

## Cytoprotective and Anti-genotoxic Effects of Xanthone Derivatives from *Garcinia mangostana* Against H<sub>2</sub>O<sub>2</sub> Induced PBMC Cell and Blood Leukocytes Damage of Normal and Type 2 Diabetes Volunteers<sup>†</sup>

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### Abstract

Hyperglycemia is well-known for inducing cellular oxidative damage in type II diabetes (T2D) patients. This research addressed the cytoprotective and anti-genotoxic effect of xanthone derivatives from *Garcinia mangostana* against hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-induced human peripheral blood mononuclear cell (PBMC) and blood leukocytes damage of the normal and T2D volunteers. The cytoprotective effects of an aqueous extract of xanthone (100 and 200 µg/mL) was assessed on cell viability and free radical scavenging activity using the trypan blue exclusion method on PBMC cells. Malondialdehyde (MDA) levels and lactate dehydrogenase (LDH) activity were measured as cellular oxidative damage markers and estimated from culture medium of PBMCs of normal and T2D volunteers. The anti-genotoxicity was assessed as the protective effect of xanthone against H<sub>2</sub>O<sub>2</sub>-induced DNA damage of blood leukocytes of the normal volunteers following comet assay technique. Xanthone and Gallic acid (control) concentrations 100, 200 and 100 µg/mL significantly ( $P < 0.05$ ) protected from H<sub>2</sub>O<sub>2</sub> (20 mM)-induced oxidative damage of PBMCs. It was confirmed by increased cell viability and free radical scavenging activity coupled with the decreased MDA and LDH levels in cell culture medium compared to H<sub>2</sub>O<sub>2</sub> (20 mM)-treated group. In H<sub>2</sub>O<sub>2</sub> (40 mM)-induced blood leukocytes of normal volunteers, different concentration xanthone (50 - 500 µg/mL) significantly ( $P < 0.05$ ) improved the anti-genotoxicity effect compared to negative/positive control group by lowering comet formation. Xanthone treatments on PBMCs and blood leukocytes of the normal and T2D volunteers could attenuate the H<sub>2</sub>O<sub>2</sub>-induced cellular oxidative damage and cell death *via* exhibiting antioxidant and free radical scavenging activities.

**Keywords:** Xanthone, *Garcinia mangostana*, cytoprotective activity, anti-genotoxicity, type II diabetes

### Introduction

Diabetes is one of the leading diseases of disability and death in the world [1-3]. According to the WHO (2000), approximately 2.8 % of the global population are affected by diabetes and this will rise to 4.4 % by 2030 [4]. The excessive formation of highly reactive molecules such as reactive nitrogen species (RNS) and reactive oxygen species (ROS) causes healthy cell death through loss of function and structure. Oxidative stress is associated with more than 50 diseases, most commonly diabetes mellitus [5].

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Free radical formation mediates non-enzymatic protein glycation, glucose oxidation, increase lipid peroxidation (LPO), and insulin resistance in the diabetic condition [6]. In late diabetic condition, free radical formation also plays an important role in damaging lipids, proteins, and DNA. Several scientific studies have shown a relationship between diabetes and oxidative stress through assessing numerous DNA damage and lipid peroxidation biomarkers [7].

Since the ancient time, people have believed in plants as the source of alternative medicine safer and effective than synthetic drugs [8]. Thus, natural product scientists have always an intention to find out natural alternative medicine from available sources such as vegetables, herbs, and fruits. *Garcinia mangostana*, a plant found in tropical rainforest area in South Asian countries such as Thailand, Indonesia, Malaysia, Philippines, Sri-Lanka. It possesses traditional medicinal properties against abdominal pain, dysentery, wound infections, suppuration, chronic ulcer and other disease [9].

Xanthone, a biologically active polyphenolic compound, isolated from *G. mangostana* have been shown to be a potent antioxidant, anti-inflammatory, and antidiabetic activities [10]. About sixty-eight xanthenes such as  $\alpha$ -mangostin,  $\beta$ -mangostin,  $\gamma$ -mangostin, garcinone C, garcinone D, 8-Desoxygartanin, and mangostenone E have been identified in *G. mangostana* [11]. In human umbilical vein endothelial (ECV304) cell line, the methanolic fraction of *G. mangostana* exhibited cytoprotective effect by lowering H<sub>2</sub>O<sub>2</sub>-induce ECV304 cell damage. *G. mangostana* extract significantly increased the free scavenging activities (OH<sup>•</sup>, O<sup>2•-</sup>, and NO radicals), decreased LPO in cell-free systems, improved cell viability, and reduced intracellular ROS production [12]. In addition, 5' benzophenones xanthone isolated from *Hypericum annulatum* suppressed the epirubicin-induced K-562 cell cytotoxicity [13].

Therefore, this study was to evaluate the cytoprotective and anti-genotoxic effects of xanthone derivatives from *G. mangostana* against H<sub>2</sub>O<sub>2</sub>-induce PBMCs and blood leukocytes damage of the normal and T2D volunteers.

## Materials and methods

### Chemicals and reagents

Xanthone isolated from *Garcinia mangostana* was purchased from Asia & Pacific Quality Trade Co., Ltd. Bangkok, Thailand. ABTS (2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulphonic acid) and Folin-Ciocalteu's phenol (FCR) reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and Millipore Corporation (Billerica, MA, USA), respectively.

