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### Research Articles

#### A COMPARATIVE STUDY OF ORAL OLANZAPINE AND ORAL HALOPERIDOL ON GLUCOSE TOLERANCE LEVELS IN PATIENTS WITH SCHIZOPHRENIA

Authors: **G N S Sangeetha Lakshmi**

Int J Med Res Health Sci. 2015;4(3):477-482 | [Abstract](#) | [Download \(.pdf\)](#) | DOI: 10.5958/2319-5886.2015.00092.2

#### SEROPREVALENCE OF HEPATITIS B IN A TERTIARY CARE CENTRE IN BIJAPUR, KARNATKA: A TWO YEARS PROSPECTIVE STUDY

Authors: **Preeti B. Mindolli, Manjunath P. Salmani**

Int J Med Res Health Sci. 2015;4(3):483-485 | [Abstract](#) | [Download \(.pdf\)](#) | DOI: 10.5958/2319-5886.2015.00093.4

#### HISTOPATHOLOGIC AND CYTOMORPHOLOGIC CORRELATION IN LEPROSY

Authors: **Sharayu A Sarode, Anil T Deshmukh**

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Authors: **Fathima Nusrath, Baderuzzaman, Anees Syeeda, N Parveen, Siraj M, Ishaq M**

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Authors: **Tamboli Kshitij S, Avachat Subhada S, Tamboli Suchit S**

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Authors: **Christofer Thomas, Praveen K Kodumuri, Saranya P**

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**Authors:** Srinivasagopalan, Nappinnai, Solayappan

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**Authors:** Ravindra HN, Patel Krupa D

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**Authors:** Goda Jatin B, Patel Shailesh M, Parmar Ajay M, Agarwal GC

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**Authors:** Yuri M. Iryanov, Nikolay A. Kiryanov, Olga V. Dyuriagina, Tatiana Yu. Karaseva, Evgenii A. Karasev

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**Author:** Angadi Netravathi B, Hiremath Shrishail V, Suranagi Vijayalakshmi V

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**Author:** Ashok S Komaranchath, L. Appaji, K.C. Lakshmaiah, Mangesh Kamath, Rekha V Kumar

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**Author:** Sidrah, Tandrapad Priyanka, Asiya Naaz, Lakshmi Chaitanya G, Sridevi D

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**Author:** Kostov Rostislav V

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**Author:** Lusiana Darsono, Meilinah Hidayat, Maesaroh Maesaroh, Nurul Fauziah, Wahyu Widowati

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**Author:** Mathew Ninan, Doye George, P Sukumaran, Gunanidhi Sahu, RN Mania, Philip Mathew

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**Author:** Rajarajeswari D, Ramalingam, Sharmila T, Prasad Naidu M, Naidu JN

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**Authors:** Uma Thombarapu, Prabha Devi Kodey, GangadharaRao Koneru

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**Authors:** Chauhan Sandhya, Kariwal Peeyush, Kumari Anita, Vyas Shaili

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**Authors:** Rakshitha Gowda BH, Meera KS , Mahesh E

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**Authors:** Sudhir Modala, Usha Dhar, K V Thimmaraju, B J Pradeep Kumar, Bandi Hari Krishna

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**Authors:** Henal Javeri, Tulsi Thampan, Krusha Shah, Neeta Misra, Rahul R Kunkulol

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## Research article

### EX VIVO STUDY OF GARCINIA MANGOSTANA L. (MANGOSTEEN) PEEL EXTRACT AND XANTHONES AS ANTI-ADIPOGENESIS IN HEPG2 CELL MODEL

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## ABSTRACT

**Background:** Anti-adipogenesis is one of proposed mechanism for anti-obesity. Adipogenesis regulation of obesity is important, so identification of anti-adipogenic activity is a potential strategy to find anti-obesity agent.

