

Antidiabetic activity of thin film containing astaxanthin-loaded nanoemulsion using carboxymethylcellulose sodium polymer on alloxan-induced diabetic rabbit

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ABSTRACT

The present study was conducted to evaluate the potency of thin film containing astaxanthin-loaded nanoemulsion (FDT-As-NE) in lowering blood glucose levels on alloxan-induced diabetic rabbit (ADR). Astaxanthin nanoemulsion (As-NE) was prepared using self-nanoemulsifying method, followed by incorporated into the carboxymethylcellulose sodium matrix polymer using a solvent casting method to form a thin film. The evaluation of FDT-As-NE was performed by chemical, physical, and mechanical characterizations. The administration of thin film was done by an intraoral route. New Zealand albino rabbits were induced with alloxan to get experimental diabetic animals. The antidiabetic activity was carried out in three groups of treatment. Group I was ADR treated by FDT-As-NE, Group II was ADR treated by pure astaxanthin, while Group III was normal control. The measurement of fasting means blood glucose levels was carried out in 0 days (before treatment) and after 14 days of treatment. The histopathological analysis of the pancreas was also examined. Data were statistically evaluated using Kruskal–Wallis statistical test. $P < 0.05$ was considered statistically significant. FDT-As-NE had good physical and mechanical characteristics that suitable for intraoral administration. Group I reduced elevated blood glucose levels compared to Group II ($P < 0.01$). Histopathological examination of pancreatic tissue for a Group I showed the normal condition of pancreatic β -cell, suggesting the absence of any pathological lesions. These results suggest that thin film containing astaxanthin-loaded nanoemulsion administered by an intraoral route potentially useful for reducing glucose levels.

Key words: Alloxan-induced diabetic, astaxanthin, intraoral, nanoemulsion, thin film

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INTRODUCTION

Diabetes mellitus is a metabolic disease which diagnosed based on blood plasma hyperglycemia. In general, in diabetes mellitus patients occur tissue damage and dysfunction of

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pancreatic β -cells.^[1-3] Alloxan is a diabetogenic agent that used to examine the antidiabetic effect in studies involving diabetes. Alloxan-induced diabetes can be done by alloxan administration into animals.^[4]

Astaxanthin is a lipophilic xanthophyll compound which is a red fat-soluble pigment and act as antioxidant. As super antioxidant, astaxanthin has many shortcomings in its use by the oral route; among others, the bioavailability is low due to low solubility in the gastrointestinal tract.^[5] One of the uses of astaxanthin as a super-antioxidant is its activity as an antidiabetic. Astaxanthin can reduce oxidative stress in β -pancreatic cells and can improve blood glucose and serum insulin levels.^[6] Another study showed that astaxanthin helps improves glucose metabolism in diabetes mellitus patients.^[7] However, in those studies, astaxanthin was administered without any preparations that could cover its shortcomings.

Thus, in this study, we offered a new dosage form of astaxanthin for intraoral route of administration as a strategy to increase the effectiveness administration of astaxanthin. In this case, the effectiveness of the dosage form was demonstrated from its antidiabetic activity as our focused. Astaxanthin was loaded into the nanoemulsion using a combination between oil phase, surfactant and co-surfactant that had been optimized in our previous studies.^[8] Thin film preparation was performed using solvent casting method using Carboxymethylcellulose sodium (CMC-Na) polymer as a matrix system. Chemical, physical, and mechanical evaluations of FDT-As-NE were carried out for intraoral purposes. Furthermore, antidiabetic activity was carried out by giving the FDT-As-NE for 14 days to assess the potential effect of this dosage form in lowering blood glucose levels on alloxan-induced diabetic rabbit (ADR). The antidiabetic activity of FDT-As-NE was compared to ADR treated by pure astaxanthin and ADR untreated. The histopathology of pancreatic tissue in ADR after treatment was also assessed.

SUBJECTS AND METHODS

Study materials

Astaxanthin (Astareal[®] L10) was purchased from Fuji Chemical Industries (Japan). Sunflower oil was purchased from Jan Dekker International (Netherland). Polyoxy-35-castor oil (Kolliphor[®] RH40) was purchased from BASF (Indonesia). Polyethylene Glycol (400) (PEG 400) was purchased from Merck, Tbk (Indonesia). CMC-Na (Cellogen[®] FSH) was purchased from Dai-Ichi Kogyo Seiyaku Co., LTD (Japan). Alloxan was purchased from Merck, KGaA (Germany). All chemicals were of analytical grade.