### Determination of total phenolic content (TPC) and total antioxidant capacity (TAC) of xanthone

The total phenolic content of the xanthone extract was determined using the Folin-Ciocalteu's method [14] and gallic acid was used as a standard. The phenolic content of xanthone was expressed as "gallic acid equivalents (mg of GAEs/g extract)". In addition, ABTS scavenging activity was determined using cation decolorization method [15], whereas Trolox was used as a standard. The total antioxidant capacity of xanthone was expressed as "Trolox equivalent antioxidant capacity ( $\mu$ M TEAC/g extract)".

### Blood samples collected from the normal and T2D subjects

Blood samples were collected from 3 normal and 3 T2D subjects followed by venipuncture using heparinized tubes at Thasala Hospital (Nakhon Si Thammarat, Thailand), after approval by the committee of human ethic, Walailak University (no. 006/2015). T2D status was determined in accordance to WHO (1985) criteria, whereas a fasting blood glucose (FBG) of 126 mg/dL and/or 2 h (or random), and FBG 200 mg/dL with or without 75 g oral glucose tolerance test (OGTT) based on the presence/absence of signs and symptoms. All subjects were healthy, non-smokers, without a history of alcohol or drug abuse, or other recent medical history. A tube of 20 mL venous blood was collected from each subject and kept in heparinized tubes for subsequent PBMC isolation.

For the anti-genotoxicity assessment (Comet assay), 3 mL venous blood sample was collected and instantly kept on ice to avoid heat-induce cell damage. Briefly, the blood samples were pre-treated with

xanthone (50 - 1000  $\mu\text{g}/\text{mL}$ ) with/without 40 mM  $\text{H}_2\text{O}_2$ , while PBS and  $\text{H}_2\text{O}_2$  (40 mM) separately served as the negative and positive controls, respectively.

#### **Isolation of PBMC cell**

PBMCs were separated from 20 mL heparinized venous blood by density gradient centrifugation method using ficoll (Sigma-Aldrich, St. Louis, MO, USA). Firstly, blood samples were diluted with cold PBS (pH 7.4, 0.1 M, 0.9 % NaCl) at a ratio of 1:1. In another centrifuge tube, diluted blood was layered on to ficoll at a ratio of 5:1 and centrifuged at  $2125\times g$  for 20 min using a swing centrifuge (Sorvall Legend XTR Centrifuge, Thermo Fisher Scientific, Waltham, MA, USA). The buffy coat of PBMC was collected in another tube followed by washing with PBS twice using centrifugation at  $1518\times g$  for 10 min. The total number of cells were calculated using the trypan blue dye exclusion method [16].

#### **Cytotoxicity screening of xanthone and $\text{H}_2\text{O}_2$ on PBMC cell**

PBMCs were incubated for 3 h with various concentrations of xanthone and  $\text{H}_2\text{O}_2$  in a 96 well tissue culture plate at a density of  $1\times 10^6/100 \mu\text{L}/\text{well}$ . The cell viability was subsequently calculated using the trypan blue dye exclusion method. The  $\text{IC}_{50}$  concentrations of xanthone (50 - 1600  $\mu\text{g}/\text{mL}$ ) and  $\text{H}_2\text{O}_2$  (5 - 20 mM) were assessed from linear curve of scatter plot [17]. The equation of  $\text{IC}_{50}$  value are given below:

$$Y = mx + C \quad (1)$$

where, Y = % of inhibition (50%), x = unknown concentration, C = Constant, and m= Coefficient.

#### **Assessment of free radical scavenging activity of xanthone on PBMC from normal and T2D volunteers**

For the assessment of free radical scavenging activity of xanthone, 100  $\mu\text{L}$  PBMC (density  $1\times 10^6$  cells) of the normal and T2D patients were added in to wells of a 96 well plate. Then, 100  $\mu\text{L}$  of different concentration of xanthone or gallic acid 100 mg/mL were added in the wells. Cells were subjected to exposure to 20  $\mu\text{L}$   $\text{H}_2\text{O}_2$  (20 mM) exposure for 3 h. After incubation, the free radical scavenging activity was estimated by a cell viability test using the trypan blue exclusion technique [17].

#### **Determination of malondialdehyde (MDA) and lactate dehydrogenase (LDH) in culture medium**

PBMCs ( $1\times 10^6$  cells) from the normal and T2D volunteers were added in to well of a 96 well plate and treated with xanthone or gallic acid prior exposure to  $\text{H}_2\text{O}_2$  (20 mM) for 3 h. The medium was collected and was centrifuged at  $250\times g$  for 10 min. The supernatant was carefully removed and transferred to another microplate for assay of MDA levels and LDH activity.

To determine MDA levels, 150  $\mu\text{L}$  of cell supernatants were added with 25  $\mu\text{L}$  of 0.2 % butylated hydroxytoluene (BHT) and 600  $\mu\text{L}$  0.1 % trichloroacetic acid (TCA) in an Eppendorf tube. This mixture was mixed and centrifuged at 4  $^\circ\text{C}$ ,  $4000\times g$  for 15 min. Then, 300  $\mu\text{L}$  of each the supernatant was added with 600  $\mu\text{L}$  0.5 % thiobarbituric acid (TBA) in 20 % TCA and samples were incubated at 80  $^\circ\text{C}$  for 30 min in water-bath. Subsequently, the samples were immediately cooled and centrifuged at  $13,500\times g$  for 5 min to separate to TBA precipitate. The absorbance of the supernatants was measured at 532 nm and 600 nm using a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA) [18]. A MDA standard was used as a calibrator, and the data presented in  $\mu\text{M}/\text{L}$ .