**Aim:** The aim of this study is to evaluate the anti-adipogenesis potential of *Garcinia mangostana* L. peel extract (GMPE) compared to xanthones in HepG2 cells line as model. **Material and Methods:** GMPE was performed based on maceration method using distilled ethanol 70% as the solvent. The level of triglyceride and cholesterol and the inhibitory activity of triglyceride (TG) and cholesterol (CHOL) in HepG2 cells were assayed and determined as the anti-adipogenesis parameter. **Results:** The most active substance to lower the triglyceride level was showed by GMPE in every concentration followed by the garcinone-C, -mangostin, garcinone-D and -mangostin respectively. The highest activity to decrease the cholesterol level was showed by GMPE and followed by -mangostin, -mangostin, garcinone-c, garcinone-d respectively. **Conclusion:** GMPE posses the anti-adipogenesis potential in inhibiting TG and CHOL synthesis was better than any other xanthone (-mangostin, -mangostin, garcinone-C and garcinone-D).

**Keywords:** Obesity, Adipogenesis, *Garcinia mangostana* L., HepG2, Triglyceride, Cholesterol.

## INTRODUCTION

Obesity is one of the most common global metabolic disorders defined as an excessive body weight in the shape of fat accumulation.<sup>[1]</sup> Recently, the metabolic syndrome including obesity represents one of the most serious problem worldwide.<sup>[2]</sup> Obesity has a strong association with the chronic disease such as diabetes, cardiovascular diseases, hypertension, osteoarthritis, some cancer and inflammation-based pathologies.<sup>[3]</sup> At the cellular level, obesity is characterized by an excess accumulation of adipose tissue is largely comprised of fat cells.<sup>[3-5]</sup> Obesity including excessive differentiation and growth of adipocytes which leads to increase fat cell mass and number, adipogenesis, lipid accumulation and lipogenic enzyme expression and surplus energy accumulation stored as triglyceride (TG) in

adipocytes.<sup>[1]</sup> Adipose tissue growth involves in formation of new adipocytes from precursor cells, further leading to an increase in adipocyte size.<sup>[6]</sup> Adipogenesis is a multi step process involving a cascade of transcription factors and cell cycle protein regulating gene expression and leading to adipocyte development.<sup>[7]</sup> The number of studies to prevent and treat obesity continues to rise.<sup>[8]</sup> Developing anti-obesity drugs that are efficacious and have minimal side effects become a pressing need.<sup>[1]</sup> Anti-adipogenesis is one of the proposed mechanisms of anti-obesity.<sup>[9,10]</sup> Adipose tissues are specialized for high capacity to accumulate TG.<sup>[11]</sup> Human hepatoma HepG2 cells is the most suitable and accessible human-derived cells that retain many of the biochemical functions of human liver parenchymal

cells for the *ex vivo* study including anti-adipogenesis screening.<sup>[12]</sup> Subjects with established obesity have an increased lipogenesis in hepatocytes (not in adipocyte) that might contribute to develop and/or retain the excessive fat mass.<sup>[13]</sup> Excess hepatic lipid accumulation is associated with nutritional factors, drugs, and multiple genetic defects in energy metabolism.<sup>[14]</sup> Many phytonutrients were investigated for their potential therapeutic properties. Some phytochemical bioactive have been shown to inhibit adipocyte differentiation and induce adipocyte apoptosis.<sup>[15,16]</sup> *Garcinia mangostana* L. (mangosteen) is a tropical fruit originated from Southeast Asia, has been used in traditional therapy in the treatment of great variety of medical conditions for decades.<sup>[17]</sup> The pericarps of this fruit have been used for many years as traditional medicine in treating sicknesses such as trauma, skin infection, abdominal pain, dysentery, and wounds.<sup>[18]</sup> The major bioactive secondary metabolites of *G.mangostana* are xanthone derivates.<sup>[19]</sup> Xanthones could be isolated from peel, whole fruit, bark, and leaves of mangosteen.<sup>[20]</sup> Xanthones were reported to have a great variety of pharmacological activities including antioxidant, antifungal, antibacteria, cytotoxic, anti-inflammation, anti-histamine, anti-HIV, and other activities.<sup>[19]</sup> The previous study confirm that mangosteen peel extract contained -mangosteen (105 ppm), -mangosteen (7.20 ppm), garcinone C (3.50 ppm), and garcinone D (9.92 ppm) based on high performance liquid chromatography (HPLC).<sup>[21]</sup> Adipogenesis regulation of obesity is important, so identification of anti-adipogenic activity is a potential strategy to find anti-obesity agent. Therefore, the aim of this study is to evaluate the anti-adipogenesis potential of *G. mangostana* peel extract (GMPE) compared to xanthones including -mangostin, -mangostin, garcinone-C, garcinone-D in HepG2 cells by using the inhibitory activity to lower triglyceride and cholesterol synthesis as the parameters.

