Effect of *Garcinia mangostana* pericarp extract on glial NF-κB levels and expression of serum inflammation markers in an obese-type 2 diabetes mellitus animal model

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Received February 24, 2021; Accepted May 17, 2021

DOI: 10.3892/br.2021.1439

Abstract. Type 2 diabetes mellitus (T2DM) is an age-related disease associated with cerebral inflammation and Alzheimer's disease. *Garcinia mangostana* pericarp (GMP) possesses antihyperglycemic, antidiabetic and anti-inflammatory effects. The aim of the present study was to evaluate the effect of GMP extract on cerebral inflammation in Wistar rats with T2DM by examining the expression levels of glial nuclear factor-κB (NF-κB), interleukin (IL)-6, tumor necrosis factor-α (TNF-α) and superoxide dismutase (SOD). A total of 36 8-10-week-old male Wistar rats were randomly divided into six groups and provided a standard diet (normal control; C1), high-fat diet (HFD) with 200 g/kg GMP extract BW/day (GMP control; C2), HFD with streptozotocin-nicotinamide (diabetic control; C3), and HFD with 100 (M1), 200 (M2) or 400 g/kg body weight (BW)/day (M3) GMP extract for Wistar rats with diabetes. GMP extract was administered for 8 weeks after induction of T2DM was confirmed. Glial NF-κB activity was assessed by immunohistochemical staining, and by measuring IL-6 levels, TNF-α levels and SOD activity in the serum using ELISA. BW significantly increased following HFD treatment. After 7 weeks, the BW remained significantly higher compared with the normal control and GMP extract-treated groups, but decreased continuously in the T2DM groups. Glial NF-κB immunoreaction in the hippocampal region was significantly higher in the diabetic Wistar rats compared with the normal control Wistar rats, and 200 g/kg BW/day GMP significantly reduced its activity. The T2DM Wistar rats showed significantly higher expression levels of serum IL-6 and TNF-α and lower activity of SOD compared with the normal control Wistar rats. Meanwhile, rats in GMP groups M1, M2 and M3 exhibited significant reductions in the levels of IL-6 and TNF-α expression, and increases in SOD activity. GMP extract treatment effectively reduced hippocampal NF-κB, IL-6 and TNF-α levels and increased antioxidant SOD activity. These results suggest that GMP extract prevents cerebral inflammation in T2DM Wistar rats.

Introduction

Type 2 diabetes mellitus (T2DM) has become a major public health issue and is associated with a high incidence of cognitive impairment and dementia disorders, particularly Alzheimer's disease (AD) (1-3). Previous studies reported that the relative risk of AD in patients with T2DM is ~2x higher than that in non-diabetic patients (4,5). The burden of patients of AD with diabetes mellitus in the future may even worsen as the prevalence of T2DM continues to increase (3). Diabetes mellitus induces some toxic effects, such as hyperglycemia, vascular dementia, brain insulin resistance and neurodegeneration, which increase the risk of cognitive impairment and dementia (1,6). Previous studies reported that cognitive impairment and dementia are related to the inflammation (7).

Obesity increases the risk of diabetes mellitus (8-10). Obese individuals exhibit pathological proliferation (hyperplasia) and enlargement (hypertropia) of adipose tissue as a response to excessive nutrition, which may reduce tissue oxygenation and cause cell hypoxia (11,12). Inflammation in adipose tissue may accelerate insulin resistance and systemic inflammation response by activating several pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor nuclear

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Key words: Garcinia mangostana pericarp, mangosteen pericarp extract, type 2 diabetes mellitus, cerebral inflammation, glial nuclear factor-κB, interleukin-6, tumor necrosis factor-α, superoxide dismutase

factor-α (TNF-α), and increasing the generation of reactive oxygen species (ROS) (11,13). The high IL-6 and TNF-α levels in diabetes mellitus have been shown by several studies in animals and humans (7,14-16). The generation of the anti-oxidative stress enzyme superoxide dismutase (SOD) has been correlated with neurodegenerative diseases (17,18).

The progression of AD has been correlated with the activity of nuclear factor-κB (NF-κB) (19-22). The NF-κB signal serves a crucial role in maintaining brain homeostasis (19). It essentially participates in synaptic plasticity and in balancing brain functions that are associated with learning and memory (19). Destruction of this signaling pathway triggers several neuronal changes, such as brain inflammation, glial activation, oxidative stress generation and apoptotic cell death stimulation, which lead to neuronal degeneration, the initial stage of AD (19-22).

