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Evaluation of Encapsulated Astaxanthin from White Shrimp Shells (*Litopenaeus vannamei*) on Hepatotoxicity

(Penilaian Astaxantin Berkapsul daripada Kulit Udang Putih (*Litopenaeus vannamei*) terhadap Kehepatoksikan)

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ABSTRACT

Recent advances in astaxanthin encapsulation have been reported, but hepatotoxic effect remains unclear. The present investigation therefore aimed to examine the effects of encapsulated astaxanthin from white shrimp shells (*Litopenaeus vannamei*) on liver toxicity. Wistar rats were divided into 6 groups as control (C), and receiving vitamin E (VE), astaxanthin commercial (AC), astaxanthin extracted from white shrimp shells (AE), astaxanthin encapsulation into powder form (AP), and blank powder (BP). The evaluation of liver in response to astaxanthin administration was then assessed in terms of biochemical parameters and histopathological features. Liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), showed no significant differences among all groups of treatment. Histopathological study showed no abnormal changes on liver tissue including hepatic inflammation. Our data demonstrated that astaxanthin encapsulation did not increase the expression of NF-κB nuclear translocation and CYP2E1 in comparison with the control group. Additionally, in this study, the consumption of astaxanthin and vitamin E resulted in a reduction in the oxidative stress index (OSI), while the levels of antioxidant enzymes, including glutathione peroxidase (GPx) and superoxide dismutase (SOD), were significantly increased compared to the control group. Our data suggested that astaxanthin encapsulation does not cause hepatic toxicity, contributing useful information in the applications of astaxanthin encapsulation technology.

Keywords: Astaxanthin; encapsulation; histopathology; liver; white shrimp shells

ABSTRAK

Perkembangan terkini dalam enkapsulasi astaxantin telah dilaporkan, namun kesan hepatotoksik masih tidak jelas. Oleh itu, penyelidikan ini bertujuan untuk mengkaji kesan astaxantin terkapsul daripada kulit udang putih (*Litopenaeus vannamei*) terhadap ketoksikan hati. Tikus Wistar dibahagikan kepada 6 kumpulan sebagai kawalan (C), serta menerima vitamin E (VE), astaxantin komersial (AC), astaxantin yang diekstrak daripada kulit udang putih (AE), astaxantin enkapsulasi dalam bentuk serbuk (AP) dan serbuk kosong (BP). Penilaian hati sebagai tindak balas kepada perlakuan astaxantin kemudiannya dinilai daripada segi parameter biokimia dan ciri histopatologi. Enzim hati, aspartat aminotransferase (AST) dan alanin aminotransferase (ALT), tidak menunjukkan perbezaan yang signifikan antara semua kumpulan rawatan. Kajian histopatologi mendedahkan tiada perubahan abnormal pada tisu hati termasuk keradangan hepatik. Data kami menunjukkan bahawa enkapsulasi astaxantin tidak meningkatkan pengekspresn translokasi nuklear NF-κB dan CYP2E1 berbanding kumpulan kawalan. Di samping itu, dalam kajian ini, penggunaan

astaxantin dan vitamin E mengakibatkan pengurangan dalam indeks tekanan oksidatif (OSI), manakala tahap enzim antioksidan, termasuk glutation peroksidase (GPx) dan superoksida dismutase (SOD), meningkat dengan signifikan berbanding kumpulan kawalan. Data kami mencadangkan bahawa enkapsulasi astaxantin tidak menyebabkan ketoksikan hepatik, lantas menyumbang maklumat berguna dalam aplikasi teknologi pengkapsulan astaxantin.

Kata kunci: Astaxantin; enkapsulasi; hati; histopatologi; kulit udang putih

INTRODUCTION

Drug delivery system has been extensively investigated to potentiate the treatment of several diseases, for example, neurological diseases and cancers (Coelho et al. 2010). Regarding encapsulation, it is the technique in which active or core material is entrapped within the distinctive different coating material or shell. Extensive studies have developed various processes to achieve the encapsulation including chemical, physicochemical, and mechanical processes based upon the desired applications (Al-kasmi et al. 2017). Notably, encapsulation of bioactive compounds or drugs is a useful technology to pack the compounds in the sealed capsules, which can protect the compounds from degradation or inactivation, improve the delivery bioavailability and stability, and allow the control of drug release. Despite many advantages of using encapsulation technology that have been demonstrated, some of them have been shown to cause hepatotoxicity (Coelho et al. 2010; Inglut et al. 2020; Sun et al. 2021; Yao et al. 2019).

Drugs, bioactive compounds, toxic substances, or even nanoparticles are metabolised mainly in the liver. In addition to the drugs metabolism, liver is also responsible for several physiological functions including macronutrient metabolism, storage of glycogen and fat-soluble vitamin, detoxification, and immune surveillance. Injury to the liver results in the alterations of basic functional unit of liver structure which consists of a majority of hepatocytes, Kuffer cells, hepatic stellate cells, and hepatic sinusoidal endothelial cells, accompanied by the impairment of hepatic function (Sun et al. 2021; Yao et al. 2019).

Astaxanthin, mostly found in marine organisms including shrimps, is a known potent antioxidant and exerts several biological activities (Chintong et al. 2019; Takasima et al. 2019; Tanasawet et al. 2020; Xiong & Chang 2020). It has been documented that astaxanthin is unstable and poor water-soluble, leading to low bioavailability and poor pharmacological activity, thus becoming unfavorable to medical and agriculture applications (Chintong et al. 2019; Sangsuriyawong et al. 2019; Takasima, Limpawattana & Klaypradit 2015). Attempts have been made to encapsulate astaxanthin to improve the stability and solubility. Our previous studies produced encapsulated astaxanthin in many forms (Sangsuriyawong et al. 2019; Takasima, Limpawattana & Klaypradit 2015; Takasima et al. 2019). Interestingly, encapsulated astaxanthin into powder form by using cryogenic incorporated freeze-drying was found to effectively reduced cognitive and memory (spatial and non-spatial) impairment as well as reduced oxidative damage and increased neurons survival in Alzheimer's animal model (Takasima et al. 2019). However, the hepatotoxicity of encapsulated astaxanthin has not yet been definitively confirmed. Therefore, the objective of this study was to examine the hepatotoxicity of encapsulated astaxanthin from white shrimp shells and the biochemical changes in Wistar rats.

