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Cytoprotective and Anti-genotoxic Effects of Xanthone Derivatives from *Garcinia mangostana* Against H_2O_2 Induced PBMC Cell and Blood Leukocytes Damage of Normal and Type 2 Diabetes Volunteers†

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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_2O_2)-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_2O_2-induce 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_2O_2 (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_2O_2 (20 mM)-treated group. In H_2O_2 (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_2O_2-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 xanthones such as α-mangostin, β-mangostin, γ-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_2O_2-induce ECV304 cell damage. *G. mangostana* extract significantly increased the free scavenging activities ($OH^{\cdot}$, O^{2-}, 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_2O_2-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 decolonization method [15], whereas Trolox was used as a standard. The total antioxidant capacity of xanthone was expressed as "Trolox equivalent antioxidant capacity (µM 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 μg/mL) with/without 40 mM H_2O_2, while PBS and H_2O_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×*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×*g* for 10 min. The total number of cells were calculated using the trypan blue dye exclusion method [16].

Cytotoxicity screening of xanthone and H_2O_2 on PBMC cell

PBMCs were incubated for 3 h with various concentrations of xanthone and H_2O_2 in a 96 well tissue culture plate at a density of 1×10^6/100 μL/well. The cell viability was subsequently calculated using the trypan blue dye exclusion method. The IC_{50} concentrations of xanthone (50 - 1600 μg/mL) and H_2O_2 (5 - 20 mM) were assessed from linear curve of scatter plot [17]. The equation of IC_{50} value are given below:

$$Y = mx + C \tag{1}$$

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 μL PBMC (density 1×10^6 cells) of the normal and T2D patients were added in to wells of a 96 well plate. Then, 100 μL of different concentration of xanthone or gallic acid 100 mg/mL were added in the wells. Cells were subjected to exposure to 20 μL H_2O_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 (1×10^6 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 H_2O_2 (20 mM) for 3 h. The medium was collected and was centrifuged at 250×*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 μL of cell supernatants were added with 25 μL of 0.2 % butylated hydroxytoluene (BHT) and 600 μL 0.1 % thrichloracetic acid (TCA) in an Eppendorf tube. This mixture was mixed and centrifuged at 4 °C, 4000×*g* for 15 min. Then, 300 μL of each the supernatant was added with 600 μL 0.5 % thiobarbituric acid (TBA) in 20 % TCA and samples were incubated at 80 °C for 30 min in water-bath. Subsequently, the samples were immediately cooled and centrifuged at 13,500×*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 μM/L.

To determine the LDH activity, 100 μL supernatants were transferred to well of a 96 well plate. 100 μL of the LDH reagent kit (purchased from Sigma-Aldrich, St. Louis, MO, USA) was added and samples were incubated for 5 min at 37 °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].

$$A450 = (A450)\ final - (A450)\ initial \quad (2)$$

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).

$$\%\ of\ Comet\ formation = (0 \times n1) + (1 \times n2) + (2 \times n2) + (3 \times n3) \quad (3)$$

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 ± 12.85 mg in term of gallic acid equivalent (GAE) (**Table 1**). The antioxidant capacity is 2874.72 ± 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	
Total phenolic content of xanthone (mean ± SEM)			**257.13 ± 12.85**

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	
Total antioxidant capacity of xanthone (mean ± SEM)			**2874.72 ± 204.20**

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

Cytotoxicity screening of xanthone and H_2O_2

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_{50}) of cell viability at the dose 714.85 µg/mL (**Figure 1A**). H_2O_2 exerts dose-dependent cytotoxic effects on PBMC (**Figure 1B**). An exposure of 20 mM H_2O_2 to untreated PBMC reduced cell viability 50 % (**Figure 1B**). Hence, 20 mM H_2O_2 was used to evaluate the protective effects of xanthone.