Potent treatments are thus required to prevent diabetes-induced suppression of neuronal function. Mangosteen (*Garcinia mangostana* Linn) of the Guttiferae family is deemed as the 'Queen of Fruits' because of its distinctive and delectable tropical taste (23,24). It is a popular fruit in tropical countries, mostly in Southeast Asia and has been used as a medicine for hundreds of years around the world (23,25). WHO has proven its safety as a traditional fruit without reported mutagenicity or teratogenicity for over 100 years, and is recommended for consumption by humans, as there are no reports of acute or chronic hepatic damage or immunological activities following consumption of fruits and commercial beverages containing pericarp (26). A previous study reported that daily intervention with 220-280 mg pericarp extract for 12 weeks, followed by a double dose for the next 12 weeks was adequately safe for oral administration in the humans (27). Mangosteen leaves, bark, whole fruit and pericarp contain secondary metabolites with potential biological effects (23,25,28,29). Xanthones are a class of polyphenolic compounds, of which α- and γ-mangosteen are the most abundant (23,27-29). This fruit reportedly exhibits various therapeutic effects, such as anti-inflammatory, anti-dyslipidemia, antioxidant, hypoglycemic and anti-obesity effects (24,30-36). The glycemic index of mangosteen fruit was considered low; however, there are ~10x more phenolic compounds and 20x more antioxidant activity in the pericarp compared with the edible part of the fruit (37,38). Thus, in the present study, the pericarp extract was used. Whether or not mangosteen pericarp extract exerts a protective effect of brain function in patients with diabetes has not been assessed previously, to the best of our knowledge.

The aim of the present study was to analyze the mechanism of cerebral inflammation by identifying the activation of glial NF-κB and the expression of serum IL-6, TNF-α and SOD under diabetic conditions. It also aimed to investigate the protective effects of *Garcinia mangostana* pericarp (GMP) extract on brain function in T2DM rats. The mechanism underlying the effects of GMP on T2DM-suppressed brain function has not been sufficiently investigated. Therefore, the effect of GMP on the brain function of obese rats with and without T2DM was assessed.

Materials and methods

Animals and treatment protocol. A total of 36 male Wistar rats, aged 2-3 months, with an average body weight of 150-200 g were used in the present study. The Wistar rats were used in the animal building with an environmental setting of 23±2˚C, 50±5% humidity, a 12-12 h light-dark cycle, and *ad libitum* access to a standard chow and tap water. Rats were randomly divided into six groups with the following interventions: Normal control (C1), administered with a standard normal diet; mangosteen control (C2), administered a high-fat diet (HFD) and GMP extract at 200 g/kg BW/day; diabetic control (C3), diabetic Wistar rats treated with HFD; mangosteen intervention 1 (M1), diabetic Wistar rats treated with HFD and 100 g/kg body weight (BW)/day GMP extract; mangosteen intervention 2 (M2), diabetic Wistar rats treated with HFD 200 g/kg BW/day GMP extract; and mangosteen intervention 3 (M3), diabetic rats treated with HFD and 400 g/kg BW/day GMP extract. GMP extract was prepared as a solution (as described below) and administered by oral gavage technique to Wistar rats. A previous study used mangosteen pericarp ethanolic extract at doses of 200, 400 and 800 mg/kg, and found that the oral intervention of 800 mg/kg decreased vasa vasorum angiogenesis through H_2O_2, HIF-1α, NF-κB and iNOS inhibition in hypercholesterolemic Wistar rats (39). Another study reported that pericarp extract at 100 mg/kg via oral gavage could protect mice from the memory degrading effects of scopolamine and improved memory retention (40). Therefore, in the present study, doses of 100, 200 and 400 mg/kg body weight of Wistar rats were used. No behavioral changes were observed, and there were no deaths of rats during the experiment, suggesting that the doses used in the present study did not cause any notable toxic effects.

The Wistar rats were allowed to acclimatize for 1 week before being treated with HFD consisting of 90% comfeed standard II, 10% pork fat and 1.25% pure cholesterol at a dose of 20 g BW/day for 6 weeks to induce obesity. The body weight was measured every week to monitor the weight gain of the animals. Streptozotocin (STZ) (Nacalai Tesque, Inc.; 45 mg/kg BW dissolved with sodium citrate buffer 1.5 ml/100 g BW) and nicotinamide (NA) (Nacalai Tesque, Inc.; 110 mg/kg BW dissolved with NaCl 1.5 ml/kg BW) were intraperitoneally administered at day 42 to induce T2DM in the rats.

In a previous study, Wistar rats showed significant impairment of memory, as well as ability and restoration of learning at 8 weeks after being diagnosed as diabetic (17). Therefore, the intervention with GMP extract in groups 3 to 6 was started at weeks 8 after a diabetic status was confirmed.