MATERIALS AND METHODS

PREPARATION OF ENCAPSULATED ASTAXANTHIN POWDER (AP)

Astaxanthin was ethanolic extracted from white shrimp shells (*Litopenaeus vannamei*) according to Takasima, Limpawattana and Klaypradit (2015). For encapsulated astaxanthin powder, it was produced from emulsion of astaxanthin as previously described (Takasima et al. 2019). Alginate (2.0 g/100 mL) and modified starch (20 g/100 mL) were mixed, and the resultant mixture was used as wall material solution. The crude astaxanthin (2% w/v) was homogenized with the wall material solution using a homogenizer (IKA T-25-Werke Gmbh & Co.KG, Staufen, Germany) at 10,000 rpm for 20 min. The emulsion was converted into powders using cryogenic incorporated with freeze drying. The obtained encapsulated astaxanthin powder was collected and kept in amber bottles.

EXPERIMENTAL ANIMALS

In this study, 48 male Wistar rats (8 weeks old, 250-300 g) were purchased from Nomura Siam International Co., Ltd. They were housed with light and dark cycles of 12 h and 12 h with *ad libitum* access to standard laboratory food and water. The animal protocols were approved by the Institutional Animal Care and Use Ethical Committee (MOE 0521.11/105) of Prince of Songkhla University. Rats were divided into 6 groups as follows: Group 1, control group (C) Group 2, vitamin E group (100 mg/kg/day) (VE); Group 3, astaxanthin commercial group (10 mg/kg/day) (AC); Group 4, astaxanthin extracted from shrimp shells group (10 mg/kg/day) (AE); Group 5, astaxanthin encapsulation into a powder form group (10 mg/kg/day)

(AP); and Group 6, blank powder group (10 mg/kg/day) (BP). At day 30th of treatment, livers were surgically collected for the biochemical assays and histological analyses.

BIOCHEMICAL INDICATORS OF LIVER FUNCTION

The liver tissues were washed with ice-cold 0.9% normal saline and dissected into small pieces on ice. Liver homogenate tissues were prepared using 0.01 M Tris-HCl buffer pH 7.4 at 4 °C and centrifuged at 14,000 × g for 30 min at 4 °C. The supernatant fluid from the liver homogenates was used for the assays. The levels of liver aspartate aminotransferase (AST) and alanine aminotransferase, (ALT) were evaluated using commercially available kits (Merck Millipore, Darmstadt, Germany) according to the manufacturer's instructions.

HISTOPATHOLOGICAL STUDY

Liver samples were collected and fixed in 10% normal buffered formalin. The fixed tissues were then processed in ascending grades of ethyl alcohol for dehydration, cleared in xylene, and embedded with paraffin. Thereafter, the tissues were sectioned in 5 µm thickness with a rotary microtome (Leica Microsystems, Germany), placed on a silane-coated slice, followed by staining with hematoxylin and eosin (H and E). The liver tissues were visualized under a light microscope (DP73, Japan) for the hepatic damage or morphological alterations.

IMMUNOHISTOCHEMICAL STUDY

Liver tissues were sectioned into 5 µm thickness and were deparaffinized with xylene, rehydrated with a series of ethanol, and blocked with 3% hydrogen peroxide for 45 min to eliminate the endogenous peroxidase. After that, the sections were permeabilized with 0.3% triton X-100 and heat-mediated antigen retrieval with boiling citrate buffer was performed prior to blocking of the unspecific binding sites with blocking solution containing 4% bovine serum albumin for 1 h. The sections were then incubated with primary antibodies namely anti-NF-κB (1: 500) and CYP2E1 (1: 500) (Abcam, Cambridge, USA) at 4 °C overnight. Subsequently, the slices were washed three times with 0.3% PBST followed by incubation with the biotinylated anti-mouse IgG secondary antibodies (1:1000) at room temperature for 1 h and the colorimetric detection with diaminobenzidine (DAB) as a chromogen. Finally, the slides were counter stained with hematoxylin to observe the nucleus and examined under a light microscope (DP73, Japan). Within the liver acinus, it is anatomically divided into three main zones which are zone 1, also known as the periportal zone, zone 2, referred to as the intermediate zone, and zone 3, known as the pericentral zone, which surrounds the central vein. The percentage of immunohistochemically labeled cells was quantified by using the following formula:

$$\% \text{ Labeled cells} = [\text{Number of positive cells counted in five separate microscopic fields (40×) / Total cells}] \times 100$$

DETERMINATION OF TOTAL PEROXIDASE (TP), TOTAL ANTIOXIDANT STATUS (TAS) AND OXIDATIVE STRESS INDEX (OSI)

The determination of total peroxide and total antioxidant status were performed according to previously described method (Sukketsiri et al. 2016). Briefly, liver samples were isolated and homogenized to obtain the supernatant, then they were incubated with ammonium ferrous sulphate buffer (FOX2) reagent for 30 min and centrifuged at 20817 g for 10 min to assess the TP level. The absorbance was measured at 560 nm and H_2O_2 was used as a standard. For TAS level, the supernatant achieved from homogenized liver was mixed with acetate buffer (0.4 mol/L) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) in acetate buffer (30 mmol/L) for 5 min. The absorbance was measured at 660 nm and the data were expressed as an equivalent of the millimolar concentration of the Trolox solution. The OSI was determined according to the following formula:

$$\text{OSI} = [\text{TP (mmol/L)/ TAS (mmol Trolox equivalent/L)}] \times 100$$

DETERMINATION OF GLUTATHIONE PEROXIDASE (GPx) AND SUPEROXIDE DISMUTASE (SOD)

To determine the major antioxidant enzyme GPx activity, the supernatant was collected from the homogenized liver tissue, followed by incubation with sodium phosphate, ethlenediaminetetraacetic acid, β-nicotinamide adenine dinucleotide phosphate hydrate, sodium azide, glutathione reductase, glutathione, DL-dithiothreitol, and hydrogen peroxide. The absorbance was measured at 340 nm. The measurement of SOD activity was performed using SOD assay kit according to the manufacturer instruction (Cayman Chemicals, MI, USA). The supernatant from liver homogenate was mixed with the diluted radical detector, followed by incubation with xanthine oxidase for 30 min. The absorbance was determined at 460 nm and compared with SOD standard curve.