## MATERIAL AND METHODS

The present study was carried out in Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, West Java, Indonesia in collaboration with Medical Research Center, Faculty of Medicine, Maranatha Christian University. HepG2 cells (human liver hepatocellular carcinoma cell line) were used as cells model after cells were induced to

differentiate as adipocyte. The laboratory experiment was performed 4 months.

**Plant material preparation and extraction:** *Garcinia mangostana* L. was collected from Cisalak-Subang, West Java, Indonesia. The plant was identified by herbarium staff, Department of Biology, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The peel were collected, chopped, and kept in drier tunnel service. Extraction was performed based on the maceration method with distilled ethanol 70% as solvent.<sup>[21-23]</sup>

**HepG2 cell culture and adipocyte differentiation induction:** HepG2 cells (human liver hepatocellular carcinoma cell line) was cultured in DMEM (Dulbecco's Modified Eagle Medium, Biowest) supplemented with 10% FBS (Fetal Bovine Serum, Biowest) and 100 U/mL penicillin-streptomycin (Biowest) then incubated for 24 hours at 37°C humidified atmosphere and 5% CO<sub>2</sub>.<sup>[14]</sup> After the cells were confluence, medium was discharged and cells were harvested after trypsin-EDTA treatment in 2500 rpm centrifuge for 4 minutes, cells then resuspended by 1 mL new medium and seeded in the 6 well plate (5 x 10<sup>5</sup> cells/well) with DMEM supplemented with 10% FBS and 100 U/mL penicillin-streptomycin then incubated for 48 hours until confluent. Medium then discharged and supplemented with starving medium (DMEM + 1% antibiotic solution) then incubated for 24 hours. Starving medium then discharged and supplemented with induction medium (DMEM, 1:2 of 1mM palmitic acid: 1mM linoleic acid, BSA, and GMPE or xanthone). Cells then incubated in 37°C humidified atmosphere and 5% CO<sub>2</sub>.

**Cells Lysate:** Cell lysate was performed according to Biorad #163-2068 kit protocol. Confluent cells after GMPE or xanthone treatment were harvested using 500 g centrifuge for 4 minutes. Supernatan was discharged and work solution was added into the pelet cell. The solution briefly was sonicated for 30 second four times. Cells then centrifuge in 16.000 rpm for 30 minutes in room temperature. Supernatant then transfered to 2 mL ependorf tube and placed in -80°C as the sample for the TG and CHOL levels assay.<sup>[24]</sup>

**Triglyceride (TG) Levels and Inhibitory Activity Assay:** The TG level was measured according to Randox TR 210 assay kit protocol. Briefly 450µL

reagent with 5µL sample was incubated in 37°C for 5 minutes. Double-distilled water (ddH<sub>2</sub>O) was used as blank and standard reagent was used as standard. Seven different concentration (2.180; 1.090; 0.545; 0.273; 0.136; 0.068 and 0.034 mmol/L) were prepared by serial dilution for standard solution. The absorbance was measured in 500 nm of wave length. The TG level was calculated based on the ratio of sample absorbance to standard absorbance multiplied by the standard concentration.<sup>[25]</sup>

