



Anti-hyperglycemic activity of encapsulated Java tea-based drink on malondialdehyde formation

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ABSTRACT

Nano-encapsulated Java tea-based beverage was previously reported capable of demonstrating the highest protective activity for Langerhans and beta-cell. The mechanism of anti-hyperglycemic activity, however, has remained unclear. This research aimed to study the mechanism of the beverage as an anti-hyperglycemic encapsulated functional drink through its role in malondialdehyde formation in *streptozotocin*-induced diabetic Sprague Dawley rats. The rats were divided into four groups: (A) normal rats as negative control, (B) untreated diabetic rats as positive control, (C) diabetic rats treated with micro-encapsulated drink (3.64 ml/200 g), and (D) diabetic rats treated with nano-encapsulated drink (3.64 ml/200 g). The intervention was conducted for 44 days. Malondialdehyde level was measured periodically every 2 weeks, body weight and blood glucose level were measured periodically every week for 6 weeks. Malondialdehyde level analysis *in vitro* was carried out as a comparison. Compared to malondialdehyde (MDA) level on positive control group, encapsulated Java tea-based beverages intervention attenuated the formation of malondialdehyde in diabetic rats from 36.63% to 51.95%. In addition, micro- and nano-encapsulated drink suppressed the fluctuation of blood glucose level and body weight. *In vitro* assessment showed that micro- and nano-encapsulated drink suppressed the formation of MDA at 5.25% and 72.16%, respectively. The anti-hyperglycemic activity of micro- and nano-encapsulated drink is shown by their ability to alleviate the MDA formation both *in vivo* and *in vitro* assessment.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease, which can cause complication (Vijay and Sharma, 2014). Oxidative stress may play a key role due to unbalance of prooxidant and antioxidant, leading to the formation of reactive and toxic species (Vijay and Sharma, 2014). It is related to the production of malondialdehyde and the inhibition of antioxidant activity (Fatani *et al.*, 2016). Chronic hyperglycemia could damage the organs and cells because of the production of reactive species (Surya *et al.*, 2014). The reactive species could be used as an indicator to study the mechanisms of degenerative diseases (Niki, 2013).

Diabetes mellitus type II leads to increased oxidation of fatty acids because of the formation of free radicals and level of oxidative stress (Madiseh *et al.*, 2016). Oxidative stress decreases the insulin sensitivity and damages beta-cells of the pancreas (Madiseh *et al.*, 2016). Multiple oxidation of polyunsaturated fatty acids by lipid peroxidation generates 4-hydroxynonenal and malondialdehyde (MDA) (Gaschler and Stockwell, 2017). MDA is a highly reactive substance as an end product of the lipid peroxidation. Its reactivity is corresponding to cell damage and degenerative diseases (Gaschler and Stockwell, 2017). The increasing level of lipid peroxidation can be prevented by retarding peroxidase formation and peroxidase toxicity (Gaschler and Stockwell, 2017). In this regard, antioxidant could inhibit those deleterious activities (Madiseh *et al.*, 2016).

Medical plants are well known for having antioxidative abilities due to their bioactive components such as phenolics and flavonoids (Krishnaiah *et al.*, 2011). The use of synthetic medicines and antioxidants has been regarded less desirable due to their possible harmful side effects; thus, copious studies on

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antioxidant activity of medical plants have been made to substitute the synthetic one (Ezgi and Vural, 2017). Their antioxidant activity may vary, depending mainly on the type of bioactive compounds and abundance (Alok *et al.*, 2014).

Java tea (*Orthosiphon aristatus* Bl Miq) is commonly known as “kumis kucing” in Indonesia. It has been used widely as a herbal medicine for its content high antioxidant activity (Noguchi *et al.*, 2013). Java tea extract-based drink was enriched with various herbal plants possessing a high antioxidant activity, such as ginger, turmeric, citrus hystrix, citrus aurantifolia, and sappan woods extract (Wijaya *et al.*, 2007). The formulated drink had a high antioxidant activity between 500 and 800 ppm AEAC. Furthermore, previous experiment using high performance liquid chromatography asserted that the bioactive compounds in this functional drink were found similar to those contained in raw material extracts (Indariani *et al.*, 2014). The drink was also found able to increase the glucose absorption in *ex vivo* assay (Wijaya *et al.*, 2010), suggesting that it shows antihyperglycemic properties. In addition, the formulated drink using simplicia extracts has been reported for its high antioxidant activity (Wijaya *et al.*, 2018).

