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Drug Safety Evaluation of Micro Encapsulated Astaxanthin for Sustained Drug Release

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Abstract:*Astaxanthin is a reddish pigment that belongs to a group of chemical called carotenoids. It occur naturally in certain algae and causes the pinkish red color in salmon, trout, lobster, shrimp and other seafood. It may be used as feed supplement and food coloring additive for salmon, crab, shrimp and chicken. It also may a role in treating various diseases like cancer, Alzheimer's disease, Parkinson's disease, diabetics etc., Hence, standardization of both microencapsulated and non-encapsulated astaxanthin drug is necessary before testing it in DEN induced mice. Astaxanthin was encapsulated using different compound like sodium alginate, calcium chloride, liposomes, chitosan and TPP. Drug standardization and qualitative analysis of heavy metals in test samples were performed. Overall result shows that microencapsulated and non-encapsulated astaxanthin drug have good quality and purity. Further, it can be used against in vitro and in vivo hepatocellular carcinoma in mice.*

Keywords: *Astaxanthin, Standardization, Heavy metals, Foreign matter, Organoleptic features*

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I. Introduction

Microencapsulation is a rapidly expanding technology in which very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of polymeric material. A large number of core materials like live cells, adhesives, flavors, agrochemicals, enzymes, pharmaceuticals etc., can be encapsulated. The scanning electron microscopy is used to reveal the structural features of microencapsulated compound [1].

Astaxanthin is a natural pigments found in many classes of algae and in aquatic world. It is found in seafood like salmon, trout, sea bream and shrimps. The richest source of natural astaxanthin is*Haematococcuspluvialis* microalgae. It is now believed to be the most effective antioxidant pigments among other carotenoids. Due its antioxidant properties, astaxanthin play a beneficial role in various health conditions such as inflammation, diabetics, certain cardiovascular diseases, anti-cancer activity etc[2]. Inflammatory diseases (e.g., inflammatory bowel disease) could increase the risk of developing different types of cancer including bladder, cervical, gastric, intestinal, esophageal, ovarian, prostate and thyroid tumors [3].Thus, standardization of astaxanthin is important to check the purity and quality of the drugs.

Standardization of drugs means confirmation of its identity and determination of its quality and purity[4]. The process of standardization can be achieved by stepwise pharmacognostic studies[5]. Standardization is a system to ensure that the medicine sold has the correct amount and will induce its therapeutic effect[6]. Need of standardization includes safe, healthy, secure, high quality and flexible types of drugs. Determination of physical, chemical, extractive values, ash residues, heavy metals and organoleptic characters plays a significant role for standardization of the indigenous crude drugs[7,8].

The term heavy metal refers to any metallic chemical element that has a relatively high density and may be toxic or poisonous even at low concentrations[9]. Heavy metals include zinc, lead, calcium, mercury etc. Heavy metals are natural components of the earth crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water & air. As trace elements, some heavy metals (e.g. Cu, Zn, Fe) are essential for maintaining the human body metabolism. There are several sources of metal poisoning which include water contamination via lead pipes, food contamination and inhalation of contaminated dust.

Heavy metals are dangerous, because they tend to bio accumulate. Bio accumulation means an increase in the content of a chemical in a biological organism over time, compared to the chemicals content in the environment. Diverse amounts of heavy metals may be found everywhere in soil, water, air, sediments, plants etc. chemicals like heavy metals once introduced to the environment by one particular method may spread to various environmental components which may be caused by the nature of interactions occurring in this natural system. Heavy metals may chemically or physically interact with the natural compounds, which change their forms of existence in the environment[10]. The environment contains a wide range of heavy metals with varying

concentration ranges depending on the surrounding geological environment and natural activities occurring or that has once occurred. These heavy metals can be Fe, Zn, Cr, Cd, Pb, Ni, Mn, Hg, etc. However, some heavy metals like Pb, Zn, Cd, Hg and Th are of great concern because of their potential effects on human health, agriculture and environment. In this study, the organoleptic characters, physio-chemical analysis, extractive values, physical properties and evaluation of heavy metal in both non-encapsulated and encapsulated astaxanthin were investigated using standard analytical techniques.