**Cholesterol Levels and Inhibitory Activity Assay:** The CHOL level assay was measured according to the Chol Kit Randox CH 200 protocol kit. Briefly 500µL mix reagent was added into 24 well plate and 5µL sample was added into the sample well. 5µL of ddH<sub>2</sub>O was used as blank. Seven different concentration (5.170; 2.585; 1.293; 0.646; 0.323; 0.162; and 0.081 mmol/L) were prepared by serial dilution for standard solution. 5µL standard solution was added into the well as standard. The absorbance was measured in 500 nm of wave length. The reaction then incubated at 37°C for 5 minutes. The absorbance was measured in 500 nm of wave length. The cholesterol concentration was calculated based on the ratio of sample absorbance to standard absorbance multiplied by the standard concentration.<sup>[26]</sup>

**Statistical Analysis:** The every treatment was done in three replication. Statistical analysis was conducted using SPSS software (version 17.0). Significant differences between the groups were determined using the Analysis of Variance (ANOVA) and Tukey Post Hoc Test. Statistical significance was set at  $p < 0.05$ . The data were presented as mean  $\pm$  standard deviation.

## RESULTS

Table 1 showed that GMPE and xanthones including -mangostin, -mangostin, garcinone-C and garcinone-D have lower TG levels in HepG2 cells compared to the triglyceride level in the cell lysate without GMPE and xanthones. The most active substance was showed by GMPE in every concentration followed by the garcinone-C, -mangostin, garcinone-D and -mangostin respectively showed by the TG inhibitory activity. High plasma TG is associated with obesity.<sup>[27]</sup>

**Table 1: Triglyceride (TG) Level and Inhibition Activity of GMPE and Xanthones in Various Concentration**

Treatment	Triglyceride (TG)	
	TG level (mg/dL)	TG inhibition %
-mangostin 250 µg/mL	24.50 $\pm$ 0.26 f	72.54 $\pm$ 0.29 k
-mangostin 125 µg/mL	25.68 $\pm$ 0.01 f	71.22 $\pm$ 0.01 k
-mangostin 62.5 µg/mL	26.97 $\pm$ 0.27 f	69.76 $\pm$ 0.30 k
-mangostin 31.25 µg/mL	46.68 $\pm$ 0.74 i	47.68 $\pm$ 0.83 h
-mangostin 15.625 µg/mL	57.60 $\pm$ 0.45 lm	35.42 $\pm$ 0.50 de
-mangostin 250 µg/mL	35.71 $\pm$ 0.11 h	59.97 $\pm$ 0.12 i
-mangostin 125 µg/mL	50.70 $\pm$ 0.83 jk	43.16 $\pm$ 0.93 fg
-mangostin 62.5 µg/mL	54.32 $\pm$ 0.37 kl	39.10 $\pm$ 0.40 ef
-mangostin 31.25 µg/mL	58.22 $\pm$ 0.45 lm	34.72 $\pm$ 0.51 cd
-mangostin 15.625 µg/mL	61.14 $\pm$ 0.02 m	31.46 $\pm$ 0.03 c
Garcinone-C 250 µg/mL	12.36 $\pm$ 0.16 e	86.15 $\pm$ 0.18 l
Garcinone-C 125 µg/mL	12.64 $\pm$ 0.02 e	85.84 $\pm$ 0.02 l
Garcinone-C 62.5 µg/mL	32.68 $\pm$ 0.11 gh	63.37 $\pm$ 0.11 ij
Garcinone-C 31.25 µg/mL	33.14 $\pm$ 1.11 gh	62.85 $\pm$ 1.24 ij
Garcinone-C 15.625 µg/mL	31.75 $\pm$ 3.78 g	64.41 $\pm$ 4.24 j
Garcinone-D 250 µg/mL	34.19 $\pm$ 0.06 gh	61.67 $\pm$ 0.07 ij
Garcinone-D 125 µg/mL	50.02 $\pm$ 0.66 ij	43.93 $\pm$ 0.74 gh
Garcinone-D 62.5 µg/mL	55.50 $\pm$ 0.54 l	37.78 $\pm$ 0.60 de
Garcinone-D 31.25 µg/mL	60.48 $\pm$ 0.16 m	32.19 $\pm$ 0.17 c
Garcinone-D 15.625 µg/mL	75.44 $\pm$ 1.54 n	15.42 $\pm$ 1.73 b
GMPE 250 µg/mL	0.00 $\pm$ 0.01 a	100 $\pm$ 0.01 p
GMPE 125 µg/mL	0.00 $\pm$ 0.02 b	100 $\pm$ 0.02 o
GMPE 62.5 µg/mL	0.00 $\pm$ 0.01 c	100 $\pm$ 0.01 h
GMPE 31.25 µg/mL	0.00 $\pm$ 0.18 d	100 $\pm$ 0.20 m
GMPE 15.625 µg/mL	26.64 $\pm$ 1.70 f	70.14 $\pm$ 1.91 k
Positive control	89.20 $\pm$ 0.69 o	0.00 $\pm$ 0.00 a