The antihyperglycemic effects of formulated Java tea-based functional drinks were investigated on the diabetic mice. The drink was reported to inhibit the incremental of blood glucose level and delay pancreatic beta cell damages. However, the development of its ready-to-drink product remained as a critical challenge due to its less stability during storage, where the antioxidant levels decreased during the storage period (Indariani *et al.*, 2014). To prevent this, encapsulation is a promising effort for its protective effect on the bioactive compounds (Ezhilarasi *et al.*, 2012). The formulated Java tea-based beverage was encapsulated to increase the stability and its functional properties (Afandi, 2014).

Previous study found that encapsulation could improve consumer acceptance since it masks bitter taste of the product. However, the antioxidant activity of encapsulated drink was lower than that of ready-to-drink. This may display barrier effects of encapsulation, contributing to reduction of the released bioactive compounds, as previously reported in *in vitro* experiment (Wijaya *et al.*, 2013).

On the other hand, encapsulated drink showed the best antihyperglycemic activity compared with ready-to-drink, based on *in vivo* assay. In that experiment, the encapsulation could also maintain body weight of the rats and suppress the blood glucose level during the experiment. Furthermore, histopathologic assay showed that encapsulated drink is beneficial for β -cells of pancreas (Rekasih, 2016).

The mechanism of the anti-hyperglycemic activity of the encapsulated drink has remained unclear. The purpose of this research is to study the mechanism of encapsulated drinks on the formation of malondialdehyde as an end product of lipid peroxidation over a longer period in experimental rats. The encapsulated drink was administered to diabetic rats, as it has the higher anti-hyperglycemic activity than that on ready-to-drink form. Diabetic rats were administered with micro-encapsulated and nano-encapsulated drink. The malondialdehyde formation is studied and compared with diabetic rats without treatment and non-diabetic rats group.

MATERIALS AND METHODS

Materials

Java tea (*Orthosiphon aristatus* Bl Miq—WCSP), turmeric (*Curcuma zanthorriza*—WCSP), ginger (*Zingiber officinale*—WCSP), and sappan wood (*Caesalpinia sappan* L—ILDIS) were obtained from Biopharmaca garden, Bogor Agricultural University, while lime (*Citrus aurantifolia*—WCSP), lemon (*Citrus limon*—TRO), and kaffir lime (*Citrus hystrix*—WCSP) were purchased from a traditional market in Bogor. All plants were identified and deposited at Biopharmaca Research Center, Bogor Agricultural University, Indonesia. The voucher numbers are BMK0076042016, BMK0042072014, BMK0043052015, BMK0120092016, BMK0202092016, BMK0201092016, and BMK0189092016, respectively. All chemicals used were analytical grade. Linoleic acid, thiobarbituric acid, tetra-metoxi propane, acetic acid, ethanol pa, and α -tocopherol were obtained from Sigma Ltd.

Method

Extraction

The extraction was carried out following the methods developed by Wijaya *et al.* (2007). The fresh Java tea (kumis kucing) leaves and sappan wood (kayu secang) were extracted by boiling them over 15 minutes in a closed pan containing as 30 g in 600 ml water and 20 g in 500 ml water, respectively. The extracts were filtered by vacuum filtration and concentrated with rotary evaporator until 1/3-part water remaining (150–200 ml). The extraction of ginger and turmeric was done without adding water. The tuber of ginger (jahe) and turmeric (temulawak) were crushed in an electric juicer followed by filtration. The filtrate was sedimented by kept in the refrigeration for 24 hours. The unsedimented part (30%) was separated in to dark bottle. Finally, the extract of java tea, sappan wood, ginger, and turmeric was pasteurized and kept in the refrigeration before used. The extraction of lime, lemon, and kaffir lime was carried out through crushing in an electric juicer and filtration without adding water. Lime, lemon, and kaffir lime extracts were prepared immediately before micro- and nano-encapsulation process.

Preparation of micro-encapsulated drink powder

A 5.9 ml extract of java tea was slowly mixed with 1.05 ml of sappan wood extract, followed by addition of ginger extract (1.52 ml), turmeric extract (0.2 ml), lime extract (3.098 ml), lemon extract (0.902 ml), and kaffir lime (1 ml). Water was added until reaching a total volume of 100 ml. The solution was homogenized added with maltodextrin (20 g); the homogenized solution was dried using a mini spray drier. The dried powder was kept in refrigerator as micro-encapsulated stock (Rekasih, 2016).

Preparation of nano-encapsulated drink powder

Chitosan (2 g) was dissolved in 100 ml of 2% acetic acid and stirred for 3 hours at 2,817.36 g. Nearly, 50 ml of emulsifier (Tween80 0.2%) was added and stirred room temperature for 30 minutes. Afterward, a linking agent (50 ml of sodium tripolyphosphate) was added. Finally, the mixture was concentrated up to 10% of initial volume, stirred for 3 hours, and

spray-dried. The dried powder was kept in refrigerator as nano-encapsulated stock (Wijaya *et al.*, 2013).

