2+}], as well as downregulate the NF-kB, MAPK pathway. In a nutshell, xanthones could be a potential candidate for the management of PbAc-induced kidney damage.

1. Introduction

Mangosteen (\textit{Garcinia mangostana} L.), a juicy white pulp with sweet and slightly acidic in taste fruit, has a pericarp of radish or dark brown color and found in the different region of Asia including Thailand, India, Malaysia, Sri Lanka, and other countries. Nowadays in Thailand, xanthones from mangosteen pericarp is being used with tea for refreshment. Traditionally, mangosteen pericarp has been used to treat various diseases such as skin disease, bacterial infection, diarrhoea, wound healing, and inflammation [1]. Xanthone (9-xanthenone or dibenzo-c-pyrene), a phenol class compound, have been identified in the pericarp of this fruit where α-mangostin, β-mangostin, γ-mangostin are abundant [1]. A growing body of pharmacological researches revealed that xanthones possess potential antioxidant, anticancer, anti-diabetes, cardioprotective, and hepatoprotective activities [2–5].

Lead (Pb) poisoning became an epidemic at Michigan State, USA through drinking water and continuous health complexity of people made it as national as well as global concern. Indeed, the possible causes of Pb intoxication include contamination of food, water, soil, cosmetics, house dust, and paint [6]. Accumulated evidence reported that Pb intoxication can causes gastrointestinal, hematological, reproductive, immunomodulatory, neurological, and renal disorders on the basis of the extent of exposure [7–12]. Pb executes its noxious effect on major organs of the body, especially on kidney by means of oxidative stress-an imbalance between oxidant and antioxidant. This condition could arise the following abnormalities such as dysregulation of intracellular [Ca\textsuperscript{2+}] homeostasis, displacement of essential metal ion from protein, and mitochondrial damage. As a result, outnumbers of oxidants brings damage to DNA, protein, and cell, therefore apoptosis of cells. Pb also intervenes the cellular permeability by modifying tight junction protein in the kidney. As a manifestation of kidney damage, increased protein urea and decreased kidney function along with the mutilated structure of nephrone have been recorded in various studies, which later on turned into chronic kidney disease (CKD) [13]. CKD can be defined as kidney damage with substantial albuminuria and kidney dysfunction persistently for a three month period [14]. Previously, it had been found that antioxidant compounds specially xanthones ameliorated the PbAc-induced cognitive impairment of neurotoxic mice by reducing oxidative stress parameters and modulating acet-ycholinesterase (AChE) dysfunction [15]. Therefore, this study first time focuses on the protective effect of xanthones derivative from \textit{G. mangostana} against PbAc-induced CKD.
2. Materials and methods

2.1. Xanthones extract preparation

The xanthones powder from *Garcinia mangostana* fruit pericarp was kindly provided by the Research excellent center for innovation and health products, Walailak University, Thailand. Other than xanthones, the powder contains carbohydrate, protein, fibre in a concentration of 77.2, 1.4, 10 gm per 100 gm of powder. In addition, the ORAC value was recorded as 79.46 unit per serving (20 g). According to recent study on xanthone, isolated xanthone (100 mg) from MVR contained α-mangostin (69.01%), γ-mangostin (17.85%), gartanin (4.13%), 8-deoxygartanin (2.95%), garcinon E (2.84%), and other xanthones (3.22%) [16]. Xanthones extract was prepared freshly prior to each experiment.

2.2. Determination of total phenolic content, antioxidant capacity and free radicals scavenging activities of xanthones

2.2.1. Total phenolic content of xanthones

Total phenolic content of the xanthones aqueous extract was determined as previously described method of Gulcin, 2005 [17]. Briefly, 12.5 μL of different concentrations (0.1, 0.25, 0.5, 1 mg/mL) of xanthones extract and distilled water (as blank) were added in the 96 well microplate. Then, 12.5 μL of Folin-Ciocallet's phenol reagent was added to each well. After 5 min, 125 μL Na2CO3 solution (~7.5%) was added to the mixture and incubated at room temperature for 30 min. The absorbance was recorded at 765 nm using a microplate reader (Multiskan GO, Thermo Fisher Scientific, Finland). Gallic acid with different concentrations ranging from 0 to 100 mg/L was used to construct the calibration curve. A dose-response linear regression was generated from Gallic acid standard curve and the phenolic content in the samples was expressed as milligram of Gallic acid equivalents per milligram of dry weight (mg of GAE/gdw).

