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Intestinal Absorption Of Astaxanthin Emulsion Formulations in Rats

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Introduction

The small intestine is the portal organ for nutrient and drug absorption. Its long length, inestimable villi and the presence of microvilli, contributing to its vast available surface area accessible for absorption[1]. Generally, all substances absorbed by intestine are through active transport phenomenon. However, most of lipid soluble compound such as carotenoid are transferred through the intestinal mucosa by passive diffusion transport system. In this system, the rate of absorption is determined by its concentration gradient across the intestinal membrane[2]. It is interesting to note that there is a limited quantity of carotenoid being absorbed through the intestinal mucosa despite no energy expanded in passive diffusion resulting in low bioavailability characteristics[3]. The same pattern can also be observed in astaxanthin ie member of xanthophyll group. Astaxanthin, also known as 3, 3'-dihydroxy-β-β'-carotene-4-4'-dione is a naturally occurring carotenoid produced from *Haematococcus pluvialis* and found abundantly in marine seafood. It possesses strong antioxidant property and has important applications in the nutraceutical, cosmetics, and food and feed industries[4]. Additionally, scientific reports indicate that astaxanthin, because of its antioxidative properties which is 10 times more potent than other carotenoids[5] have anti tumor[6] and anti-inflammatory activities[7], positive effects on blood pressure[8] as well as a cardioprotective effect[9].

Despite various benefits mentioned, astaxanthin has very low bioavailability characteristics. One of the approaches that can be used to improve the bioavailability of lipophilic entities such as astaxanthin is to incorporate them in the fine particles of oil-in water (o/w) emulsion[10], In the present study, astaxanthin emulsion droplet size was reduced to macro and nanosized in our attempt to improve astaxanthin bioavailability. This finding proved that astaxanthin administered in nanosize has the highest plasma concentration as compared to macrosized emulsion and in oil solution[10]. Unfortunately, this research only focused on oral bioavailability performance and to the best our knowledge, intestinal absorption of astaxanthin is lacking. Therefore, this research focused on the experimenting and analysing of astaxanthin in rats' intestine blood plasma. We evaluated the rate and extent of intestinal absorption of macro, nanosized emulsion and oil solution formulation of astaxanthin in rats. This study was carried out in the laboratory where the scope of work started with the preparation of the astaxanthin emulsion formulation as per method developed by Affandi et al[11] followed by *in vivo* absorption experiments on rats. All results were evaluated on every aspect to ensure the experiment's authenticity.

Methods

Preparation of astaxanthin emulsion formulation, HPLC condition & sample analysis

The astaxanthin macro and nano sized emulsion was formulated as per method developed by Affandi et al[10]. In this study, astaxanthin oil preparation was used as the reference formulation.

The HPLC system which comprised of Waters 1525 Binary HPLC Pump (Waters Corporation, Milford, Massachusetts, USA), equipped with a Waters 717 plus auto-sampler and a Waters 2475 Multi K Fluorescence Detector (Waters Corporation). The detector was operated at a wavelength of 474mm with a

flow rate of 1 mL/min. For the chromatographic separation, a Phenomenex HPLC column (Phenomenex, USA) fitted with Synergi 4pm Hydro-RP 8oA (150 x 4.6mm) and a refillable guard column (Phenomenex, USA) was used. Mobile phase consisted of methanol: ethyl acetate: water (82: 10: 8%).

Once the blood samples collected from the jugular vein, the plasma was then analysed using the following procedure: 100 µL of plasma was measured into an Eppendorf microcentrifuge (Eppendorf, USA) and deproteinised using 200 µL of mixture of ethanol and tetrahydrofuran (1:9). It took 2 minutes to vortex the plasma using the vortex mixer. After that, it was centrifuged at 14000 rpm for 20 minutes and 50 µL of the clear supernatant was then injected into the HPLC system. The astaxanthin was eluted using the isocratic gradient with methanol: ethyl acetate: water (82:10:8) as a mobile phase. Astaxanthin was detected at 474 nm and eluted at 4.1 minutes.