To determine the LDH activity, 100  $\mu\text{L}$  supernatants were transferred to well of a 96 well plate. 100  $\mu\text{L}$  of the LDH reagent kit (purchased from Sigma-Aldrich, St. Louis, MO, USA) was added and samples were incubated for 5 min at 37  $^\circ\text{C}$  taking measurements at 450 nm every 5 min. Measure the absorbance at 450 nm at initial time (A450) initial and the final measurement (A450) absorbance were recorded for calculating LDH activity. Calculated the change of measurements of the samples from (A450) initial to (A450) final for the samples compared with NADH standard curve [19].

$$A450 = (A450)_{\text{final}} - (A450)_{\text{initial}} \quad (2)$$

#### Determination of DNA damage (Comet assay)

Comet assay known as single gel electrophoresis method was performed under alkaline condition [20]. All the chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) or Merck & Co. (Germany) unless otherwise mentioned. Briefly, microscopic slides were percolated with 1 % normal agarose and kept in 4 °C for overnight. Next day, 10 µL sample mixed with 140 µL 0.5 % normal agarose, then fixed in the slide and mounted with a coverslip. After solidifying the slide at 4 °C for 15 min, gently removed the coverslip and immerse in pre-cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, ~8.0 g of NaOH, 10 % DMSO and 1 % Triton X-100, pH 10.0) for 1 h at 4°C by maintaining dark condition to overcome unwanted DNA damage.

After lysing step, slides were transferred into electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH > 13.0) for 30 min for DNA unwinding and expression of alkali labile sites; then electrophoresis was conducted for 20 min at 35 V (1.0 V/cm) and 300 mA. Later, slides were neutralized using neutralization buffer (0.4 M Trizma Hydrochloride, pH 7.5) for 15 min (3×5 min) and fixed with methanol. Every step was performed in comparatively dark place to prevent additional DNA damage. Finally, slides were stained with 40 µL ethidium bromide (20 µg/mL) and observed under fluorescence microscope (ECHLIPS E600, NIKON).

The 100 cells were counted visually from randomly selected and categorized as class 0: 0; no damage, class 1: 1; small damage with small tail, class 2: 2; moderate damage with moderate tail and class 3: 3; highly damage with a large tail. The % of comet formation was calculated from classes (0 - 3).

$$\% \text{ of Comet formation} = (0 \times n_1) + (1 \times n_2) + (2 \times n_2) + (3 \times n_3) \quad (3)$$

where, n= Number of cell.

#### Statistical analysis

The results were expressed as mean ± standard error mean (SEM) and analyzed by one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test using a commercially available software package (IBM SPSS for Windows, V. 17.0, New York, USA).  $P < 0.05$  was considered as statistically significant.

## Results

#### Total phenolic content and total antioxidant capacity of xanthone

Phenolic content of xanthone is  $257.13 \pm 12.85$  mg in term of gallic acid equivalent (GAE) (**Table 1**). The antioxidant capacity is  $2874.72 \pm 204.20$  µM in term of Trolox equivalent antioxidant capacity (TEAC) (**Table 2**).

**Table 1** Total phenolic content of xanthone.

Xanthone (µg/mL)	Xanthone (mg/1000 mL)	Phenolic content (mg GAE/L)	Phenolic content (mg GAE/g xanthone)
25	25	6.58	
25	25	6.59	271.11
25	25	7.17	
50	50	12.25	
50	50	13.17	254.44
50	50	12.75	
100	100	23.42	
100	100	25.58	245.83
100	100	24.75	
<b>Total phenolic content of xanthone (mean ± SEM)</b>			<b>257.13 ± 12.85</b>

Values are expressed as mean ± SEM of three independent experiments (N = 3). GAE; gallic acid equivalent.

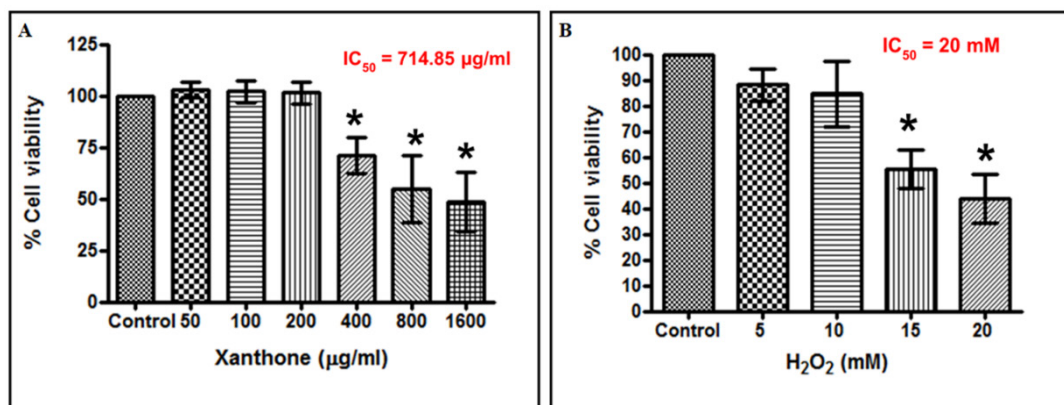
**Table 2** Total antioxidant capacity of xanthone.