2.2.2. Total antioxidant capacity (ABTS assay) of xanthones

ABTS** scavenging activity was determined according to the method of Re et al., 1999 [18]. ABTS** was produced by reacting 7 mM of ABTS in water with 4.9 mM of K3Fe(CN)6 and stored in the condition dark at room temperature for 12–18 h before use. Then, 180 μL of ABTS** (absorbance range, 0.750 ± 0.025) solution was added to 20 μL of xanthones extract (concentrations range, 0.1–1 mg/mL). Finally, absorbance was recorded at 734 nm after 3 min of mixing against distilled water (blank). The values were expressed as mM of Trolox equivalent per gram of dry weight (mM of TEAC/gdw).

2.2.3. Free radical scavenging activities of xanthones

DPPH, superoxide, hydroxyl, nitric oxide radicals scavenging activities were evaluated according to the previously published report of Rana and Tangpong, 2017 [19], whereas IC50 was calculated and it's defined the concentration of the sample required to scavenge the 50% of radicals.

3. Animals

3.1. Animal treatment

Male ICR mice (body weight 30–33 g, eight-week-old age), were obtained from the National animal research center, Bangkok, Thailand. Mice were caged and housed at room temperature (23 ± 2 °C) and humidity-controlled environment (55 ± 5%) with a 12 h light/dark cycle was maintained from 6 a.m. to 6 p.m. in the experimental lab of the Natural Product Utilization Unit of Walailak University. The cages were cleaned, washed, sterilized with 70% methanol and filled with sterilized wooden made bed twice a week. Mice were acclimatized for a week with free access to food and water prior to the experiment.

All the instructions for care and use of mice and the protocol were approved by the Animal Care and Use Committee (ACUC) of Walailak University.

Treatment strategy was conducted according to our previous study, whereas xanthones attenuated the PbAc-induced cognitive dysfunction [15]. 42 mice were separated into seven groups (n = 6). Each group received the experimental solution once a day at between 8.30 – 9.30 a.m. via oral gavages for 38 days as follows:

- Group 1: Sodium acetate (1% in drinking water) + Normal saline
- Group 2: PbAc (1% in drinking water) + Normal saline
- Group 3: Sodium acetate (1% in drinking water) + Xanthones 200 mg/kg
- Group 4: PbAc (1% in drinking water) + Xanthones 100 mg/kg
- Group 5: PbAc (1% in drinking water) + Xanthones 200 mg/kg
- Group 6: PbAc (1% in drinking water) + Vitamin E 100 mg/kg
- Group 7: Sodium acetate (1% in drinking water) + Vitamin E vehicle (mineral oil).

3.2. Blood collection and tissue sample preparation

Mice were anesthetized using Sodium Nembutal (65 mg/kgBW) and then sacrificed for collecting blood via left ventricle puncture. Afterward, the blood samples were kept in a 2.5 mL tube containing K2EDTA. Then, organs were thoroughly perfused with cold PBS (pH 7.4, 4 °C) to eradicate blood content from the tissue sample and stored at −30 °C for further use.

Kidney tissues were minced in cold PBS containing a mixture of protease inhibitors (leupeptin, pepstatin, and aprotinin) and homogenized (Sonics VCX70, USA). Thereafter, tissue suspensions were centrifuged at 15,000×g for 15 min at 4 °C. The supernatant was separated and stored at −30 °C for further enzymatic analysis.