STATISTICAL ANALYSIS

The statistical comparison was examined by using one way analysis of variance (ANOVA), followed by Duncan post-hoc comparison. All data were analyzed using SPSS statistical version 11.0 (SPSS Inc., Chicago, IL, USA) and considered as statistically significant at $p<0.05$. Data were presented as mean ± SEM.

RESULTS

The analysis of liver biomarkers AST and ALT demonstrated no significant changes in all groups of treatment. The animals that received AP did not show any change in AST and ALT levels (AST = 2.96±0.28; ALT = 4.71±0.0.06) when compared to the control group (AST = 2.44±0.27 U/mg protein; ALT = 4.34±0.19 U/mg protein) as depicted in Figure 1(A), 1(B). Oral administration of the potent antioxidant VE, AC, and AE also showed no significant differences of AST and ALT enzyme activities (AST = 2.88±0.24, 2.82±0.26, and 2.85±0.27 U/mg protein; ALT = 4.51±0.20, 4.73±0.05, and 4.77±0.07 U/mg protein). Additionally, BP group showed no significant changes of AST (2.94±0.28 U/mg protein) and ALT activities (4.75±0.12 U/mg protein).

Under a microscopic examination from a standard H and E staining, hepatic tissue of normal control group exhibited normal hepatic architectures in three major compartments including portal triads, central veins, and hepatic lobules. Each central vein was found to be surrounded by rays of hepatic cord with normal portal triads at the periphery (Figure 2(A)). Animals that received AP as well as VE, AC, and AE showed normal hepatic structure similar to those observed in the control group as shown in Figure 2(A)-2(E). There were no necroinflammatory patterns, no dilatation of hepatic sinusoids, and no fatty changes of liver cells. Fibrosis was also absent. Figure 2(F) showed no evident of liver injury in BP group.

The immunohistochemical detection of NF-κB p65 in the liver is a crucial aspect of understanding the inflammatory pathway in hepatotoxicity. In the control group, we observed mild expression and low nuclear translocation of NF-κB in the hepatocytes. The expression of NF-κB was predominantly found in zone 2 and 3 (Figure 3(A)), providing insights into the baseline of NF-κB localization in liver tissue. Additionally, zone 2 and 3, which is known as the low-oxygenated zone, are the most susceptible to injury. After the administration of AP, VE, AC, AE, and BP for 30 days, our results demonstrated no significant differences in the nuclear translocation of p65 subunit in the liver compared to the normal control as shown in Figure 3(A)-3(F) and Figure 5. This suggested that the consumption of astaxanthin by all groups did not significantly activate NF-κB signaling.

The cytochrome 2E1 (CYP2E1), a relevant enzyme that metabolizes drugs or toxins specifically found in liver, was examined by immunohistochemical analysis and demonstrated in Figure 4. In control rats, the CYP2E1 was moderately stained in the cytoplasm of normal hepatocytes in zone 2 and 3 of each hepatic lobule (Figure 4(A)). There was a similar expression pattern of CYP2E1 immunoreactivity observed in the AP, VE, AC, AE, and BP groups as demonstrated in Figure 4(A)-4(D). The percentage of CYP2E1 positive staining of AP, VE, AC, AE, and BP (24.58±0.81, 23.61±1.37, 24.35±0.91, 22.11±1.13, and 24.68±1.10%) showed no significant differences when compared to the control group (24.36±1.29%) (Figure 5).

The TP level in the liver of the experimental groups (AP, VE, AC, AE, BP) remained unchanged compared to the normal control group as shown in Figure 6(A). The TAS level of the known potent antioxidants, VE, AC, and AE, was significantly increased (9.12±0.05, 9.79±0.27, and 9.86±0.31 mmol/L, respectively) when compared to the control (5.14±0.05 mmol/L). Interestingly, the administration of AP demonstrated a significant increase in the TAS level (9.36±0.23 mmol/L) compared to the control rats. However, the rats that received BP showed no significant difference of TAS level (5.33±0.10 mmol/L) when compared to the control (Figure 6(B)). In addition, the level of OSI in the AP, VE, AC, and AE groups exhibited significant reductions to 3.05±0.22, 3.26±0.40, 3.05±0.38, and 3.06±0.24, respectively, compared to the control group (5.95±0.51) as demonstrated in Figure 6(C) ($p<0.05$). Nevertheless, BP group showed no significant difference from the control (Figure 6(C)).

The activity of antioxidant enzymes including GPx and SOD were monitored. The GPx activity in the liver tissues of rat fed with antioxidants, VE, AC, and AE (5.24±0.32, 5.51±0.15, and 5.40±0.36 U/mg protein, respectively), were significantly increased compared to the control (3.83±0.36 U/mg protein). Furthermore, the administration of AP also demonstrated significant increase of GPx activity (5.28±0.31 U/mg protein) when compared to the normal control (3.83±0.36 U/mg protein). The BP group showed no significant difference of the GPx activity (3.50± 0.26 U/mg protein, respectively) (Figure 7(A)). In addition, the tissue antioxidant metalloenzyme, SOD, activity was also examined in the liver. Rats that received VE, AC, and AE showed a significant increase in liver antioxidant enzyme, SOD, activity compared to the control (4.61±0.19, 4.51±0.16, 4.51±0.17 U/mg protein, respectively, compared to 2.48±0.18 U/mg protein). The AP treated group had significantly increased SOD level (4.73±0.29 U/mg protein); however, BP group was not significant (2.33±0.17 U/mg protein) (Figure 7(B)). Therefore, AP, AC, AE, and VE effectively prevented oxidative stress by increasing the antioxidant enzyme GPx and SOD.