In vivo intestinal absorption protocol & statistic analysis

All experiments were carried out in accordance with the guidelines of the experimental animal care and approval from UiTM Animal Research and Ethics Committee of Universiti Teknologi MARA. Absorption of astaxanthin emulsions were examined using an in situ closed-loop technique, as reported previously[3]. The rats were divided into 3 groups of 5 rats each. The male Wistar rats (body weight, 250-280g) were fasted overnight, 16 hr prior to the start of the experiment and anesthetized with intra peritoneal sodium pentobarbital (32 mg kg^{-1}). The intestine was exposed through a midline abdominal incision. After ligating the bile duct, the whole small intestine was isolated and both ends were tied off to form a closed loop. Drug emulsion was introduced into the loop through a cannulated opening in the proximal portion and clamped with forceps. The jugular vein was exposed and blood samples were collected into heparinized syringes at eight points of predetermined time interval (0, 10, 30, 60, 90, 120, 180, 240 min). The concentration of drugs in plasma was determined by using HPLC method developed by Afffandi et al[10] and the plasma concentrations-time profiles of drugs with three different formulations were plotted. Peak concentration (C_{max}) and time to reach peak concentration (T_{max}) were determined directly from plasma concentration-time curves. The area under the curve (AUC) was calculated using the trapezoidal method from zero to the final sampling time (240 min). The extent of bioavailability was calculated as follows:

$F= AUC_{(intestine)} - D_{(i.v)}/AUC_{(i.v)} . D_{(intestine)} X 100$

Where F is bioavailability (%) and D is the administered dose. The $D_{(intestine)}$ is the drug dose administered to the intestine while D(i.v) is the dose for intravenous administration.

Results were expressed as the mean and standard deviation of at least three experiments and statistical significance was performed by analysis of variances (ANOVA) with $p<0.05$, as the minimum level of significance.

Results & Discussion

Effects of emulsion droplet size on astaxanthin bioavailability

The plasma profile of astaxanthin in adult Wistar rats was analysed in order to study the effects of three different droplet size emulsions formulations on rats' intestinal absorption. As illustrated in Figure 1, it is evidently proven that astaxanthin in nanosize and macrosize emulsion represents improvement on drug absorption as compared to astaxanthin in oil solution. Although the result showed that the absorption between groups of those nanosized emulsion and macrosized emulsion were not significantly different from each other, there is a significant effects and bioavailability of the nanosized and macrosized emulsions formulations against the oil solutions. This was parallel with the facts reported by Odeberg et al[12] which indicate that the absorption of astaxanthin could be enhanced when surfactants with the right combination of lipophilic and hydrophilic were added in the formulations.

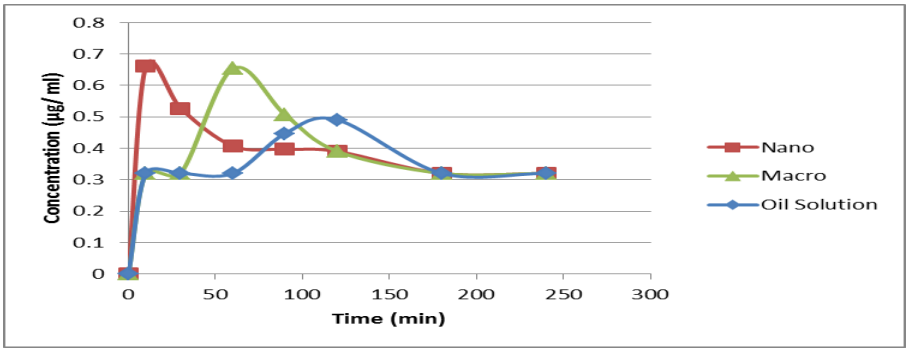

Figure 1 Rat plasma concentration versus time profile of nanosized emulsion, macro sized emulsion and oil solution of astaxanthin after administered using in-situ closed loop technique (n=9).