A portion of kidney was homogenized in cold lysis buffer containing PMSF and kept on ice. There after centrifuged at 15,000×g for 15 min, 4 °C. The resulted supernatant was then collected emerging quickly the eppendorf at liquid nitrogen first, then preserve at −80 °C for Western blot analysis.

3.3. Determination of relative kidney weight

The weight of the left kidney and body weight was recorded and calculated relative kidney weight using the following formula [20]:

Relative kidney weight (%) = \( \frac{\text{Left kidney weight (g)}}{\text{Body weight (g)}} \times 100 \)

3.4. Determination of Pb concentration in blood and kidney sample

Pb content in whole blood and tissue samples were determined using an atomic absorption spectrometry (AAS) with flameless atomization in a graphite furnace (Zeeman Atomic Absorption Spectrophotometer Z-5000, Hitachi, Tokyo, Japan) as previously described by Tangpong and Satarug, 2010 [21]. Briefly, samples were diluted at a ratio of 1:5 using a diluent-containing 0.2% of Triton-X 100 and (NH4)2HPO4 in double deionized water. The absorption wavelength was 283.3 nm. Pb levels in samples were calculated using a calibration curve of standard Pb. The result was recorded as μg/dL for blood samples and μg/g protein for kidney samples, respectively.

3.5. Evaluation of kidney function parameters

Blood urea nitrogen (BUN) and creatinine are widely used to evaluate kidney function. A bioassay system reagent kit (Hayward, CA, USA) was used to evaluate the BUN and creatinine was determined using automatic chemistry analyzer (KONE Lab20, Tokyo, Japan).
Table 1
Phenolic content, total antioxidant capacity and scavenging activities of xanthones on different free radical.

| Aqueous extract | TPC (mg GAE/g extract) | TAC (mM TEAC/g extract) | DPPH | Superoxide | Hydroxyl | Nitric oxide |
|-----------------|-----------------------|-------------------------|------|------------|----------|-------------|
| Xanthones derivative | 92 ± 5.0 | 665 ± 0.0 | 0.693 ± 0.01 | 0.463 ± 0.03 | 1.214 ± 0.00 | 1.65 ± 0.03 |

TPC = Total phenolic content; TAC = Total antioxidant capacity. The values are express as mean ± SEM of three independent experiments.

Table 2
Xanthones significantly reduced relative kidney weight but failed to reduced aggregated PbAc from the body.

| Groups | Body weight (g) | Kidney weight (g) | Ratio (%) | Blood Pb (μg/dL) | Kidney Pb (μg/g protein) |
|--------|----------------|------------------|-----------|------------------|----------------------|
| Untreated control | 41.742 ± 0.80 | 0.33 ± 0.01 | 0.80 ± 0.03 | 1.65 ± 0.11 | 1.83 ± 0.19 |
| Pb (1% in drinking water) | 39.96 ± 1.24* | 0.43 ± 0.01* | 1.09 ± 0.05* | 62.35 ± 3.92* | 49.97 ± 4.32* |
| xanthones (200 mg/kgBW) | 43.27 ± 0.86 | 0.41 ± 0.01 | 0.95 ± 0.01 | 1.69 ± 0.08 | 1.37 ± 0.26 |
| Pb + xanthones (100 mg/kgBW) | 38.11 ± 0.96b | 0.34 ± 0.02b | 0.90 ± 0.04b | 59.12 ± 3.59b | 48.45 ± 3.14b |
| Pb + xanthones (200 mg/kgBW) | 39.14 ± 0.98b | 0.33 ± 0.02b | 0.85 ± 0.06b | 58.96 ± 4.25b | 46.50 ± 2.96b |
| Pb + Vit E (100 mg/kgBW) | 37.22 ± 1.00b | 0.37 ± 0.01b | 1.00 ± 0.06 | 60.89 ± 2.45a | 48.90 ± 2.18b |
| Vehicle control | 42.60 ± 0.80 | 0.32 ± 0.01b | 0.77 ± 0.04b | 1.69 ± 0.21b | 1.56 ± 0.11b |

Values are means ± SEM (n = 6). *Significant difference at p < 0.05 compared with untreated control group. bSignificant difference at p < 0.05 compared with Pb-treated only.