Preparation of FDT-As-NE

Four milligram astaxanthin was dissolved in 1 g of oil phase containing sunflower oil, kolliphor[®] RH40, and

PEG 400 with the ratio of 1:8:1, respectively. Then, the mixture was mixed for 30 min using magnetic stirrer (IKA[®] C-MAG HS7), followed by sonication for 1 h (Krisbow[®]). Nanoemulsion (As-NE) will be formed after addition deionized water until 20 g of solution. As-NE was added slowly into the matrix system containing of 3.5% (w/v) mixture polymer of CMC-Na and PEG 400 with the ratio of 3:1, respectively. Final mixing was done by added deionized water until 100 g of mixture and the mixture was mixed using magnetic stirrer (IKA[®] C-MAG HS7) in 150 rpm for 1 h. Then, wet mixture was poured into the petri dish in diameter of 10 cm and dried by using oven (Memmert[®]) for 6 h at 40°C.

Evaluation of FDT-As-NE

Organoleptic, film thickness, and weight variation of FDT-As-NE

Organoleptic including color, odor, and clarity of thin film was visually observed. Film thickness was measured using micrometer (Mitutoyo[®]) at three locations on the film, while weight variation was determined by using analytical balance (Mettler Toledo XS204).

Folding resistance of FDT-As-NE

Folding resistance of FDT-As-NE was determined by folding the film repeatedly at the same location until the film was broken or folded up to 300 times.^[9]

Disintegration and dissolution time of FDT-As-NE

Film disintegration and dissolution time were determined visually by inserting the film in a petri dish containing 25 mL of deionized water at 37°C where the container was shaken every 10 s. The time when the film starts to break is called the disintegration time, and then the time when the film has dissolved completely is called the dissolution time.^[9]

Mechanical stress tests of FDT-As-NE Tensile strength and percent elongation

Mechanical stress tests of FDT-As-NE were performed using universal testing machine (Oriented UCT-5T). Dry film was cut into uniform sized pieces using sharp bladed cutting mold. Film (with area exposed to the stress of 4 mm × 25 mm) was sandwiched between two jaws. The load was given to the film gradually and automatically at a speed of 30 mm/min until the film was split. Test was carried out at 23°C ± 2°C and 50% ± 5% relative humidity.^[9]

Content uniformity of astaxanthin in FDT-As-NE

Content uniformity was carried out by dissolving FDT-As-NE (with size of 3 cm × 3 cm) in volumetric flask containing 10 mL of phosphate buffer pH 6.8 for 30 min. Then, the absorbance was measured by UV-Visible Spectrophotometer (Genesys[™] 10S) at the maximum wavelength of 472 nm. Content uniformity in FDT-As-NE was calculated by estimating the astaxanthin content in individual film.^[9]

Experimental animals

All of the animals used for this study were obtained from IPB University, Bogor. Female albino rabbits strain New Zealand with weights ranging from 2.0 to 2.5 kg and ages ranging from 2 to 4 months were used for the experiment. The study protocol was approved by rResearch Ethics Committee of Padjadjaran University, Bandung, Indonesia, with number of 1077/UN6.KEP/EC/2019. The animals were housed in acrylic cages with dimensions of 60 cm × 40 cm × 40 cm. The temperature in the experimental animal room was maintained at 22°C ± 2°C with relative humidity 30-70%. Artificial lighting around 800–1300 lux/m² with the sequence being 12 h light and 12 h dark. Food and water were provided ad libitum. The cage was cleaned every 2 days and every cage was filled with 3 rabbits.

Experimental design

The antidiabetic activity study was carried out after the test animals were acclimatized for 5 days. Animals were divided into three groups, each group consisting of three animals. The groups of animal are shown in Table 1.

Before induction, the blood glucose levels of each animal in three groups were determined. Induction of diabetes was done by intravenous injection for 3 times of alloxan solution in aqua pro injection (150 mg/kg body weight) into the rabbit after fasting for 10 h. After that, rabbits with blood glucose levels of >200 mg/dL were included in the study. Treatment with FDT-As-NE and pure astaxanthin were started 48 h after alloxan injection. Blood collection was carried out through the rabbit ear vein and blood glucose levels were determined before and 2 h after treatment everyday by using Accu-Chek® active blood glucose meter kit (Roche, Germany).