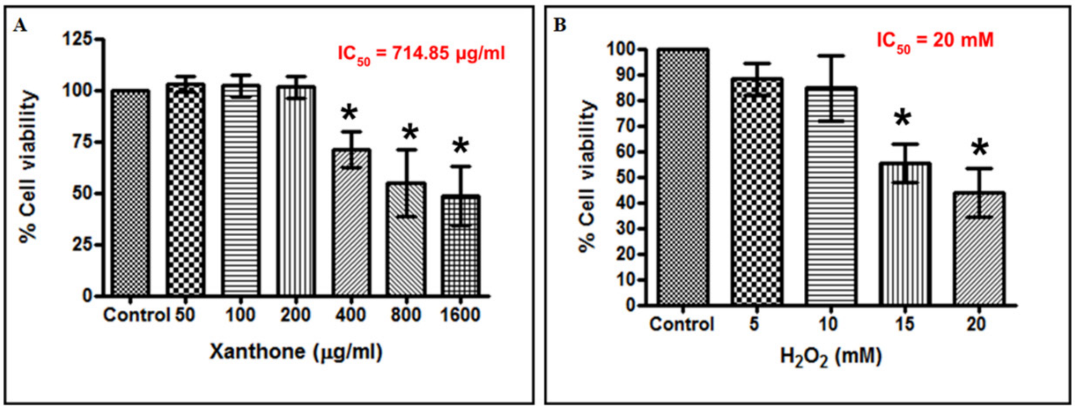

Figure 1 Cytotoxicity testing of xanthone (50 - 1600 μg/mL) (A) and H_2O_2 (5 - 20 mM) (B) in PBMC of the normal volunteers.

Free radical scavenging effect of xanthone against H_2O_2 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_2O_2 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_2O_2 and showed significantly ($P < 0.05$) increased % of cell viability compared to 20 mM H_2O_2 treated only (**Figures 2A** and **2B**). The present results indicated that xanthone and gallic acid would be able to scavenge H_2O_2 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**).

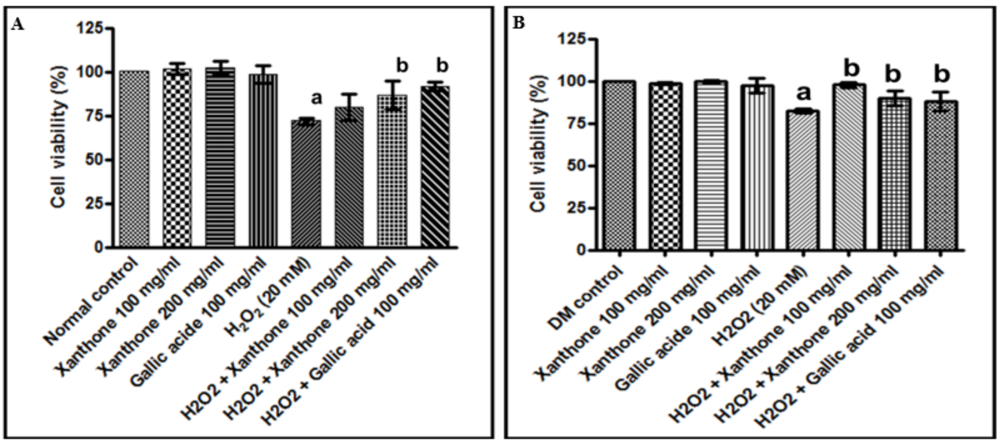

Figure 2 Free radical scavenging activity of xanthone on 20 mM H_2O_2 induced PBMC, isolated from normal volunteers (A) and T2D patients (B). Gallic acid (standard) was used to protect 20 mM H_2O_2 induced PBMC in both normal and T2D patients. Data were expressed as mean ± SEM of three independent experiments (N = 3). $^aP < 0.05$ compared with control, $^bP < 0.05$ compared to H_2O_2 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_2O_2 (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_2O_2 (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 (µM/L)	MDA T2D (µML)	LDH Normal (U/L)	LDH T2D (U/L)
Control	10.46±2.32	18.83±4.17^{c}	8.33±7.34	50.67±16.69
Xanthone 100mg/mL	12.87±2.41	23.17±4.34	14.67±2.34	52.00±14.31
Xanthone 200mg/mL	12.50±3.89	22.50±7.01	12.67±2.67	46.00±2.00
Gallic acid 100mg/mL	18.43±8.81	23.17±5.84	8.67±8.68	59.33±10.35
H_2O_2 (20 mM)	20.37±8.25^{a}	50.00±1.50^{a,c}	43.67±3.34^{a}	105.67±22.48^{a,c}
H_2O_2+Xanthone 100mg/mL	15.19±6.21	27.33±11.18^{b}	16.33±6.35^{b}	59.67±5.67^{b,c}
H_2O_2+Xanthone 200mg/mL	14.72±1.95^{b}	26.50±3.50^{b,c}	11.00±10.01^{b}	47.00±18.02^{b,c}
H_2O_2+Gallic acid 100mg/mL	17.22±7.51	31.00±13.52^{b}	9.00±9.01^{b}	48.67±13.68^{b,c}

Data are expressed as mean ± 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. ^{a}P < 0.05 compared with control; ^{b}P < 0.05 compared with H_2O_2 (20 mM) treated group; ^{c}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 µg/mL). DNA damage was induced by exposing H_2O_2 (40 mM). H_2O_2 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_2O_2 40 mM). On the other hand, xanthone exerts anti-genotoxicity by reducing H_2O_2 induced DNA damage dose dependently (P < 0.05) compared to positive control group (H_2O_2 40 mM) except 1000 µg/mL. Surprisingly, xanthone at the dose 1000 µg/mL demonstrated the toxic effect, genotoxicity, to PBMC (**Figure 3B**). Xanthone concentrations (50 - 500 µg/mL) displays promising anti-genotoxicity without producing any toxicity itself and showed the safe dose for human peripheral blood leukocytes (**Figure 3C**).