The termination of rats was performed under ether-induced anesthesia by cervical dislocation. For anesthesia, ~30% of anesthetic ether was added in a glass jar containing cotton pads with an average volume of 6.5 ml/h. Each rat was placed in the glass jar for ~4.5 min, after which cervical dislocation was performed.

The research protocols were performed in accordance with the National Institute of Health Guidelines for Animal Care (41), and has been reviewed and approved by the Medical and Health Research Ethics Committee, Faculty of Medicine Diponegoro University, Semarang Indonesia (approval no. 115/EC/H/KEPK/FK-UNDIP/VIII/2019).

Preparation of GMP extract. GMP was collected from Surabaya, and voucher specimens were deposited at the Department of Pharmacognosy and Phytochemistry, Faculty

of Pharmacy, Universitas Airlangga, Indonesia. The extract preparation was performed as described previously (39,42). Fresh pericarps of *Garcinia mangostana* (10 kg) were cut into small pieces and dried under indirect sunlight. The drying process was performed to minimize the amount of water. Then, the dried pericarps were powdered (1 kg) and extracted with 70% ethanol (4 liters, 3 times) through maceration for 24 h. The 70% ethanol solutions were evaporated using a rotary evaporator and dried in a drying machine (oven) to obtain the 70% ethanol extract (302 x g).

Blood samples and tissue collection. Blood was collected from the periorbital sinus after 10 h of fasting; ~2 ml blood was collected from the periorbital sinus and placed in a heparin tube (One Med Health Care) and centrifuged (447 x g, 4˚C) to obtain the serum. The rats were sacrificed as described above, and brain tissues were collected and placed in 10% formalin buffer solution overnight at room temperature, and subsequently embedded in paraffin a block, and sectioned into 3-4 μm thick slices.

Measurement of fasting blood glucose concentrations. Fasting blood glucose levels (mg/dl) were measured after 3 days of STZ/NA intervention to determine the diabetic status in the rats and on the last day after intervention, using the glucose oxidase phenol 4-amino phenazone method (DiaSys Diagnostic Systems GmbH, cat. no. 10 026). Each of blood serum sample (10 μl), standard solution (10 μl) and blank (10 μl) was added to the dilution solution (1 ml), vortexed and then incubated for 20 min at 20-25˚C. The absorbance was measured using a spectrophotometer (Thermo Fisher Scientific, Inc.) at a wavelength of 500 nm.

Measurement of IL-6, TNF-α and SOD levels. The expression levels of IL-6 (cat. no. M6000B), TNF-α (cat. no. MTA00B) and SOD (cat. no. DYC3419-2) were examined in rat blood serum using sandwich ELISA according to the manufacturer's protocol (R&D Systems, Inc.). Absorbance was determined using a spectrophotometer at a wavelength of 450 nm. The expression levels of IL-6, TNF-α and SOD were compared between the normal control and obese-T2DM groups, as well as between the GMP intervention groups in obese and obese-T2DM.

NF-κB immunostaining. Immunostaining was performed on the formalin-fixed paraffin-embedded samples with using a UltraTek HRP (Anti-Polyvalent) Ready-To-Use kit (cat. no. AMF080-IFU, ScyTek Laboratories, Inc.) at room temperature. The primary antibody used was a rabbit polyclonal anti-NF-κB (cat. no. ab7970; Abcam; 1:300). Slides were deparaffinized in xylene followed by rehydration using a graded series of alcohol solutions (95, 80 and 70%), treated with heat-induced epitope retrieval using Tris EDTA solution with pH 9.0 at 95˚C for 10 min, followed by Super Block (cat. no. AMF080-IFU, ScyTek Laboratories, Inc.) for 10 min, and an overnight antibody incubation at 4˚C. UltraTek Anti-Polyvalent and subsequently UltraTek HRP were both applied for 10 min each at room temperature. DAB staining was administered for 3 min. A blinded observer assessed the immunostaining for glial and neuronal cells at both hippocampi. Immuno-positive glial and neuron cells were defined with positive cytoplasm staining. The evaluation was conducted using a bright field microscope at x400 magnification (BX41; Olympus Corporation) with manual counter. All immune-positive glial cells in the parenchyma area and neuron cells in the cornu ammonis (CA)1, CA4, and dentate gyrus areas were counted.