Histopathological analysis

The pancreas organ was isolated and adherent tissue was cleared. The histopathological analysis was carried out by fixation of pancreatic tissue by using 4% formaldehyde. After that, dehydration was done by inserting pieces of tissue into the series of alcohol solution until dried out. Embedding was carried out in oven at temperature of 58°C–60°C by soaking the tissue in liquid paraffin until the tissue was hardened. Finally, the tissue was examined microscopically for any gross lesions in all the animals after coloring of the tissue using eosin.

Statistical analysis

Data comparisons were performed statistically by using Kruskal–Wallis test. Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

In this study, As-NE was prepared by using self-nanoemulsifying method which refers to the previous study.^[8]

When astaxanthin is incorporated into the nanoemulsion system, it will dissolve in the oil phase and enters the micelles system, which thermodynamically stable, with the droplet size is <100 nm.^[10] As-NE had droplets size in the nano-range (10–20 nm) with polydispersity index was <0.5 which indicates that the nanoemulsion had uniform size distribution and stable for a long period of time.^[11] Meanwhile, As-NE had zeta potential value was more than (–20) mV which indicates that As-NE was stabilized by sterically because the system contains nonionic surfactant polymer.^[10]

The thin film properties containing astaxanthin nanoemulsion (As-NE) are presented in Table 2. In intraoral thin film formulation, the concentration of polymers and plasticizers is the main ingredients that can affect the film formed. In the case of the use of film in the oral cavity, the polymer should exhibit a mucoadhesive property. CMC-Na is one of the mucoadhesive polymers and widely used in oral and topical pharmaceutical formulations. CMC-Na may also be used to stabilize emulsions. Encapsulation with CMC-Na can affect drug protection and delivery.^[12] Then, in our thin film formulation, we were chosen low molecular PEG 400, as a plasticizer. Plasticizer helps improve the film flexibility and reduces the film brittleness by reducing the glass transition temperature of the polymer.^[13]

The intraoral film must be flexible, soft, elastic, but must be strong enough against stress condition in the oral

Table 1: *In vivo* experimental design of antidiabetic activity study

Groups	Treatment
I	ADR treated by FDT-As-NE intraorally, once daily for 14 days. The dose was given equally to 0.19 mg of astaxanthin/kg rabbit weight
II	ADR treated by pure astaxanthin intraorally, once daily for 14 days. The dose was given equally to 0.19 mg of astaxanthin/kg rabbit weight
III	Normal control

ADR: Alloxan-induced diabetic rabbit, FDT-As-NE: Film containing astaxanthin-loaded nanoemulsion

Table 2: Physical and mechanical evaluations of film containing astaxanthin-loaded nanoemulsion

Parameters	Observation/results
Organoleptic	Orange, clear, and odorless film
Thickness (mm)*	0.12±0.06
Weight uniformity/sheet 3×3 cm (mg)*	107.8±0.7
Folding resistance (times)	>300
Film disintegration time(s)*	31.00±1.0
Film dissolution time(s)*	67.33±3.1
Tensile strength (MPa)*	13.16±1.16
Percent elongation (%)*	44.40±5.43
Content uniformity/sheet 3×3 cm (mg)*	1.1±0.007

*Values are given as mean±standard deviation (n=3)

cavity.^[14] The thickness of the film dosage form is related to the amount of drug entrapped in the matrix film and also affects the ease of film administration to the patient. A good film thickness should be in the range of 5–200 μm .^[15] Film weight variation describes the variation amount of the drug in the film. The greater of the film weight variation, the greater the nonhomogeneity in drug content between film units.^[16]

The mechanical properties of the film are described by percentage elongation and tensile strength values.^[17] Low tensile strength and percent elongation value indicate weak and soft film properties and vice versa.^[18] The concentration and types of polymers affect the mechanical properties of the film.^[19] FDT-As-NE with a dimension of 6 cm^2 which were made have good weight uniformity, where the deviation value was $<1\%$. Folding resistance of all films was more than 300 times, which indicates that the film had good flexibility.^[20] The formed film has good physical characteristics, especially on mechanical stress resistance.