Xanthone (µg/mL)	Xanthone (mg/1000 mL)	Antioxidant activity (µM)	Antioxidant activity (µM TEAC/g xanthone)
25	25	154.15	
25	25	149.34	3092.15
25	25	160.33	
50	50	300.53	
50	50	309.47	3064.89
50	50	309.47	
100	100	486.09	
100	100	486.09	2467.1
100	100	508.08	
<b>Total antioxidant capacity of xanthone (mean ± SEM)</b>			<b>2874.72 ± 204.20</b>

Values are expressed as mean ± SEM of 3 independent experiments (N = 3). GAE; gallic acid equivalent.

**Cytotoxicity screening of xanthone and H<sub>2</sub>O<sub>2</sub>**

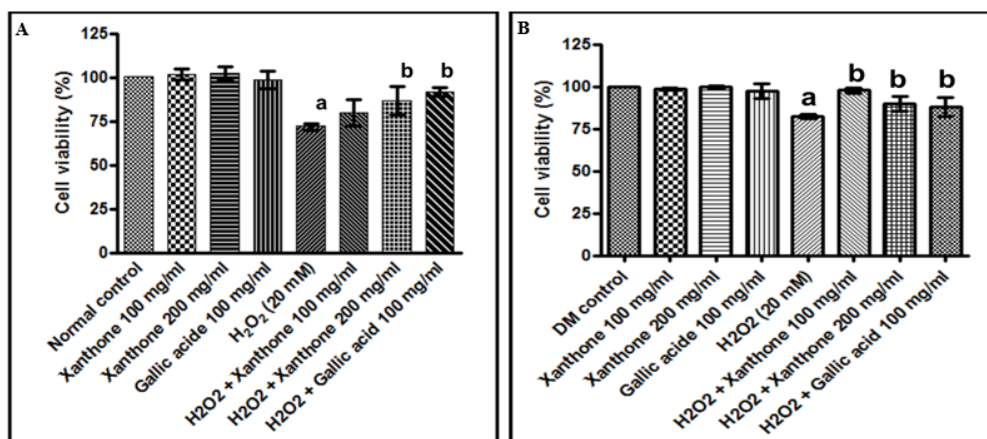
Cytotoxicity testing of xanthone concentrations from 50 - 1600 µg/mL on PBMC was determined by the trypan blue exclusion method. The amount of viable cell was significantly (*P* < 0.05) decreased after exposure of xanthone 400 µg/mL compared to control. Xanthone showed 50 % inhibition concentration (IC<sub>50</sub>) of cell viability at the dose 714.85 µg/mL (**Figure 1A**). H<sub>2</sub>O<sub>2</sub> exerts dose-dependent cytotoxic effects on PBMC (**Figure 1B**). An exposure of 20 mM H<sub>2</sub>O<sub>2</sub> to untreated PBMC reduced cell viability 50 % (**Figure 1B**). Hence, 20 mM H<sub>2</sub>O<sub>2</sub> was used to evaluate the protective effects of xanthone.



**Figure 1** Cytotoxicity testing of xanthone (50 - 1600 µg/mL) (A) and H<sub>2</sub>O<sub>2</sub> (5 - 20 mM) (B) in PBMC of the normal volunteers.

**Free radical scavenging effect of xanthone against H<sub>2</sub>O<sub>2</sub> induced PBMC cell damage of the normal and T2D volunteers**

The free radical scavenging activity of xanthone was assessed by measuring % of cell viability of PBMCs, from normal volunteers (**Figure 2A**) and T2D patients (**Figure 2B**). Exposure to 20 mM H<sub>2</sub>O<sub>2</sub> for 3 h to untreated PBMC, significantly ( $P < 0.05$ ) reduced % of cell viability compared to control. Xanthone 100, 200 mg/mL and gallic acid 100 mg/mL were used to treat PBMC induced with H<sub>2</sub>O<sub>2</sub> and showed significantly ( $P < 0.05$ ) increased % of cell viability compared to 20 mM H<sub>2</sub>O<sub>2</sub> treated only (**Figures 2A and 2B**). The present results indicated that xanthone and gallic acid would be able to scavenge H<sub>2</sub>O<sub>2</sub> generated free radical and protected normal/T2D volunteers PBMC. Exposure of xanthone 100, 200 mg/mL and gallic acid 100 mg/mL to untreated PBMC exhibited no effect on % of cell viability (**Figures 2A and 2B**).



**Figure 2** Free radical scavenging activity of xanthone on 20 mM H<sub>2</sub>O<sub>2</sub> induced PBMC, isolated from normal volunteers (A) and T2D patients (B). Gallic acid (standard) was used to protect 20 mM H<sub>2</sub>O<sub>2</sub> induced PBMC in both normal and T2D patients. Data were expressed as mean ± SEM of three independent experiments (N = 3). <sup>a</sup> $P < 0.05$  compared with control, <sup>b</sup> $P < 0.05$  compared to H<sub>2</sub>O<sub>2</sub> group.