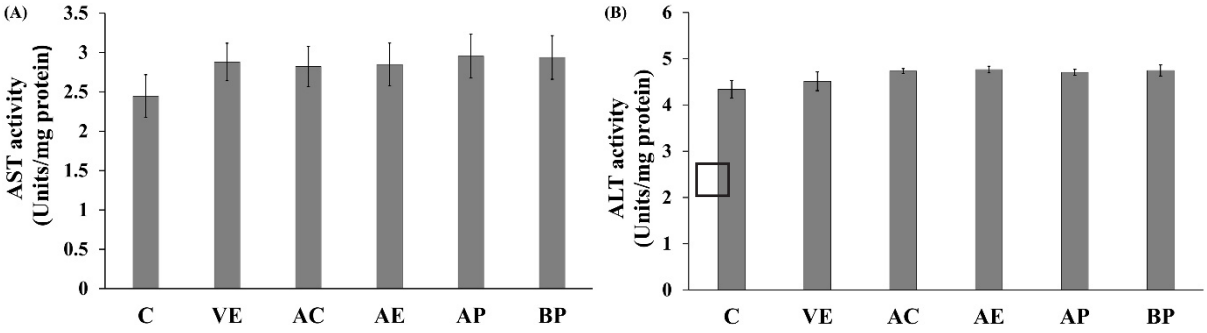

FIGURE 1. Effects of astaxanthin administration on liver enzymes (A) AST and (B) ALT. The values are expressed as mean ±SEM. $p > 0.05$ when compared to the control

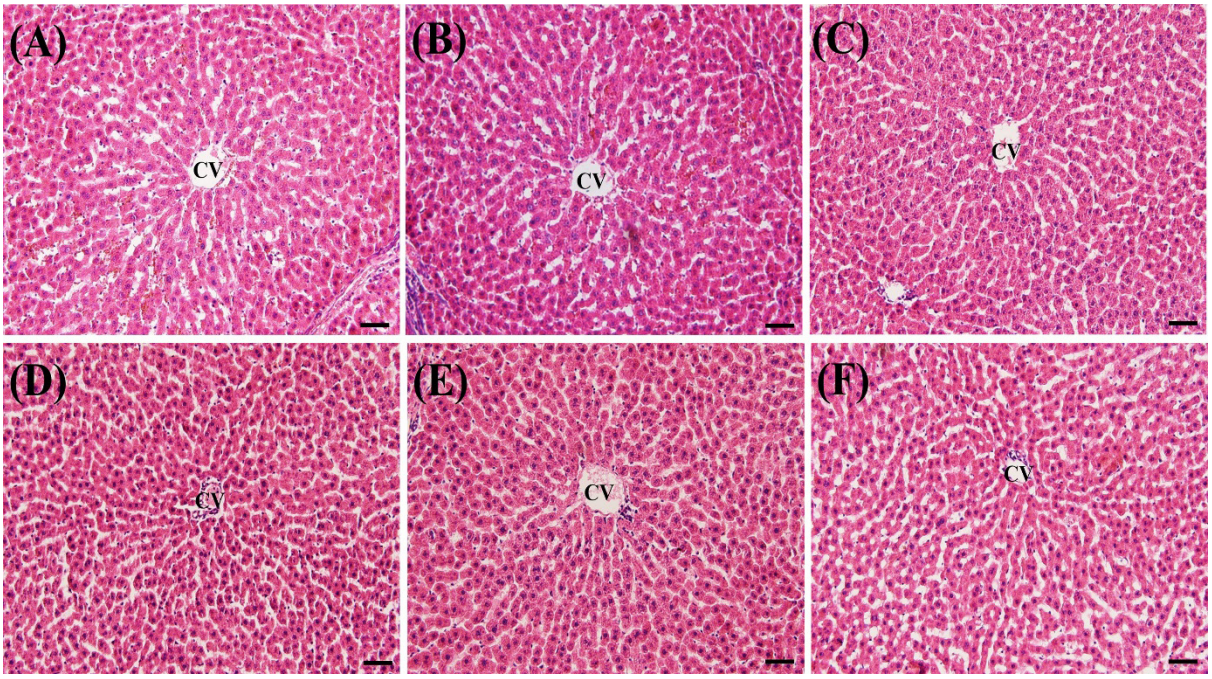

FIGURE 2. Histopathological section of liver tissue stained by hematoxylin and eosin (H and E) showing normal hepatic architectures such as central vein (CV), hepatocytes, hepatic sinusoid from (A) control group; (B) vitamin E group; (C) astaxanthin commercial group; (D) astaxanthin extracted from shrimp shells group; (E) encapsulated astaxanthin powder group; (F) blank powder