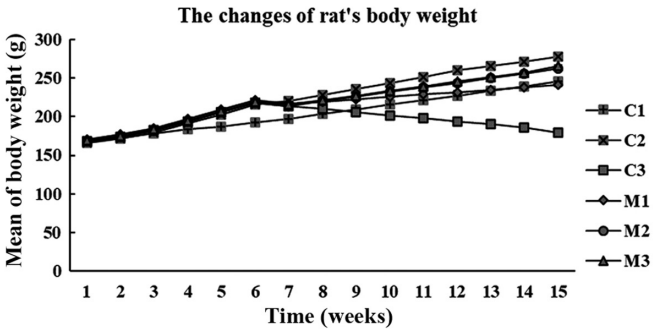

Figure 1. Changes in the mean body weight of rats between weeks 1 and 15 of treatment. HFD, high-fat diet; GMP, *Garcinia mangostana* pericarp; BW, body weight; C1, normal control rats administered with a standard normal diet; C2, mangosteen control administered a HFD and GMP extract at 200 g/kg BW/day; C3, diabetic Wistar rats treated with HFD; M1, diabetic Wistar rats treated with HFD and 100 g/kg BW/day GMP extract; M2, diabetic Wistar rats treated with HFD 200 g/kg BW/day GMP extract; M3, diabetic rats treated with HFD and 400 g/kg BW/day GMP extract.

Statistical analysis. Data are presented as the mean ± standard deviation. The distribution data of study were determined using a Shapiro-Wilk test and then analyzed statistically using a Wilcoxon Signed-Rank test to compare body weights and fasting blood glucose of the rats before and after intervention. A Kruskal Wallis test followed by a Dunn's post hoc test was used to determine differences in glial NF-κB levels, and IL-6, TNF-α and SOD expression between the control and GMP intervention groups.

Results

Changes in rat BW. The BW of the rats significantly increased in the group fed a HFD compared with the normal control rats that received a standard diet (168.31±3.48 vs. 214.5±10.37 g; P<0.001; Wilcoxon Signed-Rank test). Compared with the starting BW, after 7 weeks of GMP intervention, BW significantly increased in the normal and mangosteen control rats (198±2.43 vs. 246±2.80 g; 220±2.83 vs. 278±2.28 g, respectively; both P=0.024; Wilcoxon Signed Ranks test) but decreased continuously in the T2DM rats (214±3.29 vs. 180±3.89 g; P=0.026; Wilcoxon Signed Ranks test) (Fig. 1).

Effects of GMP extract treatment on fasting blood glucose levels in obese-T2DM rats. Fig. 2 shows the mean fasting blood glucose levels before and after GMP intervention in Wistar rats. The fasting blood glucose levels in all GMP groups (C2, M1, M2 and M3) were significantly different between the pre- and post-GMP intervention (all P<0.001; Wilcoxon Signed-Rank test). The administration of GMP at all doses effectively reduced fasting blood glucose levels in all diabetic groups, particularly at a dose of 400 mg/kg (from 277.86±1.48 to 102.45±4.78 mg/dl). This effect was also observed in an obese non-diabetic group

that was orally treated with 200 mg/kg of GMP extract (from 168.33±4.13 to 84.68±5.04 mg/dl).

Effect of GMP extract treatment on glial NF-κB expression in obese-T2DM rats. Fig. 3 shows the expression of glial NF-κB in the hippocampal area at a magnification of x400 in 10 fields of view. The number of dark brown color, absence of visible a nucleus, and irregularly shaped cells, which were considered as highly positively immunostaining glial cells, were significantly different in their number amongst the intervention groups (P=0.007; Kruskal Wallis test). The hippocampal area of the obese-diabetic rats showed significantly higher positive expression than that of the normal control group (132.9±56.41 vs. 73.6±24.44 positive glial cells; P=0.009; Dunn's test). The treatment of GMP extract, particularly at 200 g/kg BW/day, effectively reduced the number of activated glial cells (72.1±17.18 positive glial cells; P=0.016; Dunn's test; compared with the obese-diabetic group). The results of immune-positive neuronal cells was not significantly different between the groups (data not shown).

Effect of GMP extract treatment on the IL-6 and TNF-α expression of obese-T2DM rats. The T2DM rats showed significantly higher levels of IL-6 and TNF-α compared with the normal control rats (IL-6, 122.94±4.86 vs. 76.13±2.47 pg/ml, TNF-α, 14.15±0.23 vs. 6.14±0.17 pg/ml, respectively; P≤0.001; Dunn's test). GMP extract, particularly 100, 200 and 400 g/kg BW/day, significantly suppressed T2DM-induced IL-6 levels compared with the untreated rats (106.18±3.98, 98.04±5.21 and 88.25±2.36 vs. 122.94±4.86 pg/ml, respectively; P<0.001; Kruskal Wallis test) and TNF-α expression (9.15±0.12, 8.07±0.38 and 7.18±0.35 vs. 14.15±0.23, respectively; P<0.001; Kruskal Wallis test). GMP extract also effectively reduced the upregulation in IL-6 and TNF-α expression in the obese-non-diabetic rats (92.23±3.212 vs. 122.94±4.856; 7.26±0.233 vs. 14.15±0.227; respectively; both P≤0.01; Dunn's test) (Fig. 4).