Particle size characterization and polydispersity index

Sample (three drops) was dropped on an object and covered for analysis using dynamic light scattering. The measurement was done using nanoQ software version 2.2 (Makvern Instruments Ltd., UK) based on the refractive index and the viscosity of solvent according to intensity of laser beam. The laser beam was spotted on 30 different field points. The resulting data were analyzed by cumulant method (Wu *et al.*, 2012).

Animal study

The experiment was carried out at the Animal Laboratory of The Tropical Biopharmaca Research Center, Bogor Agricultural University with an approved ethical clearance (No: 003-2018 KEH TROP BRC) and guidelines are described in The Animal Ethics Committee, Bogor Agricultural University. Sprague Dawley male rats (250–300 g) were obtained from the animal experimental facility of National Agency of Drugs and Food Control Badan Pengawas Obat dan Makanan (BPOM), Republic of Indonesia. They were fed with a standard diet and water *ad libitum*, under 50%–60% of humidity, with 12-hour light. Streptozotocin (10 mg/ml) was diluted in 50 mM buffer sodium citrate (pH = 4.5) and injected intraperitoneally in the fasted rats at 45 mg/kg dosage using syringe 1 ml, 25-G. The rats were administered 10% sucrose solution for 24 hours and then they were fasted for 18 hours before checking their blood glucose level. The blood glucose level of diabetic rat was at >150 mg/dl (Safithri *et al.*, 2012; Ojiako *et al.*, 2016).

Animal experiment and treatment

The rats were divided into four groups: (A) normal rats as negative control, (B) untreated diabetic rats as positive control, (C) diabetic rats treated with micro-encapsulated drink, and (D) diabetic rats treated with nano-encapsulated drink. Each group consisted of five rats. Micro-encapsulated and nano-encapsulated powder was diluted with plain water till total amount of 100 ml and administered at 3.64 ml/200 g body weight dosage. The experiment was carried out for 6 weeks. The fasting blood glucose level and the weight of rats were checked every week, while malondialdehyde levels in the blood serum were checked every 2 weeks. The blood for malondialdehyde assessment was collected intravenously. All of the rats were fed with a same amount of diet at 15 g/day.

Measurement of malondialdehyde level in rat's blood

MDA level was measured using thiobarbituric acid (TBA) based on MDA-TBA serum formation via the nucleophilic mechanism. A standard curve was created using tetra-metoxi propane (TMP) by preparation of six different concentrations of TMP, namely 0, 0.9, 1.8, 3.6, 4.5, and 6 µg/ml. One milliliter of TBA (concentration 1% in acetic acid 50%) was added to 4 ml TMP and heated for 60 minutes at 95°C after which brought back to room temperature, followed by adding 5 ml n-butanol:pyridine (15:1). The mixed solution was centrifuged for 15 minutes at

704.34 g. Finally, the absorbance was measured by a UV-Vis spectrophotometer at 532 nm of wavelength (Yagi, 1987).

A 1.2 ml of H₂SO₄ was added to 0.3-ml blood serum at the room temperature. After 10 minutes, 0.15 ml of phosphotungstate acid 10% was added and centrifuged after 5 minutes at 704.34 g for 20 minutes. The sediment was separated from the solution. A 1.2 ml of H₂SO₄ was added again into the solution and left for 10 minutes. Furthermore, a 0.15 ml of phosphotungstate acid 10% was added, and after 5 minutes, it was centrifuged at 704.34 g for 20 minutes. The sediment was separated from the solution. A 0.5 ml of TBA 1% in 50% acetic acid was added to the solution, and then heated at 95°C for 60 minutes. The solution was cooled to the room temperature after which 2.5 ml of n-butanol:pyridine (15:1) was added, continuously centrifuged at 704.34 g for 15 minutes. The absorbance was measured at 532 nm of wavelength with a UV-Vis spectrophotometer (Ohkawa *et al.*, 1979).

Fasting blood glucose level

Fasting blood glucose levels were checked on the day before injection of *streptozotocin* (day 0). On days 2, 9, 16, 23, 30, 37, and 44 after injection, blood glucose levels were checked using electronic glucometer kit (Morakinyo *et al.*, 2015). The data were recorded in mg/dl of blood and represented in percentage of changes.

Body weight changes

During the experiment, the rat body weight was observed every week. The data were recorded in percentage of changes.