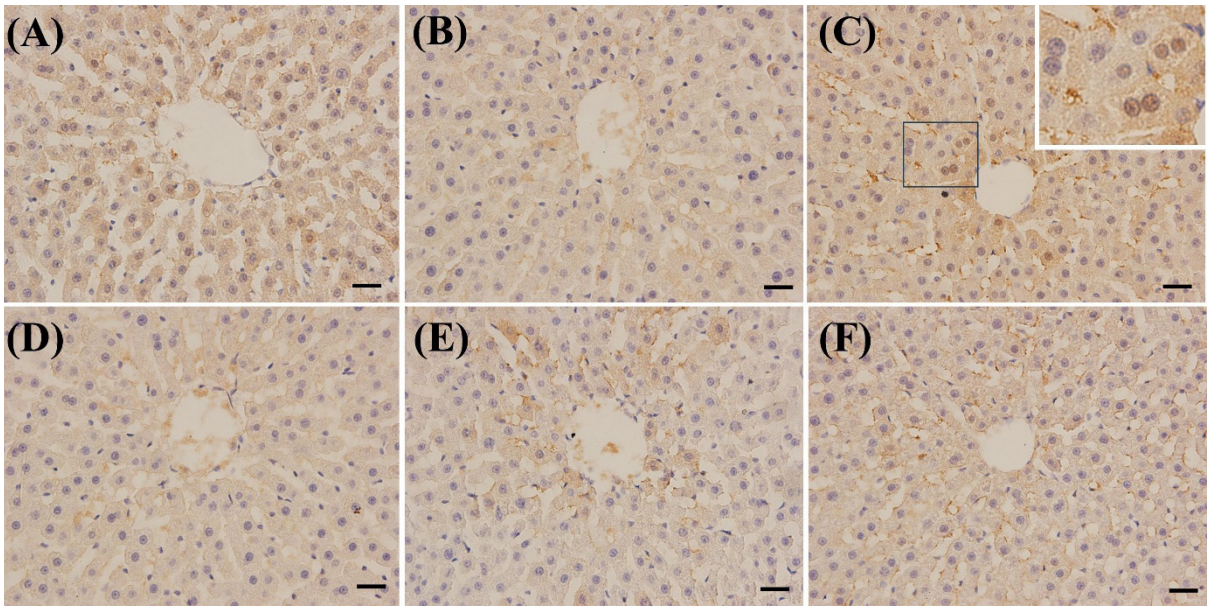

FIGURE 3. Immunohistochemical localization of NF-kB p65 subunit in rat liver sections from (A) control group; (B) vitamin E group; (C) astaxanthin commercial group; (D) astaxanthin extracted from shrimp shells group; (E) encapsulated astaxanthin powder group; (F) blank powder group. Positive staining for NF-κB was demonstrated by the presence of a brownish coloration as depicted in the inset (IHC, DAB, Scale bar = 25 μm)

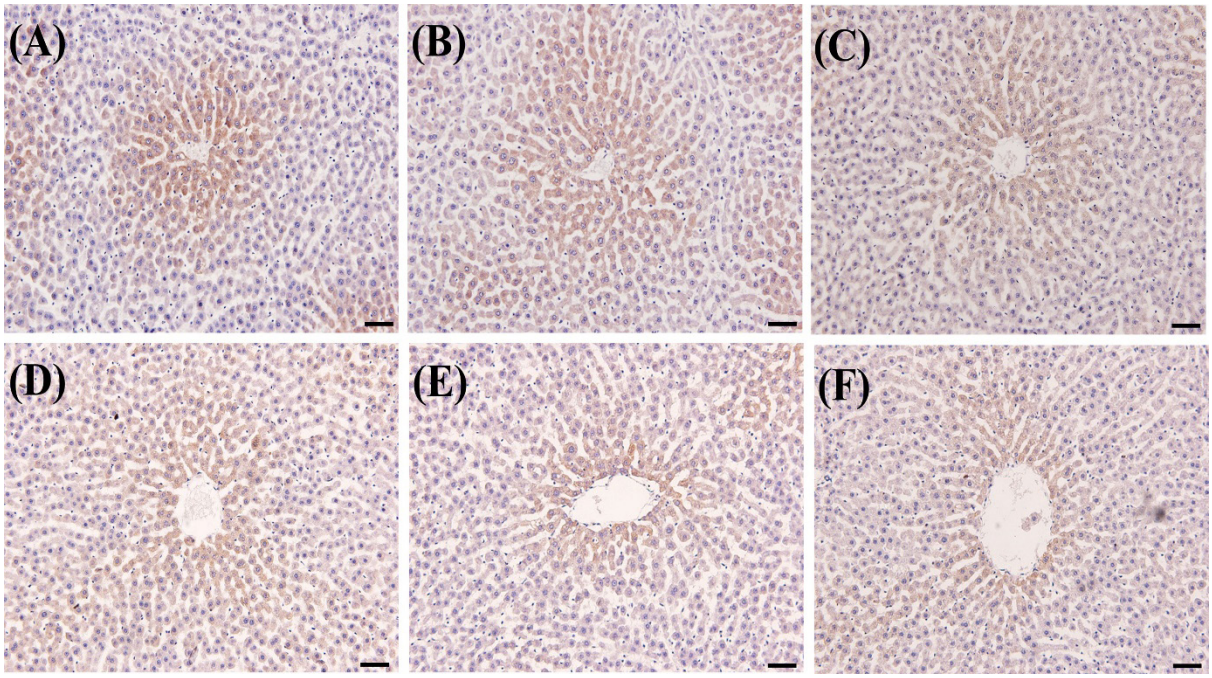

FIGURE 4. Immunohistochemical localization of CYP2E1 in rat liver sections from (A) control group; (B) vitamin E group; (C) astaxanthin commercial group; (D) astaxanthin extracted from shrimp shells group; (E) encapsulated astaxanthin powder group; (F) blank powder group (IHC, DAB, Scale bar = 50 μm)

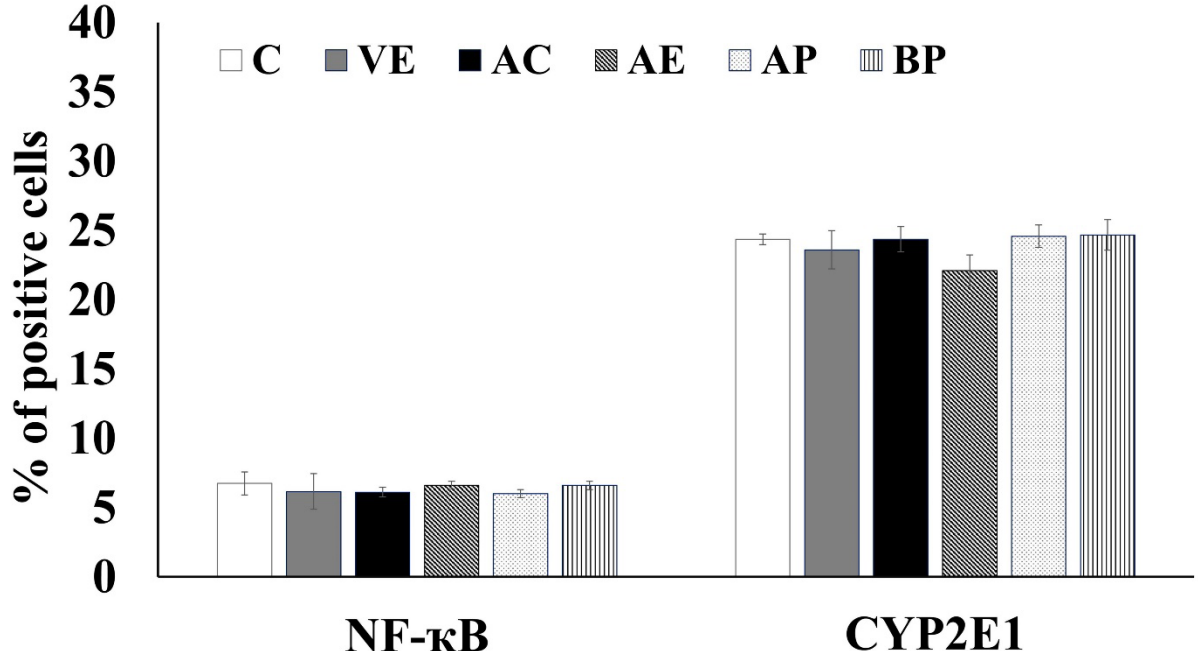

FIGURE 5. Percentage of positive staining of NF-kB (p65) and CYP2E1 after the administration of astaxanthin are expressed as mean ± SEM. $p > 0.05$ when compared to the control