Table 3
Xanthones effectively restored kidney function.

| Groups | BUN (mg/dL) | Creatinine (mg/dL) |
|--------|-------------|-------------------|
| Untreated control | 30.19 ± 2.01 | 0.23 ± 0.03 |
| Pb (1% in drinking water) | 39.04 ± 3.40a | 0.45 ± 0.05a |
| xanthones (200 mg/kgBW) | 29.65 ± 1.65b | 0.23 ± 0.03b |
| Pb + xanthones (100 mg/kgBW) | 28.38 ± 1.46b | 0.26 ± 0.09b |
| Pb + xanthones (200 mg/kgBW) | 26.96 ± 1.76b | 0.24 ± 0.03b |
| Pb + Vit E (100 mg/kgBW) | 26.33 ± 2.02b | 0.27 ± 0.06b |
| Vehicle control | 27.96 ± 2.44b | 0.26 ± 0.07b |

Values are means ± SEM (n = 6). *Significant difference at p < 0.05 with respect to untreated control group. bSignificant difference at p < 0.05 with respect Pb treated only.

3.6. Determination of lipid peroxidation in RBC, plasma and kidney tissue

Thiobarbituric acid reactive substances (TBARS), a secondary by-product of lipid peroxidation (LPO), is widely investigated as a marker to assess the magnitude of oxidative stress. TBARS assay in RBC, plasma and kidney tissue was evaluated according to the protocols of Ceci et al., 2014; Goulart et al., 2005; Jain, 1989 with slight modification [22–24]. Briefly, 100 μL of samples (RBC, plasma, and kidney homogenate) were mixed with 20% of trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 15 min. Then, 15% of thiobarbituric acid (TBA) was added to the supernatant. Afterward, the mixture was boiled at 100 °C in a water bath for 30 min. After centrifugation at 3000 rpm for 15 min, the supernatant was collected and measured the absorbance at 595 nm. The lipid peroxide levels were compared with a standard curve of Malondialdehyde (1, 1, 3, 3-tetraethoxypropane). The results were expressed as μM/g Hb for RBC, μM/L for plasma, and μM/g protein for kidney sample.

3.7. Determination of antioxidant enzyme activities in kidney sample

Superoxide dismutase (SOD) activity was determined using a slightly modified method of Marklund et al. [25]. Briefly, 1 mL of pyrogallol solution (0.2 mM) was mixed with an equal volume of Tris-EDTA buffer (pH 8.2) to auto-oxidize the pyrogallol. After that, immediately 50 μL of sample was mixed with reaction mixture to inhibit the autoxidation. Then, the absorbance was measured at 420 nm. SOD activity was expressed as units/mL, while one unit of SOD activity defines the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation.

Catalase activity was determined according to the protocol of Weydert and Cullen, 2010 [26]. The absorbance of the reaction mixture was measured at 240 nm using an UV–vis spectrophotometer (JASCO V-630, Japan). Catalase activity was expressed as Katal/mg of protein.

3.8. Determination of inflammatory markers using ELISA assay and Western blot analysis

Plasma TNF-α level was determined using a colorimetric sandwich enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) protocol. Inflammation markers of kidney tissues were determined using Western blot analysis. Briefly, protein levels were determined using Bradford reagent (Bio-Rad, USA) and run through SDS-polyacrylamide gel electrophoresis at 100 V (Bio-Rad Laboratories, Inc., California, USA). Then, proteins were transferred to a nitrocellulose membrane (Roche Diagnostics Corporation, Indianapolis, IN, USA) at 100 V, 4 °C for 2 h. After that, the membrane

Table 4
Effect of xanthones on the histopathological scoring of different treatment groups.