**MDA and LDH released from PBMC in culture medium**

MDA is a lipid peroxidation marker that indicates the over production of free radicals and LDH release from a cell after cellular damage. Exposure to H<sub>2</sub>O<sub>2</sub> (20 mM) induced cellular oxidative damage in PBMC which indicated MDA and LDH activity increased significantly ( $P < 0.05$ ) compared to the control group. However, pre-treatment of PBMCs with xanthone and gallic acid ameliorated H<sub>2</sub>O<sub>2</sub> (20 mM) induced cellular damage consequently significantly reduced MDA level and LDH activity ( $P < 0.05$ ) (**Table 3**). Xanthone 100, 200 mg/mL or gallic acid 100 mg/mL themselves no effect on MDA and LDH activity released from PBMC of the normal/T2D volunteers (**Table 3**).

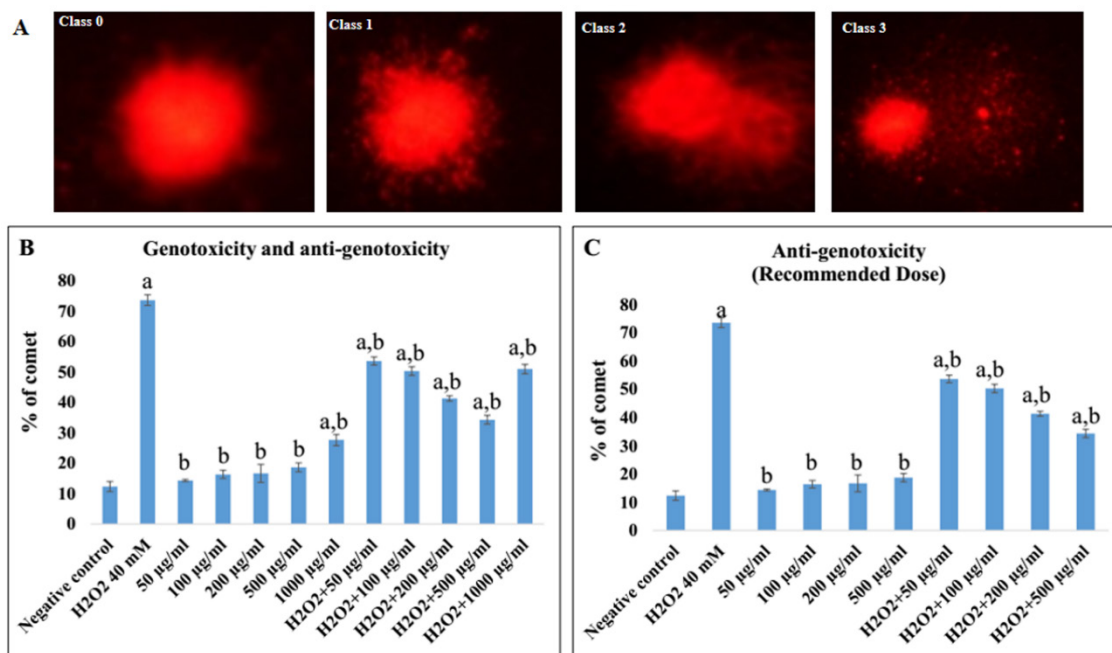
**Table 3** Amount of malondialdehyde (MDA) and lactate dehydrogenase (LDH) activity released from PBMCs.

Treatment (N = 3)	MDA Normal ( $\mu\text{M/L}$ )	MDA T2D ( $\mu\text{M/L}$ )	LDH Normal (U/L)	LDH T2D (U/L)
Control	10.46 $\pm$ 2.32	18.83 $\pm$ 4.17 <sup>c</sup>	8.33 $\pm$ 7.34	50.67 $\pm$ 16.69
Xanthone 100mg/mL	12.87 $\pm$ 2.41	23.17 $\pm$ 4.34	14.67 $\pm$ 2.34	52.00 $\pm$ 14.31
Xanthone 200mg/mL	12.50 $\pm$ 3.89	22.50 $\pm$ 7.01	12.67 $\pm$ 2.67	46.00 $\pm$ 2.00
Gallic acid 100mg/mL	18.43 $\pm$ 8.81	23.17 $\pm$ 5.84	8.67 $\pm$ 8.68	59.33 $\pm$ 10.35
H <sub>2</sub> O <sub>2</sub> (20 mM)	20.37 $\pm$ 8.25 <sup>a</sup>	50.00 $\pm$ 1.50 <sup>a,c</sup>	43.67 $\pm$ 3.34 <sup>a</sup>	105.67 $\pm$ 22.48 <sup>a,c</sup>
H <sub>2</sub> O <sub>2</sub> +Xanthone 100mg/mL	15.19 $\pm$ 6.21	27.33 $\pm$ 11.18 <sup>b</sup>	16.33 $\pm$ 6.35 <sup>b</sup>	59.67 $\pm$ 5.67 <sup>b,c</sup>
H <sub>2</sub> O <sub>2</sub> +Xanthone 200mg/mL	14.72 $\pm$ 1.95 <sup>b</sup>	26.50 $\pm$ 3.50 <sup>b,c</sup>	11.00 $\pm$ 10.01 <sup>b</sup>	47.00 $\pm$ 18.02 <sup>b,c</sup>
H <sub>2</sub> O <sub>2</sub> +Gallic acid 100mg/mL	17.22 $\pm$ 7.51	31.00 $\pm$ 13.52 <sup>b</sup>	9.00 $\pm$ 9.01 <sup>b</sup>	48.67 $\pm$ 13.68 <sup>b,c</sup>

Data are expressed as mean  $\pm$  SEM of 3 independent experiments (N = 3). The data were analyzed by one-way analysis of variance (ANOVA) followed by Turkey's post-hoc test. <sup>a</sup> $P < 0.05$  compared with control; <sup>b</sup> $P < 0.05$  compared with H<sub>2</sub>O<sub>2</sub> (20 mM) treated group; <sup>c</sup> $P < 0.05$  compared between T2D with normal group.