Effect of GMP extract treatment on SOD generation in obese-T2DM rats. The T2DM rats exhibited significantly lower levels of SOD compared with the normal control rats (28.43±3.18% vs. 89.46±2.16%; P≤0.001; Dunn's test). GMP extract, particularly at 100, 200 and 400 g/kg BW/day, significantly attenuated the T2DM-induced reduction in SOD expression compared with the untreated T2DM rats (45.34±4.50%, 68.14±3.18%, 75.74±2.75 vs. 28.43±3.18%, respectively; P<0.001; Kruskal Wallis test) in the blood serum. GMP extract also effectively increased SOD generation in the obese-non-diabetic rats (81.37±4.712 vs. 28.43±3.18%, P≤0.001; Dunn's test) (Fig. 5).

Discussion

The aim of the present study was evaluate the protective effects of GMP extract on brain tissues against inflammation, particularly the hippocampus, which is involved in the early stages of neurocognitive impairment, such as Dementia and AD, and the prevalence of these diseases is higher in diabetic patients (4,5). The results of the present study showed that the expression of NF-κB was upregulated in the glial cells of the obese-diabetic rats, and that the IL-6 and TNF-α expression, as well as SOD generation in the blood serum were increased. These results indicated that obesity with diabetes can stimulate systemic and cerebral inflammation. NF-κB is an inflammatory factor that can be released by activated glial cells and is associated with white matter astrogliosis and cerebral inflammation (43). It also serves an important role in synaptic signaling in the nervous system that maintains learning and memory functions (44,45). The NF-κB pathway is activated by numerous stimuli, including ligands of the TNF receptor families, pattern-recognition receptors, cytokine receptors and receptors of B-cells and T-cells (46). It is involved in the expression of IL-6 and TNF-α, which are secreted by glial cells in high amounts and related to the production of several inflammatory cytokines (44,47). Inhibition of NF-κB expression in reactive astrocytes exerts an effect on vascular cognitive impairment, such as repairing gliosis and axonal loss, maintaining the integrity of white matter structure and improving memory function (43). Compared with glial cells, immune-positive neuronal cells were not significantly observed in this study, suggesting that glial cells serve a crucial role in the early stage of T2DM-induced brain inflammation. Thus, upregulation of NF-κB expression in hippocampal glial cells may serve as an early indicator of the pathogenic process of cognitive impairment in the brain, prior to symptoms of cognitive function impairment in the rats.

The activation of IL-6 and TNF-α expression has been reported in patients with T2DM with mild cognitive impairment, and increases in their levels has been shown to be related to aging in humans (7,48). The high levels of TNF-α in the cerebrospinal fluid and high levels of IL-6 in the blood serum of patients with AD indicate that these two pro-inflammatory cytokines participate in the early stages of AD through inflammatory mechanisms (49,50). Other studies reported high expression levels of IL-6 and TNF-α in the brain micro vessels in diabetic mice with AD, suggesting an increase in

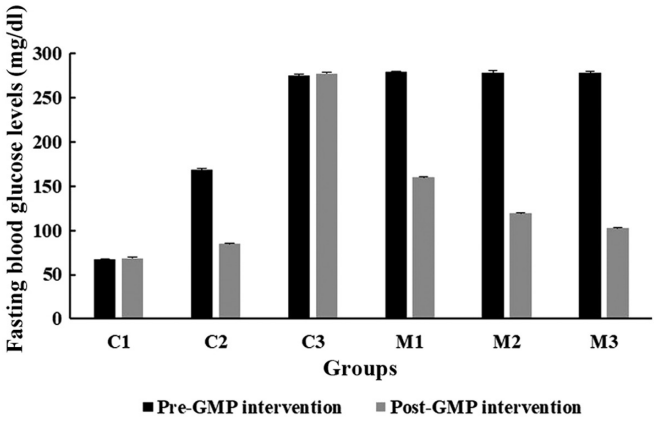

Figure 2. Comparison of fasting blood glucose levels before and after GMP intervention in the C1, C2, C3, M1, M2 and M3 groups. HFD, high-fat diet; GMP, *Garcinia mangostana* pericarp; BW, body weight; C1, normal control rats administered with a standard normal diet; C2, mangosteen control administered a HFD and GMP extract at 200 g/kg BW/day; C3, diabetic Wistar rats treated with HFD; M1, diabetic Wistar rats treated with HFD and 100 g/kg BW/day GMP extract; M2, diabetic Wistar rats treated with HFD 200 g/kg BW/day GMP extract; M3, diabetic rats treated with HFD and 400 g/kg BW/day GMP extract.