| Groups | Hydropic degeneration changes in tubules | Glomerular damage | Inflammatory cellular infiltration |
|--------|----------------------------------------|------------------|----------------------------------|
| Untreated control | 0 | 0 | 0 |
| Pb (1% in drinking water) | + + + | + + + | + + |
| xanthones (200 mg/kgBW) | 0 | 0 | 0 |
| Pb + xanthones (100 mg/kgBW) | + | + | + |
| Pb + xanthones (200 mg/kgBW) | + | + | + |
| Pb + Vit E (100 mg/kgBW) | + | 0 | 0 |
| Vehicle control | 0 | 0 | 0 |

Here, 0: absent; +: mild; + +: moderate; + + +: severe.
was blocked with TBST solution (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.02% Tween 20 containing in 5% skimmed milk) for 1 h. Afterward, the membrane was incubated with the primary antibody of TNF-α, COX-2, and iNOS (Cell Signaling Technology, Massachusetts, USA) for overnight at 4 °C. Next, the membrane was washed with TBST solution (3 × 5 min) and incubated with peroxidase-conjugated secondary antibody (Cell Signaling Technology, Massachusetts, USA) at room temperature. After washing with TBST solution (3 × 5 min), the band of protein was detected by enhanced chemiluminescence detection kit (Bio-Rad, USA) and the result was expressed as the ratio with the β-actin band (Cell Signaling Technology, Massachusetts, USA) [27].

3.9. Determination of kidney tissues histology

Kidney tissues were fixed with 4% formaldehyde, embedded on paraffin and sectioned using a microtome to obtained 6 μm section. After deparaffinization and hydration, the sections were stained with hematoxylin and eosin, examined and photographed under a light microscope (Olympus EX51, Olympus Corporation, Tokyo, Japan).

3.10. Assessment of renal apoptosis by TUNEL assay

Frozen kidney tissues were sectioned into 7 μm using microtome (Leica Microsystem CM1950, Wetzlar, Germany). Thereafter, the whole assay was conducted according to the protocol supplied by “In situ apoptosis detection kit (Abcam, Cambridge, UK)” to investigate the extent of apoptosis in the kidney section. TUNEL positive cell was counted and expressed as a number of cells per 100 fields.

4. In silico studies of xanthones

4.1. In silico prediction of activity spectra (PASS) analysis

According to the literature survey, α-mangostin, β-mangostin, γ-mangostin are predominant among phytoconstituents present in the pericarp of the mangosteen fruits [1]. These three phytoconstituents were analyzed to predict antioxidant and anti-inflammatory effect, Nrf-2, Hemeoxygenase and catalase stimulating and Ca-regulatory activity using by online PASS analysis program (Way2Drug). This program predicts more than 4000 kinds of biological activity based on the structure of compound, i.e. structure activity relationship (SAR). The analyzed data were presented as Probable activity (Pa) and Probable inactivity (Pi) with the values in between 1.000 to 0.000 [28]. Compounds which exhibited higher Pa value than Pi (Pa > Pi) was considered as biological active and Pa > 0.2 was regarded to have pharmacological potential.

4.2. In silico molecular docking study

The molecular docking study was accomplished following the description of Uddin et al., 2018 [28] using a Mestro module of Scodinger suit v11. Briefly, crystal structures of proteins were retrieved from the protein data bank as PDB format and imported into protein preparation wizard of Mestro v11. These protein structures were then preprocessed allowing the bond order, addition of hydrogen, creation of disulfide bond. Finally, the minimization was carried out by applying the OPLS5 force field, setting maximum heavy atom root-mean-square-deviation (RMSD) to 0.30 Å.

To prepare the ligand, 2D structures of α-, β-, γ-mangostin compounds were saved from PubChem as SDF format. Later on, input into
ligprep wizard of Mestro v11. Then, the 3D structures were prepared with default setting by applying OPLS5 force field, pH 7.0 ± 2.0 to generate the ionization states, allowing possible 32 stereoisomers per ligand.