Effects of emulsion droplet size in C_{max}, T_{max} and $AUC_{0-\infty}$,

Figure 1 shows that astaxanthin formulation administered in nanosize has the highest astaxanthin concentration followed by the macrosized emulsion and oil solution. Table 2 concluded that by decreasing the droplet size of emulsion resulted in increasing amount of C_{max}. However, there were no significant differences of C_{max} between the groups of formulation. This may be due to long interval between blood sampling which were 0, 10, 30, 60, 90, 120, 180 and 240 minutes. It was suggested that the interval time should be shorter between blood sampling to avoid any missed point where the astaxanthin peak might appear in the chromatograms. For example, nanosized emulsion formulations, the absorption was faster between the other two formulations of emulsion. Thus, it is assumed that the C_{max} of nanosized astaxanthin concentration may appear between the intervals of 10 minute to 30 minute.

Observation continues with the effects of decreasing the droplet size emulsion to $AUC_{0-\infty}$. By decreasing the droplet size emulsion resulted in increasing in $AUC_{0-\infty}$ (Table 1).The mean value of $AUC_{0-\infty}$ nanosized astaxanthin emulsion was 93.75 hr.ng/ml while for macrosized emulsion and oil solution were 93.42 and 87.19 hr.ng/ml respectively. There was a significance difference between the $AUC_{0-\infty}$ of nanosized emulsion and AUC om of oil solution (P = 0.03) and also between AUC 0-00 macrosized emulsion and $AUC_{0-\infty}$ of oil solution (P = 0.03). However, there was no significance difference of AUC 0-00 between nanosized emulsion and macrosized emulsion formulations. From the results obtained on $AUC_{0-\infty}$and C_{max}, it confirms that by decreasing the size of droplet emulsion, it improves the absorption of astaxanthin in the intestine. The advantage of decreasing the size of emulsion include in increasing their surface area where it allows effective absorption of the molecule into the circulatory system. Thus, enhancing the bioavailability of the drugs[13]. It is proven from the data of T_{max} (Table 1). Nanosized emulsion had the shortest T_{max} value of 10 min while macrosized with 60 min and oil solutions with average of 100 min.

Table 1 Individual numerical value of C_{max}, T_{max} and $AUC_{0-\infty}$, of three types of formulations tested on nine animals (n = 9)

	Nano			Macro			Oil		
N / Time	1	2	3	1	2	3	1	2	3
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.64	0.69	0.65	0.32	0.32	0.32	0.32	0.32	0.32
30	0.51	0.55	0.52	0.32	0.32	0.32	0.32	0.32	0.32
60	0.41	0.41	0.40	0.61	0.75	0.60	0.32	0.32	0.32
90	0.41	0.39	0.39	0.49	0.58	0.45	0.32	0.56	0.46
120	0.39	0.39	0.39	0.39	0.39	0.39	0.63	0.45	0.39
180	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
240	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
AUC	93.67	94.62	92.95	92.73	96.60	90.94	89.90	88.86	82.81
C_{max}	0.64	0.69	0.65	0.61	0.75	0.60	0.65	0.56	0.56
T_{max}	10	10	10	60	60	60	120	90	90

Conclusion

This study confirmed that the bioavailability of astaxanthin could be enhanced in intestinal absorption of Wistar rats with the reduction of oil droplet size. The nanosize emulsion shows an increase in C_{max} value and $AUC_{0-\infty}$ value compared to macrosize and oil solution. Another trend could be observed from this experiment was that the decreasing of T_{max} values between the three emulsion formulations. The T_{max} value decreased with reduction of the emulsion globule size. The nanosize emulsion shows a decreased in T_{max} value compared to macrosize and oil solution formulations.

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