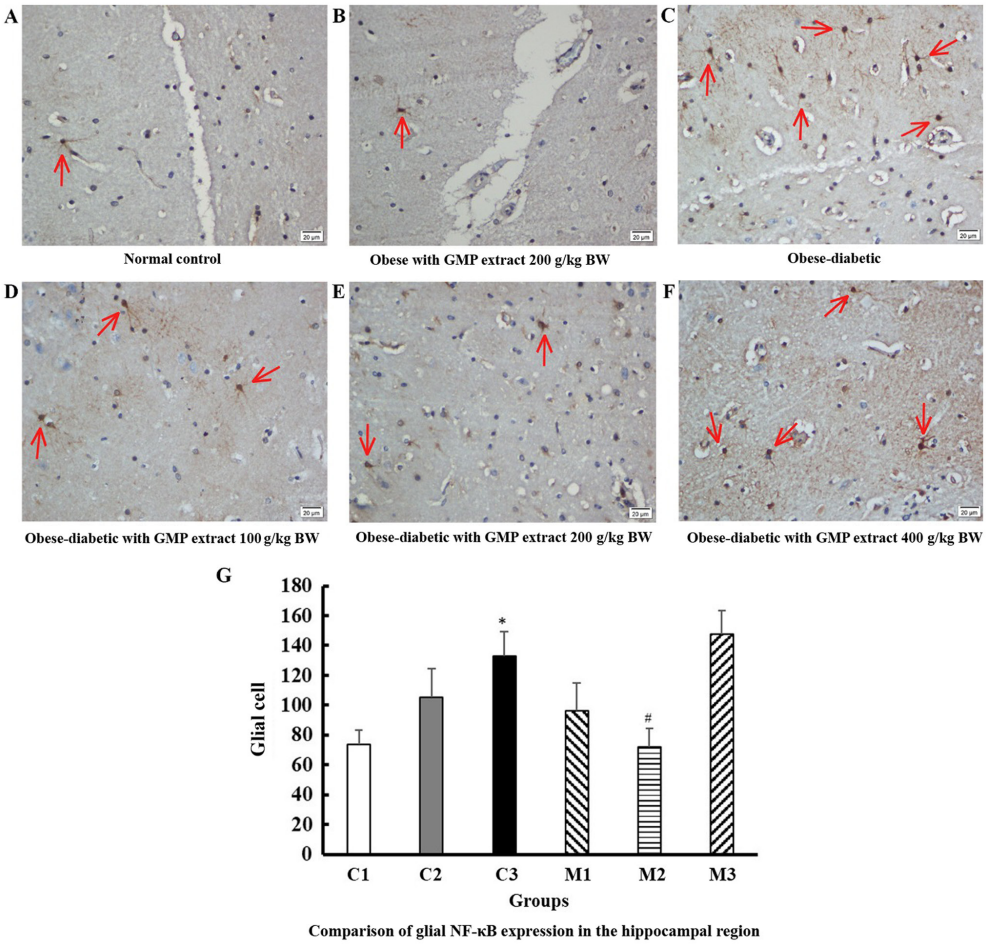

Figure 3. NF-κB expression in glial cells in the hippocampal region. NF-κB expression in glial cells (red arrow) in the (A) control group, (B) obese rats treated with *GMP*, (C) obese-diabetic rats, and obese-diabetic groups treated with (D) 100, (E) 200 and (F) 400 g/kg BW/day GMP extract. NF-κB stained glial cells appeared dark brown in color, without a visible nucleus and were irregularly-shaped cells. The number of stained cells was counted in 10 fields of view at a magnification of x400. (G) Comparison of glial NF-κB expression in the hippocampal region shows a significant difference before (C3) and after (M2) intervention with 200 g/kg BW/day *GMP* in obese-diabetic rats. Error bars indicate the standard error of the mean. *P<0.001 vs. C1; #P<0.05 vs. C3. NF-κB, HFD, high-fat diet; GMP, *Garcinia mangostana* pericarp; BW, body weight; C1, normal control rats administered with a standard normal diet; C2, mangosteen control administered a HFD and GMP extract at 200 g/kg BW/day; C3, diabetic Wistar rats treated with HFD; M1, diabetic Wistar rats treated with HFD and 100 g/kg BW/day GMP extract; M2, diabetic Wistar rats treated with HFD 200 g/kg BW/day GMP extract; M3, diabetic rats treated with HFD and 400 g/kg BW/day GMP extract.