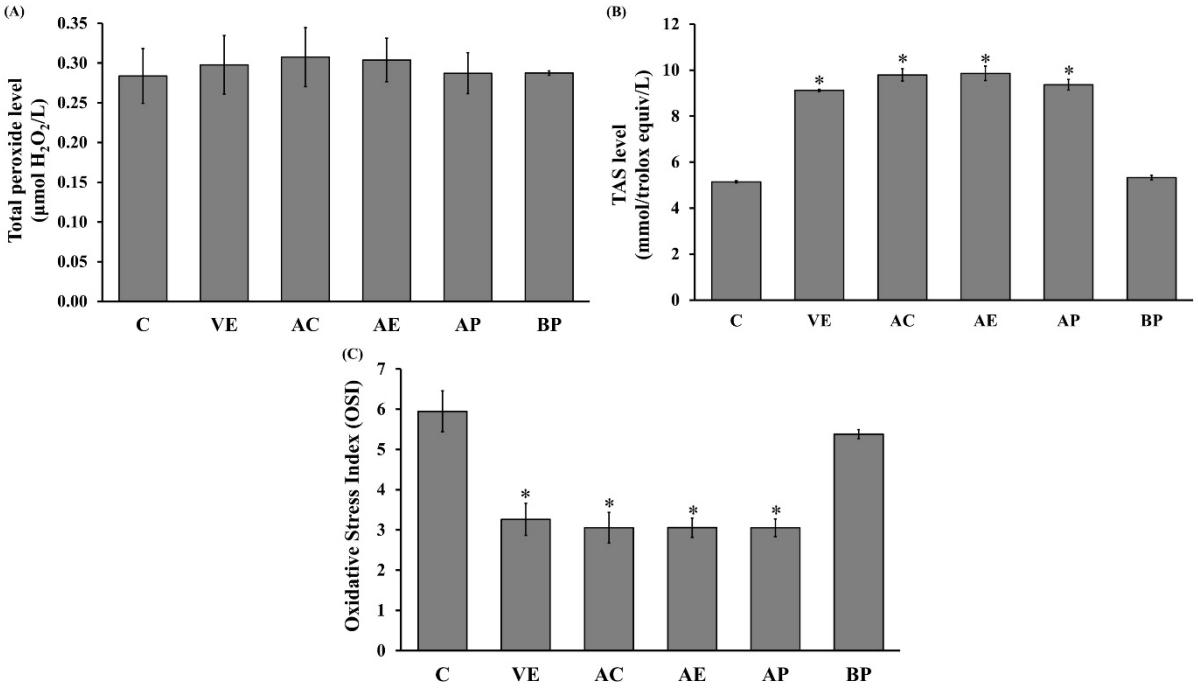

FIGURE 6. Effects of astaxanthin administration on (A) total peroxidase (TP), (B) total antioxidant status (TAS), and (C) oxidative stress index (OSI) in the rat livers. The values are expressed as mean ±SEM. * $p < 0.05$ when compared to the control

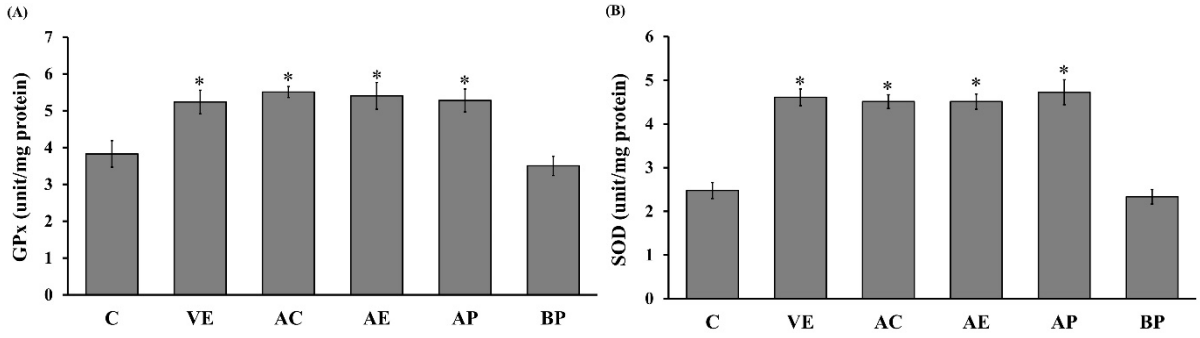

FIGURE 7. Effects of astaxanthin administration on (A) glutathione peroxidase (GPx) and (B) superoxide dismutase (SOD) in the rat liver. The values are expressed as mean ±SEM. * $p < 0.05$ when compared to the control

DISCUSSION

Astaxanthin has one of the most powerful antioxidant capacities, which is widely found in algae, yeast, and crustaceans including shrimps. Several studies demonstrated that shrimp shells are also an excellent source of astaxanthin. In Thailand, there is abundant marine industries which produce shrimp waste, with predominantly Pacific white shrimp (*Litopenaeus vannamei*) due to its potential for scale-up. Astaxanthin from white shrimp shells has been previously reported for several pharmacological effects such as on inflammatory diseases and cancers (Kuedo et al. 2016; Takasima et al. 2019; Tanasawet et al. 2020). It has been reported that astaxanthin has chemical instability and poor oral bioavailability due to its highly conjugated and double bond structure as well as lipophilicity, which considerably limit its pharmacological functionality (Ambati et al. 2014; Martinez-Alvarez, Calvo & Gomez-Estaca 2020). Several attempts have been made to minimize these disadvantages. We recently developed a method to encapsulate astaxanthin obtained from white shrimp shells. The method involves microencapsulation using a wall material consisting of alginate and modified starch. The astaxanthin was then subjected to cryogenic incorporated freeze-drying to obtain a powdered form. By using this encapsulation technology, encapsulated astaxanthin demonstrated a significant reduction of oxidative stress, and improved memory and cognition in amyloid-β induced Alzheimer model (Takasima et al. 2019). However, hepatotoxicity is the major toxicological concerns of using encapsulation technology prior to subjection to humans.