**Genotoxicity and anti-genotoxicity of xanthone in human peripheral blood leukocytes**

Human peripheral blood leukocytes were exposed to xanthone at 5 different concentrations (50 - 1000  $\mu\text{g/mL}$ ). DNA damage was induced by exposing H<sub>2</sub>O<sub>2</sub> (40 mM). H<sub>2</sub>O<sub>2</sub> caused DNA damage by forming comet and were evaluated by comet score (**Figure 3A**). Xanthone itself exhibited no genotoxicity compared to the positive control (H<sub>2</sub>O<sub>2</sub> 40 mM). On the other hand, xanthone exerts anti-genotoxicity by reducing H<sub>2</sub>O<sub>2</sub> induced DNA damage dose dependently ( $P < 0.05$ ) compared to positive control group (H<sub>2</sub>O<sub>2</sub> 40 mM) except 1000  $\mu\text{g/mL}$ . Surprisingly, xanthone at the dose 1000  $\mu\text{g/mL}$  demonstrated the toxic effect, genotoxicity, to PBMC (**Figure 3B**). Xanthone concentrations (50 - 500  $\mu\text{g/mL}$ ) displays promising anti-genotoxicity without producing any toxicity itself and showed the safe dose for human peripheral blood leukocytes (**Figure 3C**).



**Figure 3** H<sub>2</sub>O<sub>2</sub> induced DNA-damage evaluation by comet formation in human peripheral leukocytes. Class 0 = no damage; Class 1 = mild damage; Class 2 = moderate damage and Class 3 = highly damage, respectively; microscope 400× magnification (A). Genotoxic and anti-genotoxic effects of xanthone (50 - 1000 µg/mL) (B); Anti-genotoxic effects of xanthone (50 - 500 µg/mL) (C). Data were expressed as mean ± SEM of 3 independent experiments (N = 3). <sup>a</sup>P < 0.05 compared to negative control, <sup>b</sup>P < 0.05 compared to H<sub>2</sub>O<sub>2</sub> treatment.

## Discussion

Plants are the prime source of bioactive phytochemicals, partly protect the cellular systems from oxidative damage [21]. Our approach investigated the cytoprotective and anti-genotoxicity activities of xanthone derivatives from *G. mangostana* against H<sub>2</sub>O<sub>2</sub>-induced PBMC and blood leukocytes damage of the normal/T2D volunteers. Previous studies reported that *G. mangostana* and its xanthone derivatives possesses potent antioxidant and free radical scavenging activities [22-23]. We found that phenolic content of xanthone was 257.13 ± 12.85 mg GAE/g dry weight (Table 1) and ABTS antioxidant capacity was 2874.72 ± 204.20 µM TEAC/g dry weight (Table 2).

We also observed that H<sub>2</sub>O<sub>2</sub> can cause cellular oxidative damage and the IC<sub>50</sub> value was 20 mM (Figure 1B) evaluated by cell viability study. The toxicity of H<sub>2</sub>O<sub>2</sub> on PBMC damage and death were ameliorated by pre-treatment of xanthone. On the contrary, xanthone itself did not produce cytotoxicity on PBMC up to 200 µg/mL concentration and the IC<sub>50</sub> value was less than 800 µg/mL (Figure 1A). This may be due to the presence of high phenolic content and free radical scavenging activity of xanthone. The previous study reported that xanthone reduced the H<sub>2</sub>O<sub>2</sub> induced PBMC damage assessed by MTT assay [24]. In our study, xanthone concentrations 100 and 200 µg/mL were used to estimate the scavenging activity against H<sub>2</sub>O<sub>2</sub> (20 mM) induced PBMC toxicity. Xanthone concentrations 100, 200 µg/mL and gallic acid 100 µg/mL treatments protected from H<sub>2</sub>O<sub>2</sub> (20 mM) induced PBMC damage and significantly (P<0.05) increased the cell viability compared to H<sub>2</sub>O<sub>2</sub> (20 mM) exposure group (Figure 2). Sattayasai and coworkers revealed that crude extract of *G. mangostana* lowered the SK-N-SH cell damage, which was induced by H<sub>2</sub>O<sub>2</sub> and polychlorinated biphenyls (PCBs) [25]. *Raphanus sativus* (a phenolic extract) modulated the cell toxicity (MTT assay) and DNA damage (Comet assay) of H<sub>2</sub>O<sub>2</sub> induced human



lymphocytes by improving antioxidant and free radical scavenging activities [26]. *Gentiana dinarica* and mangiferin xanthone from *Mangifera indica* exhibited the radio-protective effect against H<sub>2</sub>O<sub>2</sub> induced human lymphocytes, estimated by lowered MDA level in culture medium compared to H<sub>2</sub>O<sub>2</sub> treated group [27,28]. Here, xanthone treatment groups significantly ( $P < 0.05$ ) revised the high MDA levels of H<sub>2</sub>O<sub>2</sub> induced PBMC of the normal and T2D volunteers compared to H<sub>2</sub>O<sub>2</sub> (20 mM) exposure group (Table 3). Improvement of cell viability (**Figures 2A** and **2B**) indicate the less amount of LDH released from the cell in culture medium [29]. Our research showed that xanthone prevented the H<sub>2</sub>O<sub>2</sub> induced cellular oxidative damage of both PBMCs (isolated from the normal and T2D volunteers), evidence from less LDH released in culture medium ( $P < 0.05$ ) compared to H<sub>2</sub>O<sub>2</sub> (20 mM) treated group (**Table 3**). Furthermore, our research further revealed that MDA product and LDH activity were significantly ( $P < 0.05$ ) higher in T2D patients in compared with normal volunteers (**Table 3**). This result suggests that T2D patients are more susceptible to oxidative damage than normal volunteers by various exposures e.g. H<sub>2</sub>O<sub>2</sub>. Above results indicate the protective effect of xanthone against oxidative damage of the normal volunteers and T2D patients.