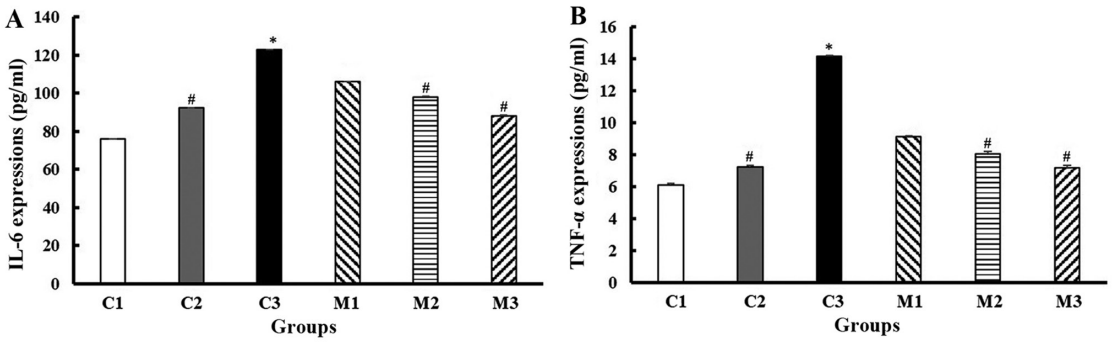

Figure 4. Upregulation of IL-6 (A) and TNF-α expression (B) in the obese-diabetic control group (C3) and its suppression in GMP intervention at 100 (M1), 200 (M2), and 400 (M3) g/kg BW/day. C1 and C2 are the control groups of the normal and obese rats treated with 200 g/kg BW/day GMP extract, respectively. Error bars indicate the standard error of the mean. *P<0.001 vs. C1; #P<0.05 vs. C3. TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; HFD, high-fat diet; GMP, *Garcinia mangostana* pericarp; BW, body weight; C1, normal control rats administered with a standard normal diet; C2, mangosteen control administered a HFD and GMP extract at 200 g/kg BW/day; C3, diabetic Wistar rats treated with HFD; M1, diabetic Wistar rats treated with HFD and 100 g/kg BW/day GMP extract; M2, diabetic Wistar rats treated with HFD 200 g/kg BW/day GMP extract; M3, diabetic rats treated with HFD and 400 g/kg BW/day GMP extract.

the pathogenesis of cognitive dysfunction under diabetic conditions by promoting inflammatory mechanisms (14). The expression of IL-6 in the plasma has an inverse association with the volume of hippocampal gray matter, which is a critical

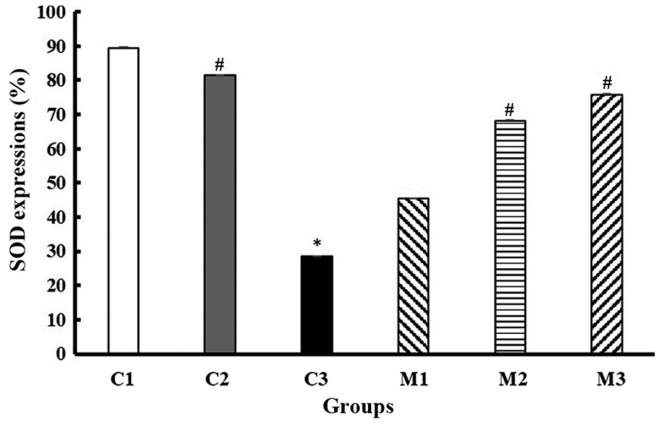

Figure 5. Detection of SOD generation in the obese-diabetic control group (C3) and its suppression in the 100 (M1), 200 (M2), and 400 (M3) g/kg BW/day GMP intervention groups. C1 and C2 are the control groups of the normal and obese rats treated with 200 g/kg BW/day GMP extract, respectively. Error bars indicate the standard error of the mean. *P<0.001 vs. C1; #P<0.05 vs. C3. SOD, superoxide dismutase; HFD, high-fat diet; GMP, *Garcinia mangostana* pericarp; BW, body weight; C1, normal control rats administered with a standard normal diet; C2, mangosteen control administered a HFD and GMP extract at 200 g/kg BW/day; C3, diabetic Wistar rats treated with HFD; M1, diabetic Wistar rats treated with HFD and 100 g/kg BW/day GMP extract; M2, diabetic Wistar rats treated with HFD 200 g/kg BW/day GMP extract; M3, diabetic rats treated with HFD and 400 g/kg BW/day GMP extract.

structure involved in memory and cognitive functions (51). The results of the present study are in agreement with previous studies, indicating that IL-6 and TNF-α inflammatory cytokines are important biological markers of cognitive impairment in T2DM, particularly in the initial stage (14,49,50). Therefore, they may serve as potential biomarkers for the early detection of neurocognitive impairment in diabetic patients.