Following prepared protein was then processed to receptor grid generation by applying OPLS5 force field. Using the ligand docking module of Mestro v11, the grid generated structure of protein and ligand are allowed to dock (flexible docking). And, the score of docking was then exported as excel file. The lowest value (negative) of the docking score indicates the best docking.

5. Statistical analysis

Data were expressed as mean ± standard error mean (SEM). Variable among the groups were analyzed by One-way analysis of variance (ANOVA) followed by Newman–Keuls, post hoc test using GraphPad Prism software (version 5, USA). The statistically significant level was set up at $p < 0.05$.

6. Results

6.1. Total phenolic content, antioxidant capacity, and free radical scavenging activities of xanthones

The results of phenolic content, antioxidant capacity, and different reactive species scavenging activities by xanthones are presented in Table 1. The result showed that the total phenolic content of xanthones was 92 ± 5.0 mg GAE/g of extract while the total antioxidant capacity was 665 ± 0.0 mM TEAC/g of extract. Xanthones also had the ability to scavenge different reactive free radicals. Antioxidative effects of xanthones in terms of inhibition concentrations (IC$_{50}$) to scavenge the radicals from DPPH, superoxide, hydroxyl and nitric oxide were found to be 0.693 ± 0.01 mg/mL, 0.463 ± 0.03 mg/mL, 1.214. ± 0.00 mg/mL, and 1.65 ± 0.03 mg/mL, respectively.

6.2. Effect of xanthones on relative kidney weight and Pb deposition

The relative kidney weight is summarized in Table 2 which demonstrated that Pb caused a significant ($p < 0.05$) increment of relative kidney weight with reduced body weight. Pb treatment significantly ($p < 0.05$) increased the Pb concentration, both in the blood (62.35 ± 3.92 μg/dL) and tissue (49.97 ± 4.32 μg/g protein), which was managed to be reduced by the co-treatment with xanthones. Surprisingly, xanthones and vitamin E failed to lower the increased Pb deposition in a significant manner.

6.3. Effect of xanthones on kidney function parameters

Biochemical analyses showed that Pb interfered the kidney function parameters particularly BUN and creatinine. Lead treated mice were found to show significantly higher ($p < 0.05$) BUN and creatinine values, compared with the normal control group, which were significantly ($p < 0.05$) and dose-dependently modulated by a 38-day xanthone administration (Table 3).

6.4. Effect of xanthones on oxidative stress-related parameters

The lipid peroxidation (LPO), considered as oxidative stress marker,
was significantly ($p < 0.05$) increased in RBC, plasma and kidney samples of Pb induced mice compared to normal control. Reduced activity of antioxidative enzymes SOD and CAT due to PbAc treatment has been reversed significantly ($p < 0.05$) with xanthone treatment. A 38 days treatment of PbAc-treated mice with xanthones significantly ($p < 0.05$) reduced the TBARS level in different samples than that of PbAc-treated mice (Fig. 1).

6.5. Effect of xanthones on inflammatory parameters

Fig. 2 revealed that PbAc significantly ($p < 0.05$) induced the inflammation in normal mice compared to untreated normal control group. Co-treatment with xanthones or Vitamin E reduced the plasma TNF-α concentration (Fig. 2A) and protein expression of TNF-α, COX-2, and iNOS in kidney tissue (Fig. 2B, C, 2D). No significant difference for the expressions of TNF-α, COX-2, and iNOS was noted in normal control group and xanthone alone.

6.6. Effect of xanthones on renal pathology and apoptosis

Alterations of tissue architecture were scored as severe, moderate and mild damage by PbAc treatment. Fig. 3 and Table 4 displays that co-treatment of xanthones or vitamin E restores the PbAc induced severe hydropic tubular degeneration, glomerular damage, and inflammatory cellular infiltration. The histopathological screening also showed that PbAc significantly ($p < 0.05$) induced the cellular apoptosis reflected by a higher number of TUNEL positive cells (Fig. 4). Co-treatment with xanthones significantly ($p < 0.05$) decreased the TUNEL positive cell number compared to PbAc treated group.