Comet assay, a very sensitive, rapid, economical biomarker for DNA breakage detection and the genotoxicity assessment of food additives. Human peripheral blood leukocytes was chosen as the carrier of toxic pollutants [30]. Carvalho-Silva and authors found that hydroalcoholic fraction of mangosteen concentrations up to 640 µg/mL exerted the anti-genotoxic effect against H<sub>2</sub>O<sub>2</sub> induced DNA damage, evaluated by comet assay, micronucleus counting, and salmonella/microsome test [31]. These study supports our result that aqueous extract of xanthone (concentrations up to 500 µg/mL) can significantly reduce the H<sub>2</sub>O<sub>2</sub> induced genotoxicity. The higher concentration of xanthone (1000 µg/mL) showed genotoxicity itself by acting as pro-oxidant as well as damage the cell (**Figures 3B** and **3C**) [32]. Vanillic acid, a phenolic compound available in vegetable, exhibited the genotoxicity at higher concentration on human lymphocytes [33]. Therefore, the safe and recommended dose of xanthone is up to 500 µg/mL (**Figure 3C**).

## Conclusions

In conclusion, xanthone exerted cytoprotective and anti-genotoxic effect against H<sub>2</sub>O<sub>2</sub> induced PBMC and blood leukocytes of the normal/T2D volunteers by suppressing MDA level, LDH release, and comet positive cell formation. Xanthone could be beneficial dietary supplements for prevention and management of oxidative stress induced diseases. Further researches and clinical trials are warranted to evaluate the in-depth molecular mechanism, to fix the suitable dose for normal subject and patient, and to ensure the clinical safety.

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## References

- [1] CD Mathers and D Loncar. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* 2006; **3**, e442.
- [2] American Diabetes Association (ADA). Diagnosis and classification of diabetes mellitus. *Diabet. Care* 2010; **33**, 62-9.
- [3] J Bhutani AND S Bhutani. Worldwide burden of diabetes. *Indian J. Endocrinol. Metab.* 2014; **18**, 868-70.
- [4] S Wild, G Roglic, A Green, R Sicree and H King. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabet. Care* 2004; **27**, 1047-53.

- [5] U Asmat, K Abad and K Ismail. Diabetes mellitus and oxidative stress: A concise review. *Saudi Pharm. J.* 2016; **24**, 547-53.
- [6] AC Maritim, RA Sanders, JB Watkins. Diabetes, oxidative stress, and antioxidants: A review. *J. Biochem. Mol. Toxicol.* 2003; **17**, 24-38.
- [7] OR Ayepola, NL Brooks and OO Oguntibeju. *Oxidative Stress and Diabetic Complications: The Role of Antioxidant Vitamins and Flavonoids*. In: O Oguntibeju (Ed.). Antioxidant-Antidiabetic Agents and Human Health. Rijeka, InTech, 2014.
- [8] F Ke, PK Yadav and LZ Ju. Herbal medicine in the treatment of ulcerative colitis. *Saudi. J. Gastroenterol.* 2012; **18**, 3-10.
- [9] N Karim, N Jeenduang and J Tangpong. Anti-Glycemic and anti-hepatotoxic effects of mangosteen vinegar rind from *Garcinia mangostana* against HFD/STZ-Induced type II diabetes in mice. *Polish J. Food Nutr. Sci.* 2018; **68**, 163-9.
- [10] N Karim, N Jeenduang and J Tangpong. Renoprotective effects of xanthone derivatives from *Garcinia mangostana* against high fat diet and streptozotocin-induced type II diabetes in mice. *Walailak J. Sci. & Tech.* 2018; **15**, 107-16.
- [11] YW Chin and AD Kinghorn. Structural characterization, biological effects, and synthetic studies on xanthones from mangosteen (*Garcinia mangostana*), a popular botanical dietary supplement. *Mini Rev. Org. Chem.* 2008; **5**, 355-64.
- [12] K Nuttavut, H Youn-Hee and M Primchanien. Antioxidant and cytoprotective activities of methanolic extract from *Garcinia mangostana* Hulls. *Sci. Asia* 2007; **33**, 283-92.
- [13] G Momekov, PT Nedialkov, GM Kitanov, DZ Zheleva-Dimitrova, T Tzanova, U Girreser and M Karaivanova. Cytoprotective effects of 5 benzophenones and a xanthone from *Hypericum annulatum* in models of epirubicin-induced cytotoxicity: SAR-analysis and mechanistic investigations. *Med. Chem.* 2006; **2**, 377-84.
- [14] O Kaisoon, S Siriamornpun, N Weerapreeyakul and N Meeso. Phenolic compounds and antioxidant activities of edible flowers from Thailand. *J. Funct. Foods* 2011; **3**, 88-99.
- [15] R Re, N Pellegrini, A Proteggente, A Pannala, M Yang and C Rice-Evans. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 1999; **26**, 1231-7.
- [16] L He, CK Wong, KK Cheung, HC Yau, A Fu, HL Zhao, KM Leung, AP Kong, GW Wong, PK Chan, G Xu and JC Chan. Anti-inflammatory effects of exendin-4, a glucagon-like peptide-1 analog, on human peripheral lymphocytes in patients with type 2 diabetes. *J. Diabetes Investig.* 2013; **4**, 382-92.
- [17] UR Kuppusamy, YL Chong, AA Mahmood, M Indran, N Abdullah and S Vikineswary. *Lentinula edodes* (Shiitake) mushroom extract protects against hydrogen peroxide induced cytotoxicity in peripheral blood mononuclear cells. *Indian J. Biochem. Biophys.* 2009; **46**, 161-5.
- [18] R Ceci, MR Beltran Valls, G Duranti, I Dimauro, F Quaranta, M Pittaluga, S Sabatini, P Caserotti, P Parisi, A Parisi and D Caporossi. Oxidative stress responses to a graded maximal exercise test in older adults following explosive-type resistance training. *Redox Biol.* 2014; **2**, 65-72.
- [19] G Holmgren, J Synnergren, Y Bogestal, C Ameen, K Akesson, S Holmgren, A Lindahl and P Sartipy. Identification of novel biomarkers for doxorubicin-induced toxicity in human cardiomyocytes derived from pluripotent stem cells. *Toxicology* 2015; **328**, 102-11.
- [20] MN Rana and J Tangpong. *In vitro* free radical scavenging and anti-genotoxic activities of *Thunbergia laurifolia* aqueous leaf extract. *J. Health Res.* 2017; **31**, 127-33.
- [21] RH Liu. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.* 2003; **78**, 517-20.
- [22] S Tjahjani, W Widowati, K Khiong, A Suhendra and R Tjokropranoto. Antioxidant properties of *Garcinia mangostana* L (Mangosteen) rind. *Proc. Chem.* 2014; **13**, 198-203.
- [23] MP Phyu and J Tangpong. Neuroprotective effects of xanthone derivative of *Garcinia mangostana* against lead-induced acetylcholinesterase dysfunction and cognitive impairment. *Food Chem. Toxicol.* 2014; **70**, 151-6.