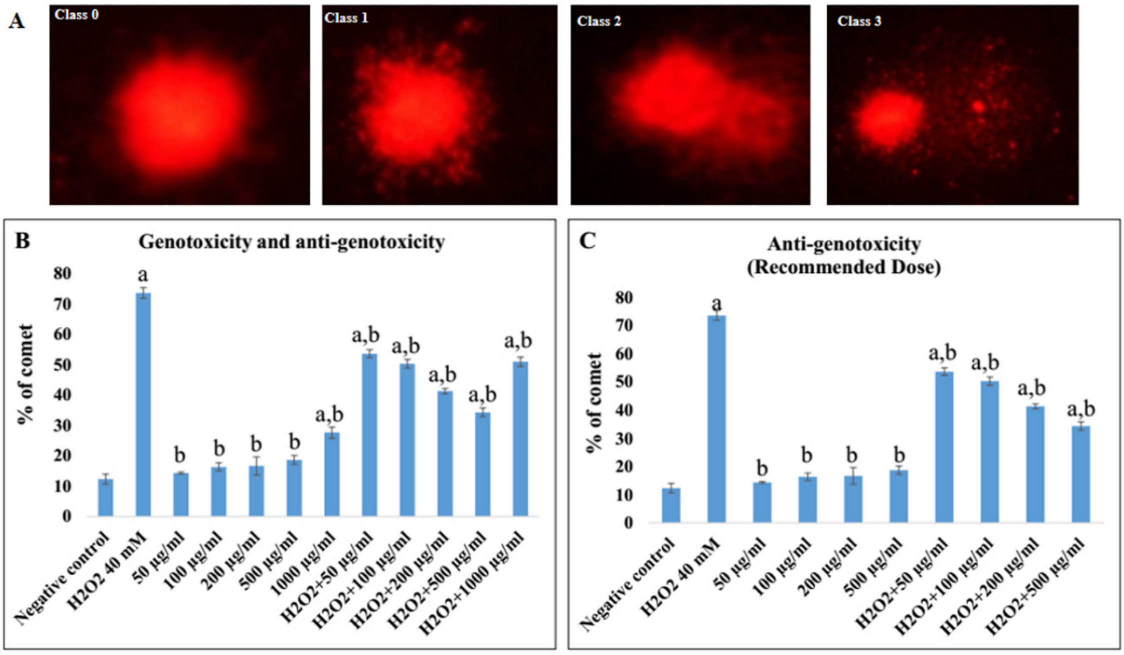

Figure 3 H_2O_2 induced DNA-damage evaluation by comet formation in human peripheral leukocytes. Class 0 = no damage; Class 1 = mild damage; Class 2 = moderate damage and Class 3 = highly damage, respectively; microscope 400× magnification (A). Genotoxic and anti-genotoxic effects of xanthone (50 - 1000 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). $^aP < 0.05$ compared to negative control, $^bP < 0.05$ compared to H_2O_2 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_2O_2-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_2O_2 can cause cellular oxidative damage and the IC_{50} value was 20 mM (**Figure 1B**) evaluated by cell viability study. The toxicity of H_2O_2 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_{50} 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_2O_2 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_2O_2 (20 mM) induced PBMC toxicity. Xanthone concentrations 100, 200 μg/mL and gallic acid 100 μg/mL treatments protected from H_2O_2 (20 mM) induced PBMC damage and significantly (P<0.05) increased the cell viability compared to H_2O_2 (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_2O_2 and polychlorinated biphenyls (PCBs) [25]. *Raphanus sativus* (a phenolic extract) modulated the cell toxicity (MTT assay) and DNA damage (Comet assay) of H_2O_2 induced human lymphocytes by improving antioxidant and free radical scavenging activities [26]. *Gentiana dinarica* and mangiferin xanthone from *Mangofera indica* exhibited the radio-protective effect against H_2O_2 induced human lymphocytes, estimated by lowered MDA level in culture medium compared to H_2O_2 treated group [27,28]. Here, xanthone treatment groups significantly ($P < 0.05$) revised the high MDA levels of H_2O_2 induced PBMC of the normal and T2D volunteers compared to H_2O_2 (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_2O_2 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_2O_2 (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_2O_2. 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_2O_2 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_2O_2 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_2O_2 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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