Liver plays a key role in detoxification and drug metabolism. The liver biochemical markers consisting of AST and ALT have been used as a useful indicator for liver damage in the diagnosis of hepatoxicity (Meunier & Larrey 2019). AST, present in both mitochondria and cytoplasm of hepatocytes, is a transaminase enzyme that metabolizes the amino acid aspartate and alpha-ketoglutarate. Additionally, ALT is involved in amino acid metabolism and gluconeogenesis. It is one of the specific indicatives for liver damage due to it mainly concentrated in the cytosol of hepatic cells rather than others (Kobayashi, Suzuki & Sugai 2020). Injury to the liver results in cellular membrane damage, subsequently causes the leakage of these enzymes into the extracellular spaces and thus increased AST and ALT activity. It has been reported that there is a direct association between AST and ALT elevation with the amount of liver injury which possibly caused by drugs or toxins (Giannini, Testa & Savarino 2005; Kobayashi, Suzuki & Sugai 2020).

In this present study, the treatments of AP, AC, AE, VE, and BP for 30 days demonstrated no significant elevation of AST in conjunction with ALT activities compared to the control group, suggesting that encapsulated astaxanthin did not cause liver injury.

At the microscopic level, histopathological assessment of encapsulated astaxanthin treated rats demonstrated normal hepatic architectures similar to those observed in the control group. Liver tissues from the animals in all groups of treatment exhibited the presence of hepatocytes arranged in the form of hepatic cords radiated from the central vein and extending to the portal triads, which were separated by hepatic sinusoids indicating the normal lobular structure of the rat liver. The patterns of liver injury from any chemical toxins or drugs were frequently observed as hepatocellular pattern, pattern of steatosis, cholestatic pattern, and mixed patterns (Kleiner 2017). Liver damage led to the loss of hepatic structure, degeneration of hepatocytes, infiltration of inflammatory cells, hepatic sinusoid dilatation, and necrosis (Gasmi &

Kleiner 2020; Greuter & Shah 2016). Nevertheless, the current study showed that none of these following patterns were observed in all groups of treatment.

Many types of hepatic injury are caused by the generation of oxidative stress, which occurred due to the imbalance between prooxidant and antioxidant system (Chen et al. 2020; Cichoz-Lach & Michalak 2014). Prooxidants, i.e., superoxide radicals, hydrogen peroxide, or other peroxide derivatives, alter the cellular structures, including DNA, proteins, and lipids. These alterations lead to the modification of cell membranes, cellular receptors, and enzymes. The antioxidant systems which include GPx and SOD, combat the unfavorable effects of prooxidants to balance this homeostasis (Arauz, Ramos-Tovar & Muriel 2016; Chen et al. 2020; Cichoz-Lach & Michalak 2014). The current data showed that the administration of astaxanthin encapsulation reduced the OSI, measured by a ratio between pro-oxidant and TAS. Additionally, astaxanthin encapsulation significantly enhanced the antioxidant activity of GPx and SOD. In consistent with this finding, Takasima et al. (2019) reported that astaxanthin from white shrimp shells (AP and AE) increased the percentage inhibition of superoxide anion and GPx activity in cerebral cortex and hippocampus of $A\beta_{1\text{-}42}$- induced Alzheimer's model rats. It has also been reported that astaxanthin from white shrimp shells demonstrated the reduction of superoxide anion radicals in carrageenan-induced acute inflammation model (Kuedo et al. 2016).

In response to liver injury, oxidative stress augments the inflammation through NF-κB activation, which in turns becomes liberated from its inhibitory IkB subunit resulting in the translocation of NF- κB p65 to the nucleus (Luedde & Schwabe 2011; Qin et al. 2014). Particular attentions on the link between NF-κB and a major liver phase I drug metabolism enzyme CYP2E1 have been well documented. NF-κB has a positive correlation with CYP2E1 at both pre- and post-translational level by either binding to the promoter region or activating heme oxygenase and the stability of CYP protein (Qin et al. 2019; Qin et al. 2014; Xu et al. 2018). By using cell specific immunocytochemistry, we reported no increment of NF-κB positive nuclear stained after the administration of AP. Similar findings were also observed in CYP2E1 staining. Based on these results, the AP group appears to cause non-hepatotoxic effect, suggesting that the designed encapsulation technology provides a promising choice for astaxanthin delivery in nutraceutical and food industry.

CONCLUSIONS

The present investigation demonstrated that encapsulated astaxanthin did not cause hepatic damage *in vivo,* as confirmed by liver function tests, histological studies, and immunohistochemical studies. In addition, the encapsulated astaxanthin effectively reduced oxidative stress and increased antioxidant enzyme activity. This encapsulation technique is safe and capable to be applied to other water-insoluble compounds.

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REFERENCES

Al-kasmi, B., Alsirawan, B., Bashimam, M. & El-zein, H. 2017. Mechanical microencapsulation: The best technique in taste masking for the manufacturing scale- effect of polymer encapsulation on drug targeting. *Journal of Controlled Release* 260: 134-141.

Ambati, R.R., Phang, S.M., Ravi, S. & Aswathanarayana, R.G. 2014. Astaxanthin: Sources, extraction, stability, biological activities and its commercial application - A review. *Marine Drugs* 12: 128-152.

Arauz, J., Ramos-Tovar, E. & Muriel, P. 2016. Redox state and methods to evaluate oxidative stress in liver damage: From bench to bedside. *Annals of Hepatology* 15(2): 160-173.