Measurement of malondialdehyde in vitro

MDA *in vitro* describes the inhibition of MDA formation by nano- and micro-encapsulated powder drink. The MDA inhibition activity is shown in comparison to vitamin E activity. The assessment was carried out by three steps: standard curve preparation of TMP, incubation period of linoleic acid, and finally, the inhibition of MDA formation activity from linoleic acid by powder drink. MDA *in vitro* was carried out followed the procedures by Kikuzaki and Nobuji (1993).

The standard curve of TMP was done by provided six level concentration of TMP, namely, 0, 5, 10, 15, 20, and 25 µM. The TMP (1 ml) was reacted to 2 ml of trichloro-acid (TCA) 20% and 2 ml of TBA 1% in 50% acetic acid. The solution was heated (100°C) for 10 minutes. The solution was cooled and centrifuged for 15 minutes (704.34 g). Finally, the absorbance was measured by a UV-Vis spectrophotometer at 532 nm of wavelength.

The incubation period of linoleic acid was determined by providing 6 ml buffer phosphate 0.1 M (pH 7). Six milliliters of linoleic acid (100 mM in ethanol 99.8%) was added into the buffer phosphate, followed by adding of 3 ml demineralized aquadest. The solution was homogenized and incubated in a dark bottle in 40°C temperature. The solution was analyzed every 24 hours until the linoleic acid reached the highest absorbance. 2 ml of TCA 20% and 2 ml TBA (1% in acetic acid 50%) were added in to 1 ml of incubated linoleic acid. The solution mixed was heated for 10 minutes (100°C). The solution was cooled and centrifuged at 704.34 g for 15 minutes. The absorbance was measured by a UV-Vis spectrophotometer at 532 nm of wavelength.

The last assessment was powder drink activity due to inhibit the formation of MDA from linoleic acid. Nano- and micro-encapsulated powder drink was provided into six different concentrations, namely, 0, 25, 50, 75, 100, and 200 ppm. A 200 ppm of alpha-tocopherol (Vitamin E) control positive also was provided as a comparison. Moreover, a blanco solution was provided by adding 2 ml of buffer phosphate 0.1 M (pH 7) into 3 ml ethanol 99.8%. One milliliter of sample solution was provided, following by adding 2 ml of TCA 20% and 2 ml of TBA (1% in acetic acid 50%) and heated for 10 minutes (100°C). The solution was cooled and centrifuged at 704.34 g for 15 minutes. The absorbance was measured by a UV-Vis spectrophotometer at 532 nm of wavelength.

Statistical analysis

Data were evaluated using analysis of variance in SPSS version 16 (SPSS Institute, Inc., Chicago, IL). Duncan test was used to determine significant difference at $p < 0.05$.

RESULTS AND DISCUSSION

Particle properties of encapsulated drink

The nano-encapsulated drink powder had particle size of 217.7 nm and polydispersity index (PDI) at 0.796 (Fig. 1), while the value was 1,785 nm and 0.985 for micro-encapsulated drink powder. In term of nano-encapsulated drink powder, the value was in accordance with FDA (2012) regulations, i.e., 0–1,000 nm. PDI is defined as monodispersity level and particle distribution, which ranges from 0 to 0.7 (Jardim *et al.*, 2015). Particle size on previous research was 537.8 nm (Rekasih, 2016) and 224.3 nm

(Wijaya *et al.*, 2013). The difference in particle size is caused by the difference in deacetylation degree of the encapsulant used.

In our experiment, the diacetylation degree of chitosan was >85%, which is different from previous studies, i.e., >90% (Wijaya *et al.*, 2013) and >75% (Rekasih, 2016). Deacetylation degree affects the strength of sizing. Higher levels of deacetylation would result in smaller and uniform particles (Minagawa *et al.*, 2007). In addition, a decreased particle size positively correlated to bioavailability of the bioactive compounds in metabolic system (Ozturk *et al.*, 2014). The encapsulated drink is distributed faster to the cell and targeted organs for increasing of solubility (Arpagaus *et al.*, 2017).

Malondialdehyde level

The MDA level was checked in the rat blood serum on days 2, 16, 30, and 44 after induction. MDA level of non-diabetic rats was stable at <4.0 $\mu\text{M}/\text{ml}$ during 6-weeks maintenance (Table 1). Yagi (1987) reported that MDA level for healthy and unhealthy was at ± 3.42 and >6.40 $\mu\text{M}/\text{ml}$, respectively. The MDA level in diabetic positive rats B, C, and D (Table 1) was fluctuated during 44-day experiment. MDA was formed due to *streptozotocin* activity as free radical. MDA is formed when the amount of free radical is higher than that of antioxidant (Annadurai *et al.*, 2014), which involved lipid peroxidation.