II. Materials and Methods

Materials

Astaxanthin (RudraBioventuresPvt Ltd, Bangalore) was used as drug and coating materials such as calcium chloride, sodium alginate, chitosan and liposomes were purchased from Sigma Aldrich. All solvents used were of analytical grade.

Preparation of microspheres of astaxanthin

Astaxanthin was encapsulated using four different agents by ionotropic gelation method[11]. In first method, microencapsulated astaxanthin was prepared by using sodium alginate and calcium chloride [ME1][12,13]. In second method, microencapsulated astaxanthin was prepared using sodium alginate and chitosan [ME 2][14]. In third method, chitosan – Tripolyphosphate was used to produce microencapsulated astaxanthin [ME 3][15,16]. In fourth method, liposome encapsulated astaxanthin was carried out by the method followed by [17] [ME 4]. These samples were subjected to drug standardization. The detailed procedure was given below.

I. Study of Organoleptic Characters

The organoleptic characters like color, odor, taste, texture and appearance using the sensory organs of our body was studied and foreign matter was also conducted for both microencapsulated and non-encapsulated astaxanthin[18].

II. Physio-Chemical Analysis

Determination of Moisture content was done to check the degradation of products, Total ash, Acid insoluble ash, water insoluble ash were also performed to judge the identity and purity along with extractive values which indicates the chemical constitutes of the drug[19].

III. Determination of Physical Characteristics

Physical characteristics of microencapsulated and non-encapsulated astaxanthin were determined which includes Bulk density, tapped density, compressibility index, pH, and Hausner ratio that indicate the flow properties of the drug[18].

IV. Pharmacological evaluation

In both the drug, the presence of Bitterness value, swelling index and foaming indexwas done. The pharmacological activity of drug that has been applied to evaluate and standardize the drug[20,21].

V. Toxicological evaluation

Qualitative detection of Heavy metals: Samples were tested for the presence of heavy metals like arsenic, cadmium, chromium, lead and mercury[22.23].

III. Results and Discussion

Microencapsulation is a quickly extending innovation and there are different methods which are accessible for microencapsulation of carotenoid pigments. In our preliminary study, encapsulation of astaxanthin was implemented and characterized. SEM view of encapsulated astaxanthin was predicted in which all the encapsulated beads formed showed minimum size of 1.522 to 15.21 µm. FT-IR analysis showed the presence of aldehyde, ketone, amines, related to both chemicals used and astaxanthin. The percentage yield, drug content and *In vitro* drug release was also performed in both Stimulated gastric fluid and Stimulated intestinal fluid of ME 1, ME 2, ME 3 and ME 4 which showed better results[11].

Further, the *in vitro* antioxidant, anti-inflammatory and antidiabetic activity of both encapsulated and non-encapsulated astaxanthin was also carried along with commercially available standard drug. The overall result showed that the better *in vitro* activity was gained by liposomal encapsulated astaxanthin (ME 4) than all other encapsulated drug (ME 1, ME 2 and ME 3). Studies have shown that astaxanthin as an antioxidant pigment contains specific anti-inflammatory properties and through this antioxidant property, astaxanthin seems to be more effective against various diseases[24-26]. So, standardization of encapsulated astaxanthin was necessary before testing its anticancer activity against hepato cellular carcinoma in DEN induced mice.

Standardization of drug was conducted to check the safety, quality and efficacy of the drug. Standardization involves many factors and all the task has been carried out in our present study.

I. Study of Organoleptic Characters

Table no 1: Study of organoleptic characteristic of test samples

Organoleptic character	Non-encapsulated astaxanthin	Microencapsulated astaxanthin			
		ME 1	ME 2	ME 3	ME 4
Appearance	Powder form	Beads form	Beads form	Beads form	Creamy form
Color	Dark red	Light red	Moderate red	Dark red	Light red
Odor	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Tasteless	Tasteless	Tasteless	Tasteless	Sour
Texture	Smooth	Rough	Rough	Rough	Smooth oily

The organoleptic characters of non-encapsulated and encapsulated astaxanthin was predicted which is represented in **Table no 1.** Non-encapsulated astaxanthin was in powder form which is dark red in color and produce Characteristic odor with tasteless and smooth in texture. Microencapsulated astaxanthin such as ME 1, ME 2 and ME 3 were in beads form which differ in color (light red, moderate red and dark red) and ME 4 was creamy in nature with light red color. All the microencapsulated astaxanthin produces Characteristic odor and they were tasteless and ME 4 produce sour taste. The texture of ME 1, ME 2 and ME 3 were rough in nature whereas ME 4 was smooth oily in nature.