Chen, Z., Tian, R., She, Z., Cai, J. & Li, H. 2020. Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. *Free Radical Biology and Medicine* 152: 116-141.

Chintong, S., Phatvej, W., Rerk-Am, U., Waiprib, Y. & Klaypradit, W. 2019. *In vitro* antioxidant, antityrosinase, and cytotoxic activities of astaxanthin from shrimp waste. *Antioxidants* 8: 128.

Cichoz-Lach, H. & Michalak, A. 2014. Oxidative stress as a crucial factor in liver disease. *World Journal of Gastroenterology* 20(25): 8082-8091.

Coelho, J.F., Ferreira, P.C., Alves, P., Cordeiro, R., Fonseca, A.C., Gois, J.R. & Gil, M.H. 2010. Drug delivery systems: Advanced technologies potentially applicable in personalized treatments. *The EPMA Journal* 1(1): 164-209.

Gasmi, B. & Kleiner, D.E. 2020. Liver histology: Diagnostic and prognostic features. *Clinics in Liver Disease* 24(1): 61-74.

Giannini, E.G., Testa, R. & Savarino, V. 2005. Liver enzyme alteration: A guide for clinicians. *Canadian Medical Association Journal* 172(3): 367-379.

Greuter, T. & Shah, V.H. 2016. Hepatic sinusoids in liver injury, inflammation, and fibrosis: New pathophysiological insights. *Journal of Gastroenterology* 51: 511-519.

Inglut, C.T., Sorrin, A.J., Kuruppu, T., Vig, S., Cicalo, J., Ahmad, H. & Huang, H.C. 2020. Immunological and toxicological considerations for the design of liposomes. *Nanomaterials* 10(2): 190.

Kuedo, Z., Sangsuriyawong, A., Klaypradit, W., Tipmanee, V. & Chonpathompikunlert, P. 2016. Effects of astaxanthin from *Litopenaeus vannamei* on carrageenan-induced edema and pain behavior in mice. *Molecules* 21: 382.

Kleiner, D.E. 2017. Histopathological challenges in suspected drug-induced liver injury. *Liver International* 38(2): 198-209.

Kobayashi, A., Suzuki, Y. & Sugai, S. 2020. Specificity of transaminase activities in the prediction of drug-induced hepatotoxicity. *The Journal of Toxicological Sciences* 45(9): 515-537.

Luedde, T. & Schwabe, R.F. 2011. NF-kB in the liver-linking injury, fibrosis and hepatocellular carcinoma. *Nature Reviews Gastroenterology & Hepatology* 8(2): 108-118.

Martinez-Alvarez, O., Calvo, M.M. & Gomez-Estaca, J. 2020. Recent advances in astaxanthin micro/nanoencapsulation to improve its stability and functionality as a food ingredient. *Marine Drugs* 18(8): 406.

Meunier, L. & Larrey, D. 2019. Drug-induced liver injury: Biomarkers, requirements, candidates, and validation. *Frontier in Pharmacology* 10: 1482.

Qin, J.D., Cao, Z.H., Li, X.F., Kang, X.L., Xue, Y., Li, Y.L., Zhang, D., Liu, X.Y. & Xue, Y.Z. 2014. Effect of ammonium pyrrolidine dithhiocarbamate (PDTC) on NF-kB activation and CYP2E1 content of rats with immunological liver injury. *Pharmaceutical Biology* 52(11): 1460-1466.

Qin, L., Kang, X., Li, X., Wang, T., Liu, F., Jia, J., Jin, Z. & Xue, Y. 2019. NF-kB-mediated regulation of rat CYP2E1 by two independent signaling pathways. *PLoS ONE* 14(12): e0225531.

Sangsuriyawong, A., Limpawattana, M., Siriwan, D. & Klaypradit, W. 2019. Properties and bioavailability assessment of shrimp astaxanthin loaded liposomes. *Food Science and Biotechnology* 28(2): 529-537.

Sukketsiri, W., Chonpathompikunlert, P., Tanasawet, S., Choosri, N. & Wongtawatchai, T. 2016. Effects of *Apium graveolens* extract on the oxidative stress in the liver of adjuvant-induced arthritic rats. *Preventive Nutrition and Food Science* 21(2): 79-84.

Sun, T., Kang, Y., Liu, J., Zhang, Y., Ou, L., Liu, X., Lai, R. & Shao, L. 2021. Nanomaterials and hepatic diseases: Toxicokinetics, disease types, intrinsic mechanisms, liver susceptibility, and influencing factors. *Journal of Nanobiotechnology* 19(1): 108.

Takasima, T., Chonpathompikunlert, P., Sroyraya, M., Hutamekalin, P., Limpawattana, M. & Klaypradit, W. 2019. Effects of astaxanthin from shrimp sell on oxidative stress and behavior in animal model of Alzheimer's disease. *Marine Drugs* 17(11): 628.

Takasima, T., Limpawattana, M. & Klaypradit, W. 2015. Astaxanthin encapsulated in beads using ultrasonic atomizer and application in yogurt as evaluated by consumer sensory profile. *LWT-Food Science and Technology* 62: 431-437.

Tanasawet, S., Sukketsiri, W., Chonpathompikunlert, P., Klaypradit, W., Sroyraya, M. & Hutamekalin, P. 2020. Apoptotic effect of astaxanthin from white shrimp shells on lung cancer A549 cells. *Tropical Journal of Pharmaceutical Research* 19(9): 1835-1842.

Xiong, F. & Chang, M.X. 2020. Astaxanthin and its effects in inflammatory responses and inflammation-associated diseases: Recent advances and future directions. *Molecules* 25(22): 5342.

Xu, L., Yu, Y., Sang, R., Li, J., Ge, B. & Zhang, X. 2018. Protective effects of taraxasterol against ethanol-induced liver injury by regulating CYP2E1. Nrf2/HO-1 and NF-kB signaling pathways in mice. *Oxidative Medicine and Cellular Longevity* 2018: 8284107.

Yao, Y., Zang, Y., Qu, J., Tang, M. & Zhang, T. 2019. The toxicity of metallic nanoparticles on liver: The subcellular damages, mechanisms, and outcomes. *International Journal of Nanomedicine* 14: 8787-8804.

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