- [24] L Chanudom and J Tangpong. Scavenging activities and protective effects of *Syzygium cumini* (L.) Skeels on H<sub>2</sub>O<sub>2</sub> induce oxidative stress in normal human peripheral blood mononuclear cells. *J. Health Res.* 2015; **29**, 315-22.
- [25] J Sattayasai, P Chaonapan, T Arkaravichie, R Soi-Ampornkul, S Junnu, P Charoensilp, J Samer, J Jantaravinid, P Masaratana, B Suktitipat, J Manissorn, V Thongboonkerd, N Neungton and P Moongkarndi. Protective effects of mangosteen extract on H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity in SK-N-SH cells and scopolamine-induced memory impairment in mice. *PLoS One* 2013; **8**, e85053.
- [26] SS Beevi, LN Mangamoori and LV Reddy. Protective effect of *Raphanus sativus* on H<sub>2</sub>O<sub>2</sub> induced oxidative damage in human lymphocytes. *World J. Microbiol. Biotechnol.* 2010; **26**, 1519-25.
- [27] S Petrovic, A Leskovac and G Joksic. Radioprotective properties of *Gentiana dinarica* polyphenols on human lymphocytes *in vitro*. *Curr. Sci.* 2008; **95**, 1035-41.
- [28] GC Jagetia and VA Venkatesha. Effect of mangiferin on radiation-induced micronucleus formation in cultured human peripheral blood lymphocytes. *Environ. Mol. Mutagen.* 2005; **46**, 12-21.
- [29] S Pal, PB Pal, J Das and PC Sil. Involvement of both intrinsic and extrinsic pathways in hepatoprotection of arjunolic acid against cadmium induced acute damage *in vitro*. *Toxicology* 2011; **283**, 129-39.
- [30] NP Singh, CE Ogburn, NS Wolf, G van Belle and GM Martin. DNA double-strand breaks in mouse kidney cells with age. *Biogerontology* 2001; **2**, 261-70.
- [31] R Carvalho-Silva, ACF Pereira, RPS Alves, TN Guecheva, JAP Henriques, M Brendel, C Pungartnik and F Rios-Santos. DNA protection against oxidative damage using the hydroalcoholic extract of *Garcinia mangostana* and alpha-mangostin. *Evid. Based Compl. Alternat. Med.* 2016; **2016**, 8.
- [32] C Martin-Cordero, AJ Leon-Gonzalez, JM Calderon-Montano, E Burgos-Moron, M Lopez-Lazaro. Pro-oxidant natural products as anticancer agents. *Curr. Drug Targets* 2012; **13**, 1006-28.
- [33] MG Erdem, N Cinkilic, O Vatan, D Yilmaz, D Bagdas and R Bilaloglu. Genotoxic and anti-genotoxic effects of vanillic acid against mitomycin C-induced genomic damage in human lymphocytes *in vitro*. *Asian Pac. J. Cancer Prev.* 2012; **13**, 4993-8.