\*The data are presented as mean  $\pm$  standard deviation. Different letters in the same column (a-o and its combination) indicate significant differences among the means of groups (GMPE and xanthones in various concentrations) based on Tukey's pos hoc comparison ( $P < 0.05$ ).

The highest CHOL inhibition activity was showed by GMPE followed by -mangostin, -mangostin, garcinone-C and garcinone-D respectively. GMPE showed CHOL inhibition activity over 50% in the concentration above 15.625 µg/mL (Table 2). Moreover, GMPE showed 100% CHOL inhibition activity. Obesity has an association with decreasing high-density lipoprotein cholesterol (HDL-C) level.<sup>[28]</sup>



**Table 2: Cholesterol (CHOL) Level and Inhibition Activity of GMPE and Xanthones in Various Concentrations**

Samples	Cholesterol (CHOL)	
	CHOL level (mg/dL)	CHOL inhibition (%)
-mangostin 250 µg/mL	8.60 ± 0.50 ab	93.71 ± 0.37 kl
-mangostin 125 µg/mL	10.91 ± 0.03 bc	92.02 ± 0.02 k
-mangostin 62.5 µg/mL	27.96 ± 0.04 ef	79.54±0.03 ghi
-mangostin31.25 µg/mL	42.27 ± 0.08 gh	69.07 ±0.06 ef
-mangostin15.62µg/mL	44.82 ± 0.08 gh	67.21 ± 0.06 ef
-mangostin 250 µg/mL	23.10± 0.15 cde	83.11 ± 0.11 ij
-mangostin 125 µg/mL	29.00 ± 0.10 ef	78.79±0.08 ghi
-mangostin 62.5 µg/mL	29.05 ± 0.57 ef	78.75±0.42 ghi
-mangostin31.25µg/mL	35.05 ± 0.28 efg	74.36±0.20 fgh
-mangostin15.62µg/mL	36.89 ± 0.33 fg	73.01 ± 0.25 fg
Garcinone-C 250 µg/mL	24.91 ± 1.48 def	81.78 ± 1.07 hi
Garcinone-C 125 µg/mL	25.93 ± 0.03 def	81.03 ± 0.01 hi
Garcinone-C 62.5 µg/mL	52.94 ± 0.28 h	61.27 ± 0.21e
Garcinone-C31.25µg/mL	68.86 ± 0.24 i	49.62 ± 0.18 d
Garcinone-15.625µg/mL	100.00 ± 0.04 k	25.85 ± 0.04 b
Garcinone-D 250 µg/mL	26.26 ± 1.16 ef	80.79±0.85 ghi
Garcinone-D 125 µg/mL	45.44 ± 0.11 gh	66.76 ± 0.08 ef
Garcinone-D 62.5 µg/mL	74.72 ± 0.01 i	45.33 ± 0.01 d
Garcinone-D31.25µg/mL	78.13 ± 4.15 ij	42.84± 3.04 cd
Garcinone-15.625µg/mL	87.94 ± 1.03 j	35.66 ± 0.75 c
GMPE 250 µg/mL	0.00 ± 0.34 a	100.00±0.25 m
GMPE 125 µg/mL	0.00 ± 0.21 a	100± 0.14 lm
GMPE 62.5 µg/mL	13.55± 0.13 bcd	90.09 ± 0.10 jk
GMPE 31.25 µg/mL	29.03 ± 1.41 ef	78.77±1.03 ghi
GMPE 15.625 µg/mL	107.81± 12.39 k	21.12 ± 9.07 b
Positive control	136.68 ± 8.20l	0.00 ± 0.00 a

