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Safety assessment of the microalgae *Nannochloropsis oculata*

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ABSTRACT

Nannochloropsis oculata is a marine-water microalgae that is considered to be a good source of omega-3 fatty acids, specifically eicosapentaenoic acid (EPA), utilized in the production of an omega-3 oil for use as a dietary supplement. This study investigates the safety of *N. oculata* in male and female Sprague-Dawley rats administered a 0 or 10 mL/kg bw/rat *N. oculata* (10E8 viable cells/mL) suspension by oral gavage once daily for 14 consecutive days. No mortalities occurred and no signs of toxicity were observed during the study. No treatment-related effects were seen for body weight, food consumption, urinalysis, clinical chemistry, hematology, gross pathology, organ weights, or histopathology. Although statistically significant effects were noted for some endpoints, none were considered to be of toxicological significance. The *N. oculata* suspension was concluded to have no toxicity in rats, confirming that the algal strain used in the production of omega-3 oil is not pathogenic when administered orally to rats.

1. Introduction

Nannochloropsis oculata is a marine-water single-celled algae of the Eustigmatophyceae class. It is one of six species of algae found in the genus *Nannochloropsis* and was originally isolated off the coast of Scotland [1]. *Nannochloropsis* sp. has been utilized as a food source in aquaculture, providing a source of omega-3 fatty acids [2]. Recently a *N. oculata*-derived oil has been determined safe for use in dietary supplements [3]. Furthermore, the nutritional evaluation of a *Nannochloropsis* sp. found it to have high levels of protein, polyunsaturated fatty acids, and antioxidant pigments [4]. The algae is described as a phototrophic unicellular, non-zoospore producing, free-floating algae having a diameter of 2–4 μm, growing non-axenically in a temperature of 11–16 °C. The cells contain yellow-green parietal chloroplasts [1,2].

The 14-day toxicity study was performed under Good Laboratory Practice (GLP) conditions according to the OECD [5] guidelines for the testing of chemicals. The suspension containing *N. oculata* was tested orally in rats to assess its toxicity and/or pathogenicity according to a modified study of the guidelines of OPPTS 885.3050.

2. Materials and methods

2.1. Test item

N. oculata suspension (Lot No. 91020140001; Qualitas Health Inc.) is a green-colored suspension of microalgae. The source biomass, *N. oculata*, was grown in shallow, open-air, plastic-lined ponds in a proprietary growth medium consisting of food grade plant fertilizers and nutrients [4]. The algae was harvested and suspended in deionized water resulting in a live algal biomass (w/w%) of <0.2% in deionized water. Toxin analyses

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conducted on *N. oculata* indicated that no natural product toxins were detected above detection limits including microcystins/nodularin, anatoxin-a, cylindrospermopsin, paralytic shellfish toxin/saxitoxins, okadaic acid and brevetoxins & domoic acid [6]. The *N. oculata* suspension was prepared by Qualitas Health Inc. and supplied to the lab for testing as viable cultures. The suspension was stored by refrigeration (2–8 °C).

2.2. Animals and housing

A total of 40 (20 male and 20 female) 7–8 week old Sprague-Dawley rats bred in-house by Advinus Therapeutics Ltd. (Bangalore, India) were housed in standard polysulfone cages (2/cage) with stainless steel top grills. Steam sterilized corn cob was used as bedding and changed along with the cage twice a week. Cages were placed on five-tier rack. The animals were acclimatized for 5 days prior to the start of treatment. Initial mean group body weights ranged from 189 to 191 g for the male rats and 153 to 155 g for the female rats. Room temperature was maintained at 20–24 °C with a relative humidity of 65–67%, a minimum of 13.7 air changes/h and a 12 h light and 12 h dark cycle. Filtered deep-bore well water and Teklad Global 14% protein rodent maintenance diet (Harlan Laboratories, An Venray, The Netherlands) were provided ad libitum. Animal handling was performed according to the requirements of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.3. Experimental design