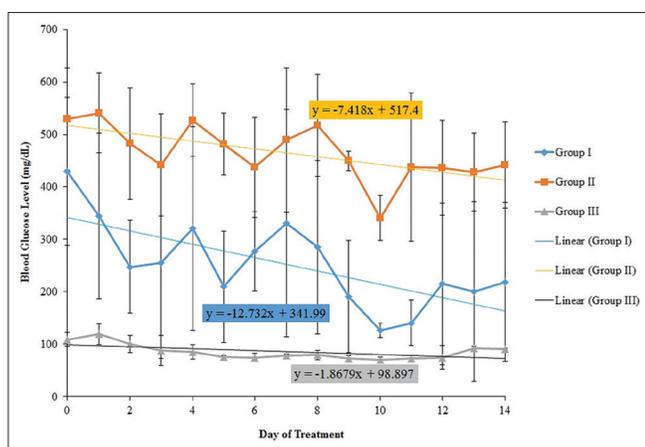


Figure 1: Effect of FDT-As-NE on fasting blood glucose level in alloxan-induced diabetic rabbit. Group I: Alloxan-induced diabetic rabbit treated by FDT-As-NE intraorally; Group II: Alloxan-induced diabetic rabbit treated by pure astaxanthin intraorally; Group III: Normal control. Data were expressed as mean \pm standard deviation ($n = 3$)

The effectiveness of FDT-As-NE was demonstrated by testing the antidiabetic activity on ADR as our focus. The effect of As-NE compared to pure astaxanthin on fasting blood glucose levels is presented in Figure 1. In this research, we have demonstrated that the treatment with astaxanthin packaged in nanoemulsion can improve glycemic control of pancreatic β -cell function in ADR. For group I, As-NE loaded into fast dissolving thin film preparation was administered intraorally, we have a premise that after the thin film dissolved, and nanoemulsion containing astaxanthin will diffuse out into the blood vessels in the mouth cavity. As-NE will enter the blood vessels through the paracellular mechanism.^[21] As a comparison, we performed treatment using pure astaxanthin (Group II) to see the effect of nanoemulsion on the effectiveness of astaxanthin. Our results showed a significant reduction ($P < 0.01$) in blood glucose levels in ADR treated with FDT-As-NE ($51.21\% \pm 20.02\%$) compared to pure astaxanthin ($15.75\% \pm 12.46\%$) after 14 days of treatment. Figure 1 shows the rate declining of blood glucose levels on ADR treated by FDT-As-NE was higher than ADR treated by pure astaxanthin. The rate of decline was indicated by the slope of the curve between periods of treatment and blood glucose levels.

A histopathological evaluation was conducted on pancreas organ. From the histopathological examinations [Figure 2] of the pancreas, in the normal control group, the islet boundaries were clear clearly visible. No necrosis or fatty degeneration observed. Similar to normal control group, sample of pancreatic tissue of group I treated by FDT-As-NE showed that Langerhans cells were normal without any lesions.

CONCLUSION

In this research, fast dissolving thin film containing astaxanthin nanoemulsion for intraoral administration was successfully developed and potentially useful for reducing blood glucose levels.

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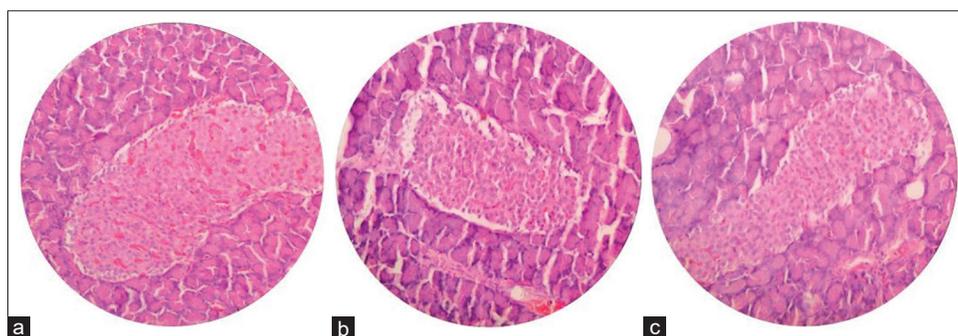


Figure 2: Histopathology of pancreatic tissue of alloxan-induced diabetic rabbit. (a) Group I was alloxan-induced diabetic rabbit treated by FDT-As-NE intraorally; (b) Group II was alloxan-induced diabetic rabbit treated by pure astaxanthin intraorally; (c) Group III was normal control

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Conflicts of interest

There are no conflicts of interest.

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