\* The data are presented as mean ± standard deviation. Different letters in the same column (a-o and its combination) indicate significant differences among the means of groups (GMPE and xanthones in various concentrations) based on Tukey's pos hoc comparison (P<0.05).

## DISCUSSION

Obesity is a risk factor for severe disease such as diabetes, atherosclerosis, coronary heart disease and several cancer.<sup>[29]</sup> Primarily, obesity is a disorder of lipid metabolism and the enzyme involved in this process could be selectively targeted to develop anti-obesity drugs.<sup>[8]</sup> Different parts of medicinal plants like stem, flower, seed, root, fruit, etc. are used to obtain pharmacologically active metabolite.<sup>[30]</sup> Mangosteen peel has been used in medicinal in both Chinese and Ayurvedic. The yellow exudate from

mangosteen peel contain xanthone as the major class of compounds including -mangostin, -mangostin, -mangostin, garcinone-C and garcinone-D along with mangostinone, tanins, and flavonoid called epicatechin.<sup>[31]</sup> In this *ex vivo* study, we evaluated the anti-adipogenesis potential of GMPE and xanthones on HepG2 cells. Our results demonstrated that GMPE and xanthones exhibited potential to decrease the level of CHOL and TG as anti-adipogenesis parameters compared to the cell without GMPE or xanthones treatment.

In present study, GMPE could lower TG level compared to the cells without GMPE or xanthones treatment. Inhibition of TG metabolism was mediated by decreasing of gene expression of FAS (Faty Acid Synthase), ACC(Acetyl-CoA Carboxylase), and malic enzyme, among other factors.<sup>[32]</sup> The decrease of TG level may result from decreasing lipid synthesis.<sup>[33]</sup> Excess fat is stored as TG in the adipose tissue.<sup>[34]</sup> The decreased levels of TG indicated the adipogenesis inhibition. The potential for suppressing of adipogenesis and reducing lipid accumulation in cells mode were showed by some xanthones including -mangostin and -mangostin.<sup>[35]</sup> -mangostin also showed its ability to inhibit FAS correlated with intracellular lipid accumulation in differentiating adipocytes and stimulated lipolysis in mature adipocytes.<sup>[18]</sup>

Adipocyte normaly contain free CHOL and will redistributed from the plasma membran to the lipid droplet as TG storage increase. Adipocyte CHOL levels will increase in proportion to TG level.<sup>[34,35]</sup> GMPE also showed have CHOL inhibitory activity in concentration dependent maner showed by the decrease HOL level compared to the cell without GMPE or xanthones treatment as anti adipogenesis parameters. Previous study also found that *G.mangostana* posses the CHOL level reduction.<sup>[36]</sup> The GMPE showed anti-adipogenic activity through suppressing proliferator-activated receptor gamma (PPAR ) expression and FAS activity.<sup>[37]</sup> Maria *et al* (2007) study observed that diets supplemented with mangosteen positively affect plasma lipid levels and plasma antioxidant activity in rats fed cholesterol-containing diets.<sup>[38]</sup> Based on our *ex vivo* study, we recommended the GMPE have beneficial effects as anti-adipogenesis agent was comparable with xanthones through the inhibition activity on CHOL and TG synthesis in HepG2 cells model.

## CONCLUSION

The GMPE posses the anti-adipogenesis potential on decreasing CHOL and TG levels in HepG2 cell better than xathones ( -mangostin, -mangostin, garcinone-C and garcinone-D). However, *in vivo* test in an animal model still needed to confirm the anti-adipogenesis activity of the GMPE and xanthones.

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**Conflict of Interest:** Nil

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