Table no 2: Determination of foreign matter in test samples

Test samples	Percentage of foreign matter (%)
Non- encapsulated astaxanthin	0
ME 1	0.01
ME 2	0.02
ME 3	0.02
ME 4	0.03

The drug should be entirely free from visible signs of contamination by moulds or insects. No abnormal odor, discoloration, slime or signs of deterioration should be detected. However, poisonous, dangerous or otherwise harmful foreign mater or residue should not be allowed. During storage, products should be kept in clean and hygienic places, so that no contamination occurs[4]. In the present study, the non-encapsulated astaxanthin shows 0% which indicates the absence of foreign materials. In microencapsulated astaxanthin such as ME 1, ME 2, ME 3 and ME 4, the percentage of foreign matters was found to be 0.01 % to 0.03 % which may be due to solvent or compound used for encapsulation purposes **(Table no 2).**

II. Physio-Chemical Analysis

Table no 3: Physio-chemical analysis of both Non-encapsulated and Microencapsulated astaxanthin

Physio-chemical content	Non-encapsulated astaxanthin	Microencapsulated astaxanthin			
		ME 1	ME 2	ME 3	ME 4
Moisture (%)	0.011 ± 0.135	0.143 ± 0.142	0.421 ± 0.125	0.941 ± 0.004	1.789 ± 0.011
Total ash (%)	0.121 ± 0.213	0.217 ± 0.114	0.204 ± 0.153	0.253 ± 0.158	0.154 ± 0.142
Acid insoluble ash (%)	0.023 ± 0.003	0.065 ± 0.021	0.035 ± 0.001	0.014 ± 0.001	0.021 ± 0.189
Water soluble ash (%)	0.078 ± 0.152	0.031 ± 0.002	0.096 ± 0.081	0.041 ± 0.151	0.076 ± 0.201

The ash remaining following ignition of drug materials is determined by three different methods which measure total ash, acid-insoluble and water-soluble ash. The total ash method is designed to measure the total amount of material remaining after ignition. Acid-insoluble ash is the residue obtained after boiling the total ash and water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water[4]. Moisture content was checked which helps in prevention of degradation of product[27].

Physiochemical analysis such as Moisture content, total ash content, acid insoluble ash and water soluble ash were determined for both non-encapsulated and microencapsulated astaxanthin which is tabulated in

Table no 3. The percentage of moisture content, total ash, acid insoluble ash and water soluble ash of non-encapsulated astaxanthin was 0.011 %, 0.121 %, 0.023 % and 0.078 % respectively which is lesser than the microencapsulated astaxanthin. The moisture content of ME 1, ME 2, ME 3 and ME 4 (i.e. 0.143%, 0.421%, 0.941% and 1.789%) was greater than the non-encapsulated astaxanthin which may be due to the use of distilled water during encapsulation process. The total ash content of microencapsulated astaxanthin ranges from 0.154%

to 0.253%. Acid insoluble ash of both non-encapsulated and microencapsulated astaxanthin was 0.023%, 0.065%, 0.035%, 0.014% and 0.021% respectively whereas water soluble ash shows 0.078%, 0.031%, 0.096%, 0.041% and 0.076% respectively.