The formation of MDA in diabetic rats fed by micro-encapsulated drink (Group C) increased (Fig. 2) during first 16 days. The MDA level noticeably increased in initial stage, then slightly decreased after day 16. The MDA level continued to decrease and reached healthy level on day 44. The dissimilar trend was observed in nano-encapsulated drink-treated diabetic

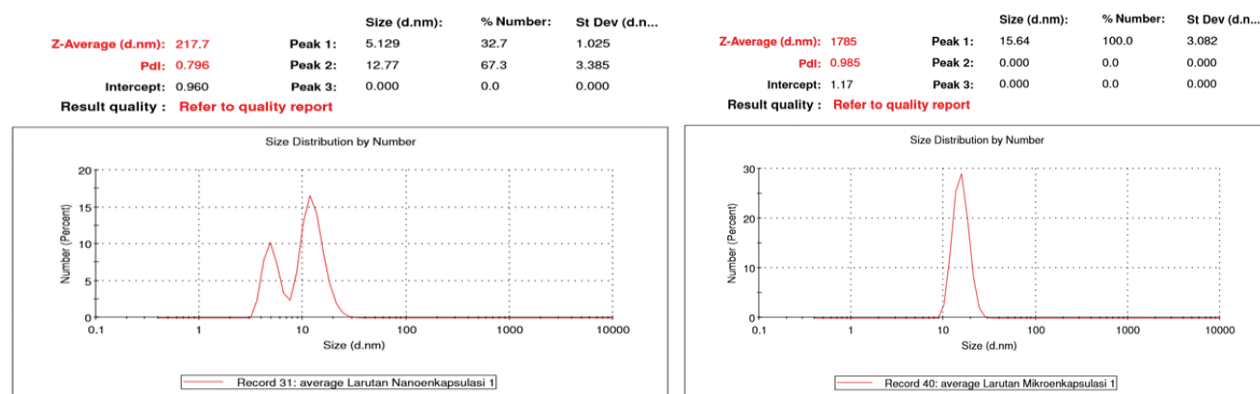


Figure 1. Particle size distribution of nano-encapsulated (a) and micro-encapsulated (b) drink powder. Particle size distribution of nano-encapsulated

Table 1. Malondialdehyde level of rats during 44 days treatment ($\mu\text{M}/\text{ml}$).

Group	Day			
	2	16	30	44
(A) Control negative	2.802 \pm 2.062a	3.969 \pm 3.427a	2.546 \pm 0.846a	2.018 \pm 1.256a
(B) Control positive	4.621 \pm 3.696b	11.241 \pm 3.939a	8.191 \pm 5.319a	7.581 \pm 5.186a
(C) Positive diabetes-micro-encapsulation	4.126 \pm 4.831b	11.227 \pm 5.307a	8.213 \pm 3.061ab	4.804 \pm 2.271b
(D) Positive diabetes-nanoencapsulation	4.285 \pm 3.522b	12.249 \pm 3.853a	5.197 \pm 1.802b	3.643 \pm 1.954b

Note: Different letters in the same column indicate significant differences ($p < 0.05$) after analysis of variance and a Duncan comparison test.

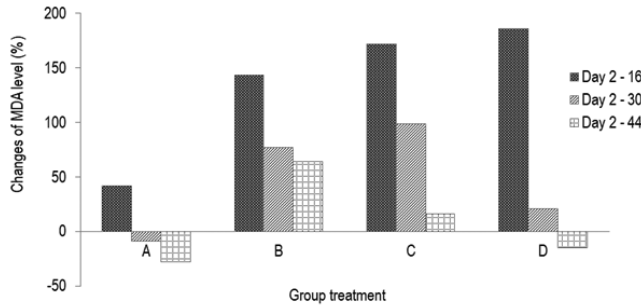


Figure 2. Changes of MDA level (%) during 44 days compared to day 2. (A) Group negative diabetic control; (B) positive diabetic control; (C) positive diabetes-micro-encapsulation; (D) positive diabetes-nano-encapsulation.

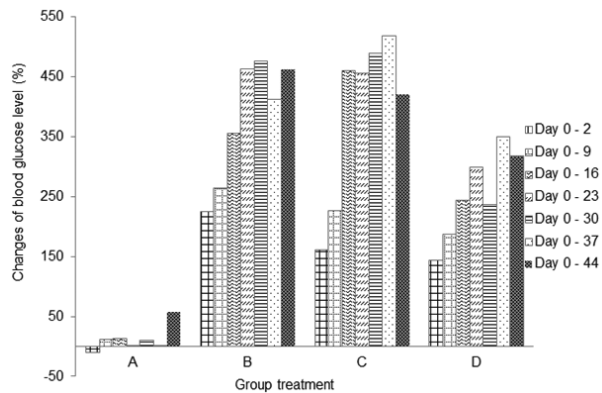


Figure 3. Changes of blood glucose level (%) during 44 days compared to day 0. (A) Group negative diabetic control; (B) positive diabetic control; (C) positive diabetes-micro-encapsulation; (D) positive diabetes-nano-encapsulation.

rats (D), which remarkably increased to the highest level within 16 days and then significantly decreased up to normal level (non-diabetic) on day 30. At this point, the concentration of MDA was not significantly different from that of MDA level on day 44. This suggests that administration of nano-encapsulated drink for diabetic rats could reach normal level of MDA level after 30 days of treatments.