Previous studies have shown an association between diabetes and increased generation of antioxidants (52-55). The generation of ROS and suppression of SOD levels are significantly increased under hyperglycemic conditions in diabetes, and their enhancement has been observed in rats with diabetes-induced severe learning and memory deficits associated with endothelial dysfunction; this result indicates their crucial roles in cognitive impairment, particularly in vascular dementia (17,18). Diabetes causes the dysfunction of endothelial tissue and increases the levels of oxidative stress, leading to diabetic neurodegeneration and encephalopathic disorders (53,56,57). Animal studies reported the decrease of eNOS expression in the vascular tissue and SOD levels in the thoracic aorta and serum in the diabetic group (17,52-55). In agreement with those previous studies, the significant impairment of SOD generation in diabetic rats was also observed in the present study.

In the last decade, *Garcinia mangostana* Linn of the Guttiferae family has been widely used as a potential preventative agent for numerous degenerative diseases, including diabetes, due to its anti-inflammatory, antioxidant and anti-hyperglycemic activities (32-35). Its pericarp contents are rich in flavonoids called xanthones, which have beneficial effects on metabolic syndrome by inhibiting α-glucosidase and post-prandial hyperglycemia, thereby reducing glucose absorption (27). The xanthone bioactive compounds (particularly in α-mangosteen) have a similar structure and chromatographic behaviors to that of flavonoids (58), and ~25% of the α-mangosteen was observed in mangosteen pericarp (59). When 20 mg/kg α-mangosteen was orally administered, the bioavailability was estimated to be just 0.4% (60), and it could reach maximum plasma levels within 63 min (61). This low bioavailability is caused by the metabolism of xanthones that takes a place in the liver and intestine, and the other compounds in mangosteen extract may obstruct the multiple CYP450 isoforms, such as CYP1A and CYP2C, and inhibit the conjugation of glucuronide and/or sulfate of α-mangosteen, which has an impact on reducing the metabolic process in the liver and intestine (62-65). The anti-inflammatory and antioxidant properties of xanthones may lower the expression of inflammatory genes, such as TNF-α, IL-6 and INF-γ inducible protein-10, as well as NF-κB in macrophages and adipocytes (30,57). Its suppressive effects on NF-κB activation are exerted by the inhibition of IκBα degradation and p65 nuclear translocation (66,67). In the present study, it was shown that the GMP extract could attenuate the activation of NF-κB expression in cerebral tissue, particularly in glial cells. The absorbed constituents of GMP extract, which included xanthones, could penetrate the blood-brain barrier and exert its effects in hippocampal glial cells, an important component of the brain that is associated with the pathogenesis of neurocognitive impairment, such as in dementia and AD. The effects of the extract also regulated the activation of TNF-α and IL-6, and increased SOD levels in the serum, indicating that the activation of NF-κB in the hippocampal tissue is concurrent with the expression of these proinflammatory cytokines and antioxidative stress in the blood. Therefore, GMP extracts may be used as a potential biomarker for the early detection of neurocognitive impairment in diabetic patients.

In conclusion, the increase in glial NF-κB levels followed by the increase in IL-6 and TNF-α expression, and the reduction in SOD activity in the serum of obese-T2DM rats indicates that obesity with T2DM increases the risk of brain inflammation, which is related to neurodegenerative disorders, such as AD. Treatment with GMP extract effectively reduced the levels of these inflammatory factors, suggesting that xanthones may potentially prevent brain inflammation in obese-T2DM rats. Further study is required to establish the advanced neuroprotective effects of xanthones in humans.

Acknowledgements

We would like to thank Mr Yulianto (Animal Laboratory of Center for Food and Nutrition Studies, Universitas Gadjah Mada) for his valuable assistance during the animal experiment.

Funding

The present study was supported by the Ministry of Research and Technology/National Research and Innovation Regency Republic of Indonesia (grant no. 201-04/UN7.6.1/PP/2020).

Availability of data and materials

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

Authors' contributions

MM wrote the manuscript. MM and YN designed the experiments, performed the animal experiments, analyzed the data, and edited the manuscript. VK analyzed the data. YP was involved in the data interpretation and writing the manuscript. NDR analyzed the data, and edited the manuscript. RW prepared and analyzed the GMP extract. SS was involved in the conception of study, the interpretation of the study results and writing the manuscript. All authors confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine Diponegoro University Semarang Indonesia (approval no. 115/EC/H/KEPK/FK-UNDIP/VIII/2019).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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