Table no 4: Determination of extractive values of both Non-encapsulated and Microencapsulated astaxanthin

Extractive values	Non-encapsulated astaxanthin	Microencapsulated astaxanthin			
		ME 1	ME 2	ME 3	ME 4
Water-soluble (%)	1.091 ± 0.021	1.973 ± 0.121	1.567 ± 0.114	1.242 ± 0.002	1.115 ± 0.041
Alcohol soluble (%)	0.782 ± 0.001	0.957 ± 0.143	0.671 ± 0.152	0.547 ± 0.071	0.841 ± 0.126
Ether-soluble (%)	0.126 ± 0.141	0.311 ± 0.158	0.269 ± 0.003	0.193 ± 0.174	0.251 ± 0.117

This method determines amount of active constituents extracted with solvents from a given of drugs[4]. Water soluble, alcohol soluble and ether soluble extractive value of non-encapsulated astaxanthin was 1.091%, 0.782% and 0.126%**.** The water soluble extractive values of microencapsulated astaxanthin such as ME 1, ME 2, ME 3 and ME 4 was found to be 1.973%, 1.567%, 1.242% and 1.115% respectively. The percentage of alcohol soluble and ether soluble extractive value of ME 1 possess 0.957% and 0.311% whereas ME 2 shows 0.671% and 0.269%. The other two microencapsulated astaxanthin ME 3 and ME 4 produces 0.547% and 0.841% of alcohol soluble and 0.193% and 0.251% of ether soluble extractive values **(Table no 4)**.

III. Determination of Physical Characteristics

Table no 5: Determination of physical characteristics of both Non-encapsulated and Microencapsulated astaxanthin

Physical characteristics	Non-encapsulated astaxanthin	Microencapsulated astaxanthin			
		ME 1	ME 2	ME 3	ME 4
Bulk density (g/ml)	0.547 ± 0.111	0.621 ± 0.145	0.628 ± 0.164	0.634 ± 0.151	0.587 ± 0.114
Tapped density (g/ml)	0.723 ± 0.132	0.812 ± 0.153	0.824 ± 0.143	0.883 ± 0.113	0.784 ± 0.157
Compressibility index (%)	24.343 ± 0.156	22.660 ± 0.163	23.786 ± 0.157	28.199 ± 0.173	25.128 ± 0.174
Hausner ratio	1.322 ± 0.001	1.308 ± 0.121	1.312 ± 0.002	1.393 ± 0.157	1.336 ± 0.108
pH range	5.6	5.8	6.0	6.0	6.2

Table no 5 indicates the Physical characteristics such as bulk density, tapped density, compressibility index, Hausner ratio and pH of non-encapsulated and microencapsulated astaxanthin. Bulk density and tapped density of non-encapsulated astaxanthin was 0.547 g/ml and 0.723 g/ml which is similar to microencapsulated astaxanthin ME 4 (i.e. 0.587 g/ml and 0.784 g/ml). Other samples ME 1, ME 2, ME 3 shows 0.621 g/ml, 0.628 g/ml, 0.634 g/ml of bulk density and 0.812 g/ml, 0.824 g/ml and 0.883 g/ml of tapped density. The compressibility index of test samples ranges from 22.660% to 28.119%. Hausner ratio of microencapsulated astaxanthin was found to 1.308 to 1.393 and non-encapsulated astaxanthin shows 1.322. The pH of non-encapsulated astaxanthin shows 5.6 which is lower than microencapsulated astaxanthin such as ME 1 (5.8), ME 2 (6.0), ME 3 (6.0) and ME 4 (6.2) which may be due the presence of sodium alginate, chitosan, TPP and liposomes.

IV. Pharmacological evaluation

Table no 6: Pharmacological evaluation of test samples: Bitterness value

Sample name	Bitterness value/g
Non-encapsulated astaxanthin	0.042 ± 0.011
ME 1	0.011 ± 0.004
ME 2	0.015 ± 0.120
ME 3	0.020 ± 0.054
ME 4	0.004 ± 0.021

Some drugs have a strong bitter taste are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice. Bitter substances can be determined chemically. However, since they are mostly composed of two or more constituents with various degrees of bitterness, it is first necessary to measure total bitterness by taste[20]. Bitterness value was founded to be high in Non-encapsulated astaxanthin (0.042 ± 0.011/g) followed by ME 3 (0.020 ± 0.054/g). Similar Bitterness value was gained for ME 1 and ME 2 i.e. 0.011 ± 0.004/g and 0.015 ± 0.120/g and lowest value was produced by ME 4 (0.004 ± 0.021/g) **(Table no 6)**.