This research clearly demonstrated that encapsulation of Java tea drink was able to normalize MDA level on blood serum of diabetic rats. A study asserted the negative correlation between antioxidant activity and MDA formation (Nikniaz *et al.*, 2015), which indicates that treatment of micro- and nano-encapsulation plays role in distributing antioxidant compounds. Micro-encapsulated drink suppressed MDA formation about 36.63%, while nano-encapsulated drink showed a better outcome about 51.95%. This suppression was associated with beneficial effects of the encapsulation on pancreatic β -cell as reported by Rekasih (2016). The decrease in MDA level was associated with inhibitory effects on lipid peroxidation since it could damage cells and DNA. In short, the lower formation of MDA contributed to less cellular damages.

There is a relation between total antioxidant status (TAS) and diabetic occurrence in human study. Diabetic patients

had a lower TAS than non-diabetic patients (Kulaksizoglu and Aylin, 2016). In addition, MDA level of uncontrolled diabetic patients was higher than that in diabetic controlled patients. The uncontrolled and controlled diabetic patients had MDA level of >10 and ± 7.5 $\mu\text{M/ml}$, respectively, while the undiabetic patients had MDA level of ± 5.0 $\mu\text{M/ml}$ (Fatani *et al.*, 2016).

Blood glucose level

The blood glucose level was checked before injection of *streptozotocin*. As appeared in Figure 3, it reached a diabetic condition after 48 hours. There was a fluctuation of blood glucose level on negative control group during the experiment. However, it was a normal level for blood glucose in non-diabetic rats. According to Annadurai *et al.* (2014), blood glucose level for diabetic rats is >150 mg/dl.

Blood glucose level in diabetic control group (B) continuously increased from days 2 to 16 (Table 2), which was significantly different ($p < 0.05$) from that on days 2 and 9. On day 16, the blood glucose level increased and demonstrated a slight increase on day 44. There was a fluctuation of blood glucose level in micro-encapsulation-treated rats (C) after day 2. No significant difference in blood glucose level was observed within 9 days, but it dramatically increased on day 16. The blood glucose level was fluctuating and statistically stable until day 44. The fluctuation is caused by the malfunction of pancreatic β -cells (Murata *et al.*, 2017) and linked with atherogenesis complications in diabetes type-2 (Xiao-Min *et al.*, 2010). In addition, the increase of blood glucose level after induction of *streptozotocin* was caused by depletion of insulin secretion (Uma *et al.*, 2014). Micro-encapsulation treatment statistically did not show a significant effect on blood glucose level, compared to diabetic control group. However, the blood glucose level on day 44 was lower than that on diabetic control group.

There was an increase in blood glucose level (Fig. 3) in nano-encapsulation-treated diabetic rats (D), but it was not as high as in micro-encapsulation group (C) and diabetic control group (B). There was no significant difference in blood glucose level from days 2 to 30, which means that it is unaltered within the first 4 weeks and stable until day 44. Our results showed that both nano- and micro-encapsulation of Java tea-based drink could suppress the incremental of blood glucose level at 45.21% and 9.89%, respectively. This is quite different from previous report of 18.16% (Rekasih, 2016).

The suppressing effect of nano-encapsulated Java tea-based beverages might be related to MDA level. Another study reported that nano-encapsulation affected the distribution of the bioactive compounds and or medicine in *in vivo* assay using diabetic rats. Metformin and thymoquinone in nano-encapsulated form had better antihyperglycemic properties in type-2 diabetic rats. It decreased the blood glucose level better than the bioactive compounds itself (Rani *et al.*, 2018). Encapsulation of curcumin enhanced its ability to inhibit alpha-amylase activity and antihyperglycemic activity during 7-days experiment (Akolade *et al.*, 2017). In addition, curcumin in nanospheres conjugated to gold showed a better activity in quenching reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Singh *et al.*, 2017).

Table 2. Blood glucose level of rats during 44 days treatment (mg/dl).