6.7. PASS analysis and docking study

In silico docking studies had revealed the possible mode of interactions of three ($\alpha$, $\beta$, $\gamma$-mangostin) constituents. According to the docking result, these phytoconstituents showed strong binding affinity with the receptors of JNK (PDB: 3OY1) and NFkB (PDB: 4KIK) (Table 5 and Fig. 5). PASS prediction of three abundant phytoconstituents ($\alpha$, $\beta$, $\gamma$-mangostin) had shown the probability to be effective as Nrf-2 and catalase stimulant, HOX1 expression enhancer, Ca-regulator, potent antioxidant and anti-inflammatory agent (Table 6). Based on sophisticated virtual screening and analysis of docking score, it could be predicted that xanthones exerted protective activity against oxidative stress and inflammation.

7. Discussion

Chronic kidney disease (CKD) has emerged as a common health burden worldwide [29] and about 11–13% of people are at stage 3 condition [30]. Kidney plays a pivotal role to excrete the waste product through urine. Thereby, kidney is the most vulnerable target organ to Pb following accumulation. Moreover, CKD is associated with Pb-toxicity [31] and also regarded as a risk factor for metabolic diseases such as diabetes, hypertension, and others [32]. The possible mechanism of kidney toxicity exerts via developing oxidative stress, which in turn causes the lipid peroxidation [6]. Indeed, CKD is characterized by renal injury initiated via the development of oxidative stress, oxidant/free radicals. In the long run, these reactive radicals triggered inflammation process and renal damage that reduce kidney performance [33]. In our current study, the interrelationship among the Pb accumulation, increased kidney/body weight ratio, kidney dysfunction, histopathology,
and inflammation had been established, while xanthones reversed these abnormalities without efficient noxious effects. Present data revealed that PbAc treatment significantly ($P < 0.05$) decreased body weight and increased kidney weight. This anomaly was in agreement with the study of Dhkil et al. [20]. The weight loss of mice could be due to the interference of Pb on the central nervous system (CNS), which in turn develop the anorexia. Co-treatment with xanthones improved the body weight and ratio of kidney/body weight in a dose-dependent manner, possibly via improving cognitive function, as reported in the previous study of Phyu and Tangpong [15].

Accumulation studies suggested that elevated Pb content attributes to oxidative stress, kidney dysfunction [34], which are consistent with our present data. Augmented LPO marker (TBARS), kidney dysfunction, declined antioxidant status, along with Pb accumulation in blood and kidney suggested that “oxidative stress” was an underlying mechanism of kidney damage. In accordance with the previous study [35], xanthones co-treatment remarkably reduced the oxidative stress but failed to limit the Pb accumulation. These results demonstrate its inability as a chelating agent, a form of antioxidant activity [36]. Analyzed histopathology and TUNNEL assay photograph revealed that PbAc caused extensive damage to kidney including mutilated glomerulus, tubular swelling & necrosis, and infiltration of inflammatory cells with a high number of apoptotic cell compare to untreated control and xanthone 200mg/kgBW groups, respectively. These tubular cells alteration is might be due to the interrupted ion pump transport by Pb, which leads to hydropic changes and exerted tubular swelling and necrosis [39]. Coherent to histopathology, Pb also induced the apoptosis possibly through ROS production and TNF-α mediated caspase-3 activation [20,37]. In contrast, co-treatment of xanthones protected nephron’s architecture and integrity by reducing cellular apoptosis as well as reversing above mentioned biochemical and enzymatic indices in a dose-response manner without exerting any potential adverse effect.