Table no 7: Swelling Index of both Non-encapsulated and Microencapsulated astaxanthin

Time (Mins)	Swelling index				
	Non-encapsulated astaxanthin	Microencapsulated astaxanthin			
		ME 1	ME 2	ME 3	ME 4

10	0.01 ± 0.121	0.08 ± 0.011	0.11 ± 0.120	0.15 ± 0.015	0.05 ± 0.002
20	0.04 ± 0.101	0.18 ± 0.015	0.25 ± 0.021	0.28 ± 0.041	0.08 ± 0.017
30	0.07 ± 0.058	0.27 ± 0.026	0.34 ± 0.016	0.37 ± 0.023	0.12 ± 0.031
40	0.11 ± 0.046	0.35 ± 0.034	0.46 ± 0.034	0.49 ± 0.071	0.16 ± 0.048
50	0.16 ± 0.015	0.42 ± 0.045	0.58 ± 0.041	0.61 ± 0.013	0.22 ± 0.064
60	0.20 ± 0.026	0.56 ± 0.008	0.71 ± 0.052	0.85 ± 0.025	0.28 ± 0.027

Swelling index was founded to be higher in microencapsulated astaxanthin than Non-encapsulated astaxanthin as the time increases. Microencapsulatedastaxanthin ME 4 possess 0.05 ± 0.002 to 0.28 ± 0.027 which tends to be similar with non-encapsulated astaxanthin that gives 0.01 ± 0.121 to 0.20 ± 0.026. Other microencapsulated astaxanthin such as ME 1, ME 2 and ME 3 was founded to be 0.08 ± 0.011, 0.11 ± 0.120 and 0.15 ± 0.015 at 10 mins which increases up to 0.56 ±.008, 0.71 ± 0.052 and 0.85 ± 0.025 after 1 hour **(Table no 7).**

Table no 8: Foaming index of both Non-encapsulated and Microencapsulated astaxanthin

Sample name	**Foaming index**
Non-encapsulated astaxanthin	Not founded
ME 1	Not founded
ME 2	Not founded
ME 3	Not founded
ME 4	<100

Many drugs contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of drugs and their extracts is measured in terms of a foaming index. Based on this, the foaming index for microencapsulated astaxanthin ME 4 was less than 100 because the height of the foam in tube is less than 1 cm which may due to the encapsulated substances cholesterol that surrounded the drug astaxanthin. The foam formation was not founded in non-encapsulated astaxanthin and microencapsulated astaxanthin such as ME 1, ME 2 and ME 3 respectively **(Table no 8)**.

V. Toxicological evaluation

Table no 9: Presence of heavy metals in Non-encapsulated and Microencapsulated astaxanthin

Test sample	**Arsenic**	**Cadmium**	**Chromium**	**Lead**	**Mercury**
Non-Encapsulated astaxanthin	Negative	Negative	Negative	Negative	Negative
ME 1	Negative	Negative	Negative	Negative	Negative
ME 2	Negative	Negative	Negative	Negative	Negative
ME 3	Negative	Negative	Negative	Negative	Negative
ME 4	Negative	Negative	Negative	Negative	Negative

Heavy metals in the test sample were determined because of the use of distilled water during encapsulation process. Contamination of drugs with heavy metals can be attributed to many causes including environmental pollution and traces of pesticides. There are different methods to identify the presence of heavy metals in drugs[28]. **Table no 9** shows the negative results to all the samples which indicate the absence of heavy metals such as arsenic, chromium, cadmium, lead and mercury in microencapsulated(ME 1, ME 2, ME 3 and ME 4) and non-encapsulated astaxanthin. The negativity results might indicate that the test samples do not contain any contaminants or heavy metals in it.

IV. Conclusion

Current research on drug standardization of Microencapsulated and Non-encapsulated Astaxanthin has been carried out to prove the quality of plant derived products as effective drug. The study ensures the stability, safety, effectiveness and acceptability of the product as a potent drug. Further, toxicity studies have to be carried out to test the efficacy and nontoxic nature of the product in mice model.

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