Group	Day							
	0	2	9	16	23	30	37	44
(A) Control negative	86.400 ± 14.046bc	78.000 ± 4.848c	97.600 ± 12.341b	98.000 ± 4.848b	87.800 ± 7.120bc	95.400 ± 4.827b	86.800 ± 6.017bc	136.600 ± 26.092a
(B) Control positive	78.400 ± 9.397c	254.800 ± 74.476b	285.600 ± 89.946b	357.200 ± 105.940ab	441.400 ± 106.741a	452.200 ± 67.865a	402.400 ± 90.930a	440.800 ± 50.771a
(C) Positivediabetes-micro-encapsulation	75.600 ± 9.209c	197.800 ± 43.008b	247.800 ± 96.113b	423.400 ± 95.560a	420.800 ± 82.612a	446.000 ± 91.263a	468.200 ± 75.194a	393.600 ± 37.427ab
(D) Positive diabetes-nanoencapsulation	86.200 ± 10.918e	211.200 ± 40.307d	248.200 ± 88.117cd	297.400 ± 49.384bc	344.200 ± 36.293ab	290.400 ± 83.692bc	388.600 ± 58.080a	360.600 ± 37.091a

Note: Different letters in the same column indicate significant differences ($p < 0.05$) after analysis of variance and a Duncan comparison test.

Table 3. Body weight changes of rats during 44 days treatment (g).

Group	Day							
	0	2	9	16	23	30	37	44
(A) Control negative	261.800 ± 21.182de	253.600 ± 18.434e	265.60 ± 16.697de	284.800 ± 20.462cd	296.400 ± 24.141cd	309.800 ± 21.277bc	323.600 ± 22.908abc	335.000 ± 25.407a
(B) Control positive	261.800 ± 25.548a	241.000 ± 27.704a	209.400 ± 23.839b	201.600 ± 23.797b	192.800 ± 21.494bcd	156.200 ± 17.094e	175.200 ± 8.319cde	169.400 ± 15.274de
(C) Positive diabetes-micro-encapsulation	265.400 ± 24.572a	243.600 ± 23.352ab	220.400 ± 26.159bc	212.600 ± 22.412cde	208.800 ± 16.543cd	189.400 ± 21.138cd	181.000 ± 31.137d	191.400 ± 27.301cd
(D) Positive diabetes-nanoencapsulation	261.400 ± 20.268a	239.800 ± 15.172ab	208.200 ± 19.435bc	193.000 ± 22.438c	187.600 ± 26.501c	191.000 ± 29.715c	193.000 ± 30.781c	192.800 ± 34.172c

Note: Different letters in the same column indicate significant differences ($p < 0.05$) after analysis of variance and a Duncan comparison test.

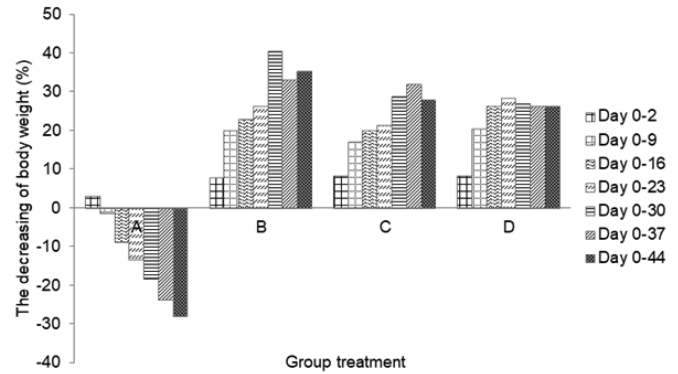


Figure 4. Changes of body weight (%) during 44 days compared to day 0. (A) Group negative diabetic control; (B) positive diabetic control; (C) positive diabetes-micro-encapsulation; (D) positive diabetes-nanoencapsulation. Minus (-) percentage describes the increasing of body weight of rats.

Body weight profile

The rat body weight was found from 250 to 300 g before the induction of *streptozotocin* and monitored during the six weeks of the experiment (Table 3). Body weight of negative control rats (A) showed a significant increase until day 44. Furthermore, there is a decrease in body weight of diabetic rats (B, C, and D) during experiment. The weight loss was caused by *streptozotocin* capable of damaging cells and DNA, reducing the weight of several organs such as liver, brain, and gonad (Cintra *et al.*, 2017). Its concentration was negatively associated with rat weight loss (Omolaoye *et al.*, 2018).

Administration of micro-encapsulated Java tea-based drink (C) showed a remarkable effect on the rat weight loss. The body weight significantly decreased during the first 9 days followed by a constant weight till day 30. The decrease reached the lowest level on day 37 and started to increase to on day 44. Nano-encapsulation treatment (D) showed a positive effect on controlling the weight loss of diabetic rats (Fig. 4). Body weight decreased significantly on day 9, and it was stable until day 44. The results suggested that treatment of nano- and micro-encapsulation on diabetic rats was beneficial in reducing their weight loss.