The potential toxicity or pathogenicity of a microorganism is generally assessed in accordance with the US Environmental Protection Agency Microbial Pesticide Test Guidelines OPPTS 885.3030 during which the survival and propagation of the microorganism in the rat are evaluated by culturing tissue samples, blood, and feces following a single high exposure and an adequate post-exposure observation period [7]. For the current study, the purpose was not only to determine whether or not the microorganism was toxic in its natural state, but to also determine its toxicity in the rat following ingestion. Furthermore, the current study was designed to determine if the organism could manage to replicate itself in organs and tissue of the host and eventually become a danger to the health of the animal. However, since *N. oculata* is a phototropic vegetable species and typically does not replicate without the presence of light, a modified version of the OPPTS study protocol is used that removed the steps to culture algae in tissue samples by using a 14-day oral administration of the test item to rats to provide information on its toxicity and pathogenicity.

Two groups of Sprague-Dawley rats ($n = 10$ rats/sex/group) were administered (via oral gavage) 0 and 10 mL/kg *N. oculata* suspension (providing a minimum of 10E8 viable cells/animal) respectively, once daily for 14 days. Vehicle control animals were administered by purified water. In order to determine the concentration of viable algal cells, 1 mL of the test material was evaluated daily and the algal cell counts were determined using a haemocytometer. Dead cells were identified by their loss of chlorophyll and were excluded from the cell count. There were no dead cells observed during the cell counts. The concentrations of viable algal cells in the *N. oculata* suspension were in the range of 1.10 × 10E8 to 1.32 × 10E8 viable algal cells/mL throughout the study (Table 1). Doses were administered using disposable plastic syringes, attached with a stainless steel metal feeding cannula. Following the treatment period, all animals were sacrificed on day 15.

Table 1
Concentration of viable algal cells during treatment.

Treatment day	Cell count (viable algal cells/mL)
1	1.21 × 10E08
2	1.28 × 10E08
3	1.32 × 10E08
4	1.25 × 10E08
5	1.27 × 10E08
6	1.14 × 10E08
7	1.18 × 10E08
8	1.23 × 10E08
9	1.12 × 10E08
10	1.15 × 10E08
11	1.10 × 10E08
12	1.16 × 10E08
13	1.20 × 10E08
14	1.16 × 10E08

Rats were observed twice daily for signs of morbidity and mortality and clinical signs until study termination. The food was analyzed and found to be below established maximum levels for heavy metals, mycotoxins (Aflatoxin B1, B2, G1 and G2), chlorinated hydrocarbons and organophosphates. The food was composed mainly of 14% protein, 4% fat and 4% fiber, as is typical for diets for this species and strain of rat. Drinking water was analyzed and found acceptable as a potable water source for the area (Bangalore, India). Body weights were recorded on day 1 prior to the test item administration and on days 4, 7, 11, and 14. Fasting body weights were recorded prior to necropsy on day 15. Food consumption was measured on days 4, 7, 11, and 14. At the end of the study, rats were fasted overnight (water allowed), anaesthetized with isoflurane, and exsanguinated.

Blood was collected from the retro-orbital sinus plexus with fine capillary tubes. An aliquot of blood was collected in tubes containing 3.2% sodium citrate solution for determination of coagulation parameters and the remaining blood was collected into k_2EDTA and lithium heparinized tubes for hematology and clinical chemistry examinations.

Prior to sacrifice, urine was collected in urine collection tubes for all rats. Each rat was placed in specially fabricated cages overnight (water allowed) and the next morning (day 18) the urine was collected for analysis. Urinalysis parameters examined in the collected samples included color, clarity, bilirubin, glucose, ketone bodies, nitrite, proteins, pH, specific gravity, urobilinogen, and volume. Urine was also subjected to microscopic examination for sediments such as crystals, epithelial cells, erythrocytes, leukocytes and casts.

All animals were fasted overnight prior to terminal sacrifice on day 19. At the scheduled termination, all