In Pb-induced group, infiltration of inflammatory cells was observed in interstitial space, which is believed to influence inflammation-mediated apoptosis via extrinsic pathway. Furthermore, activation of JNK-MAPK pathway contributes to ROS-mediated inflammation in Pb-intoxicated mice [37]. In addition, excessive ROS act as a signaling molecule to activate NF-kB, which in turn translocate to the nucleus to regulate the gene encoding inflammation-associated molecules such as TNF-α, COX-2, and iNOS [38]. Compared to untreated normal control, a sharp rise of pro-inflammatory cytokines TNF-α was observed in the circulation of PbAc-treated group. Additionally, the protein expression of TNF-α, COX-2, iNOS in tissue were also elevated in PbAc-treatment. To treat CKD patient, phenolic content or antioxidants are also recommended to suppress the oxidative stress and inflammation [39]. Our current study also uncovered that xanthones and Vitamin E effectively lessened inflammation similar to that of Wang et al., 2016 [40]. Likewise the therapeutic role of antioxidants to CKD, they are also efficient to protect kidney from PbAc [36,41] either by quenching free radicals, chain breaking mechanism against ROS [42], triggering

Fig. 4. Xanthones co-treatment reduced the TUNEL positive cell in the kidney. (A) Quantification of TUNEL positive cell in kidney section counted in a hundred fields. (B) TUNEL positive cell in kidney tissue sectioned at 7 μm and the red arrow indicates the TUNEL positive cell (original magnification x200). Data are represent as mean ± SEM (n = 3). Here, $^aP < 0.05$ compared with untreated normal control group, $^bP < 0.05$ compared with PbAc group, $^cP < 0.05$ compared with the xanthone 200 only and $^dP < 0.05$ compared with Pb + xanthone 100.
Considering the mechanism of action as described in our recently published review [6], we hypothesized that attenuation of kidney pathophysiology by xanthones was achieved through suppressing inositol-3-phosphates (IP3R) receptor to modulate the intracellular [Ca2+]i, in mediator (TNF-α) damage and apoptosis [6], whereas inhibition of pro-inflammatory mediators (i.e. SOD, CAT, HO-1, etc.), and inhibition of NF-κB (NF-κB, MAPK pathways. Thus, the current findings demonstrated that xanthones could be a potential candidate for the management of heavy metal toxicity by suppressing oxidative stress and inflammation. Further studies are also required to isolate the abundant phytoconstituents and to evaluate the mechanistic pathways.

### Ethical approval

The study was approved by the Animal Care and Use Committee (ACUC) of Walailak University, with Animal Ethics Approval Certificate Number 002/2013. All animal experiments comply with the ARRIVE guidelines and carried out following National Institutes of Health guide for the care and use of Laboratory Animals.

### Consent for publication

All authors have agreed to publish all materials belongs to this article.
Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

Funding was done by Walailak University (WU59122) to conduct the experiments.

Author’s contribution

JT was involved in designing the experiment, editing the manuscript with critically evaluating the data for maintaining the integrity of works, and approved the manuscript for submission. MNR contributed to design the experiment, conduct the experiments, analyze data and manuscript writing, MAR has supplemented the idea, revised and rearranged the manuscript, redimensioned the interpretation and restructured the data setting.

Declaration of competing interest

The authors declare that they have no competing interests.
Acknowledgment

Authors are thankful to Mohammed Sohel Chowdhury, Department of Pharmacy, International Islamic University Chittagong, Bangladesh, for his technical support regarding Schrodinger Software.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbrep.2019.100718.

List of Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| PbAc | lead acetate |
| ROS | reactive oxygen species |
| BW | body weight |
| TNF-α | Tumor necrosis factor-alpha |
| iNOS | Inducible nitric oxide synthase |
| COX-2 | Cyclooxygenase-2 |
| ELISA | enzyme-linked immunosorbent assay |
| BUN | Blood urea nitrogen |
| TBARS | Thiobarbituric acid reactive substances |
| TBST | Tris-phosphate buffer saline with Tween 20 |
| TBS | Tris phosphate saline |
| H-E | Hematoxylin-Eosin |
| TUNEL | Terminal deoxynucleotidyl transferase DUTP nick end-labeling |

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