The relationship between nano- and micro-encapsulation treatment and rat body weight may display an inhibitory activity against lipid peroxidation. Lipid peroxidation damaged the cells and DNA and reduced the ability of insulin action for glucose intake (Suarsana *et al.*, 2010). As a result, the energy is insufficient for growth of rats. Rekasih (2016) reported that micro-encapsulation and nano-encapsulation of Java tea-based beverages had effects in repairing of pancreatic β -cell. In summary, the treatment could increase the body weight while also exhibiting the repairing effects.

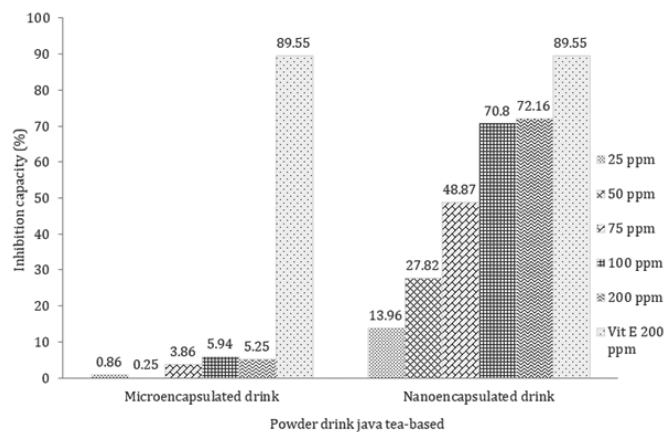
In vitro malondialdehyde level

The results demonstrated that inhibitory effects of nano-encapsulation on MDA formation were significantly different from micro-encapsulation and positive control (vitamin E 200 ppm) as presented in Table 4. In addition, we found that MDA level for negative control (0 ppm) was not significantly different from micro-encapsulation treatment. However, these values statistically

Table 4. Malondialdehyde levels produced from linoleic acid with micro-encapsulation and nanoencapsulation treatment.

Concentration (ppm)	MDA level ($\mu\text{M}/\text{ml}$)	
	Micro-encapsulation	Nanoencapsulation
0 (Negative contl)	184.352 \pm 0.332a	184.352 \pm 0.332a
25	182.775 \pm 3.005ab	158.625 \pm 10.113c
50	183.890 \pm 4.933a	133.074 \pm 5.374d
75	177.234 \pm 1.751ab	94.260 \pm 0.321e
100	173.408 \pm 4.494b	53.826 \pm 0.796f
200	174.682 \pm 5.641ab	51.323 \pm 0.653f
Vitamin E		
200 (Positive control)	19.273 \pm 3.482g	

Note: Different letters in both in the column and row indicate significant differences ($p < 0.05$) after analysis of variance and a Duncan comparison test.

**Figure 5.** Inhibition activity of drink powder against the malondialdehyde formation.

showed a difference in comparison with MDA level for positive control (vitamin E).

Furthermore, MDA level derived from linoleic acid in the nano-encapsulated drink powder was significantly different from negative control, micro-encapsulated drink powder, and positive control. Our results also found that nano-encapsulation treatment significantly resulted in lower MDA level as more drink powder was provided. In terms of nano-encapsulation treatment, the highest level of MDA was attributed to 25 ppm, while the lowest was found at 200 ppm. As depicted in Figure 5, the result clearly displayed that vitamin E at 200 ppm produced the highest inhibition activity on MDA level followed by nano-encapsulation and micro-encapsulation. In terms of MDA-inhibition activity, the *in vitro* assay showed the same result as *in vivo*, where nano-encapsulated drink showed the higher activity than micro-encapsulated drink. On diabetic rats, micro-encapsulated and nano-encapsulated drink suppressed MDA formation about 36.63% and 51.95%, respectively. Melekh *et al.* (2017) reported that there was relation between MDA level and enzymatic antioxidant capacity such as superoxide dismutase and catalase.

CONCLUSION

Nano- and micro-encapsulation of Java tea-based beverages could alleviate the MDA formation both *in vivo* and

in vitro assessment, enabling to control blood glucose level and body weight loss of diabetic rats. However, the mode of action on the MDA inhibition is still unclear, which may involve enzymatic or non-enzymatic pathways. Hence, further studies were required, focusing mainly on the inhibitory effects of encapsulation treatment on MDA formation. In addition, there is a need to investigate the anti-hyperglycemic activity of the powder drink in human study.

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