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PHARMACOLOGICAL TESTING OF BLUE-GREEN ALGAE FOR CONSTITUENTS HAVING THERAPEUTIC VALUE

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PHARMACOLOGICAL TESTING OF BLUE-GREEN ALGAE FOR CONSTITUENTS HAVING THERAPEUTIC VALUE

by

World Life Research Institute
Colton, California 92324

for the

WATER QUALITY OFFICE

ENVIRONMENTAL PROTECTION AGENCY

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ABSTRACT

The freshwater alga Aphanizomenon flos-aquae (Linnaeus) Ralfs, commonly referred to as waterbloom and pondscum, is an economic pest infesting many freshwater ponds and lakes of North America. This alga has been incriminated in fatalities in fish, fowls, and cattle.

Samples of the alga were collected at Upper Klamath Lake, Oregon, during the peak of its primary bloom on 12 June 1969. The samples were taken in Upper Klamath Lake near Bare Island, along the south side of Buck Island, and at Pelican Marina where the bloom was most luxuriant.

Acetone and ethanolic extracts were prepared for pharmacological and microbiological testing. The extracts were tested at 50 and 500 mg/kg i.p. in the rat pharmacological screen of the Atlas Chemical Industries, Inc., at Wilmington, Delaware. Antimicrobial tests were performed on Escherichia coli, Beta streptococcus, Pseudomonas aeruginosa, Mycobacterium fortuitum, Staphylococcus aureus, and Candida albicans. The results of these tests showed that there was low potency, no fractional localization of activity, no significant neurological, cardiovascular, or outstanding biological activity of any type. No antimicrobial activity was observed. However, there was evidence that the alga could be classified as "toxic" if sufficient quantities of it were ingested.

Algal extracts were tested for antitumor activity in Leukemia L-1210 and P388 in mice. The tests showed inactivity and low toxicity.

The tests conducted in these studies fail to show evidence of pharmacological properties having commercial pharmaceutical potential.

This report was submitted in fulfillment of project 16010 DOU 06/70 (contract #14-12-535) under the sponsorship of the Federal Water Quality Administration.

CONTENTS

SECTION I

CONCLUSIONS

1. In general the fractions exhibited little acute overt symptomatology and thus probably do not contain products that exert pharmacological effects similar to the type seen by drugs which ordinarily directly produce psychomotor, neurological, or cardiovascular effects. The possibility of opposing drug activities cancelling each other out exists, but it is unlikely that such an effect would be evident in all fractions or over the whole observational period. Fraction 1R was the most active, but at 500 mg/kg its effect was not outstanding.

2. Generally the activity of the fractions had a delayed onset (e.g., approximately 24 hours). This type of activity, resulting in depression of function, alertness, and appetite and decreased body weight, is characteristic of an antimetabolite action and is common to many naturally-occurring substances. Many fractionated mushroom species, for instance, produced similar effects, and this activity has been shown to be related to their antimetabolic ability. The necropsy results indicated that there was some general inflammation and delayed toxic activity.

3. In summary, there was low potency, no fractional localization of activity, no significant neurological, cardiovascular, or outstanding biological activity of any type. No antimicrobial activity was observed. However, there was evidence that the alga could be classified as "toxic" if sufficient quantities of it were ingested. The tests conducted to date fail to show evidence of pharmacological properties having commercial pharmaceutical potential.

4. On the basis of the studies, further investigation as to the commercial pharmaceutical potential of the phytochemical constituents of Aphanizomenon flos-aquae does not appear to be warranted.

SECTION II

INTRODUCTION

The freshwater alga Aphanizomenon flos-aquae (Linnaeus) Ralfs, commonly referred to as waterbloom and pondscum, is an economic pest infesting many freshwater ponds and lakes of North America. A. flos-aquae has been incriminated in fatalities in cattle which have been watered in ponds infested by this alga (Nelson, 1904). It has also been involved in fish mortalities in lakes in Iowa (Prescott, 1933). Durrell and Deem (1940) found this alga to be toxic to rabbits, guinea pigs, and chickens when they were fed A. flos-aquae or injected with extracts from this alga. This alga has also been reported to cause deaths in wild birds and domestic ducks in a water reservoir in northern Colorado. Over the years various reports have appeared from time to time concerning the toxicity of this alga to fish, fowl, and cattle.

The studies of Fitch et al. (1934), Ingram and Prescott (1954), Olson (1951), Schwimmer and Schwimmer (1955), and others have shown that toxic waterblooms involving A. flos-aquae are sporadic, and the symptoms produced and survival times of poisoned animals vary considerably. It was later determined by Gorham (1964) that a fast-death factor was present in this species of alga, but the chemical nature of the poison was not defined. In their studies on A. flos-aquae in Burton Lake, Saskatchewan, Canada, Gorham et al. (1964) found that there were both toxic and nontoxic strains of this species.

Studies conducted under the Eutrophication Program by the Pacific Northwest Water Laboratory, Federal Water Quality Administration, Corvallis, Oregon, revealed that the alga A. flos-aquae was a pest present in Upper Klamath Lake, Oregon. It was therefore considered advisable to try and find an economic application for the use of the alga involved. In view of the biological activity of A. flos-aquae it was suggested that perhaps the alga contained phytochemical constituents that might ultimately have potential value as pharmaceutical agents.

SECTION III

PURPOSE OF THE PROJECT

The objectives of this contract were to determine if the phytochemical constituents of Aphanizomenon flos-aquae from Upper Klamath Lake, Oregon, contained pharmacological properties that might have potential pharmaceutical value as a therapeutic agent.

SECTION IV

DESCRIPTION OF WORK

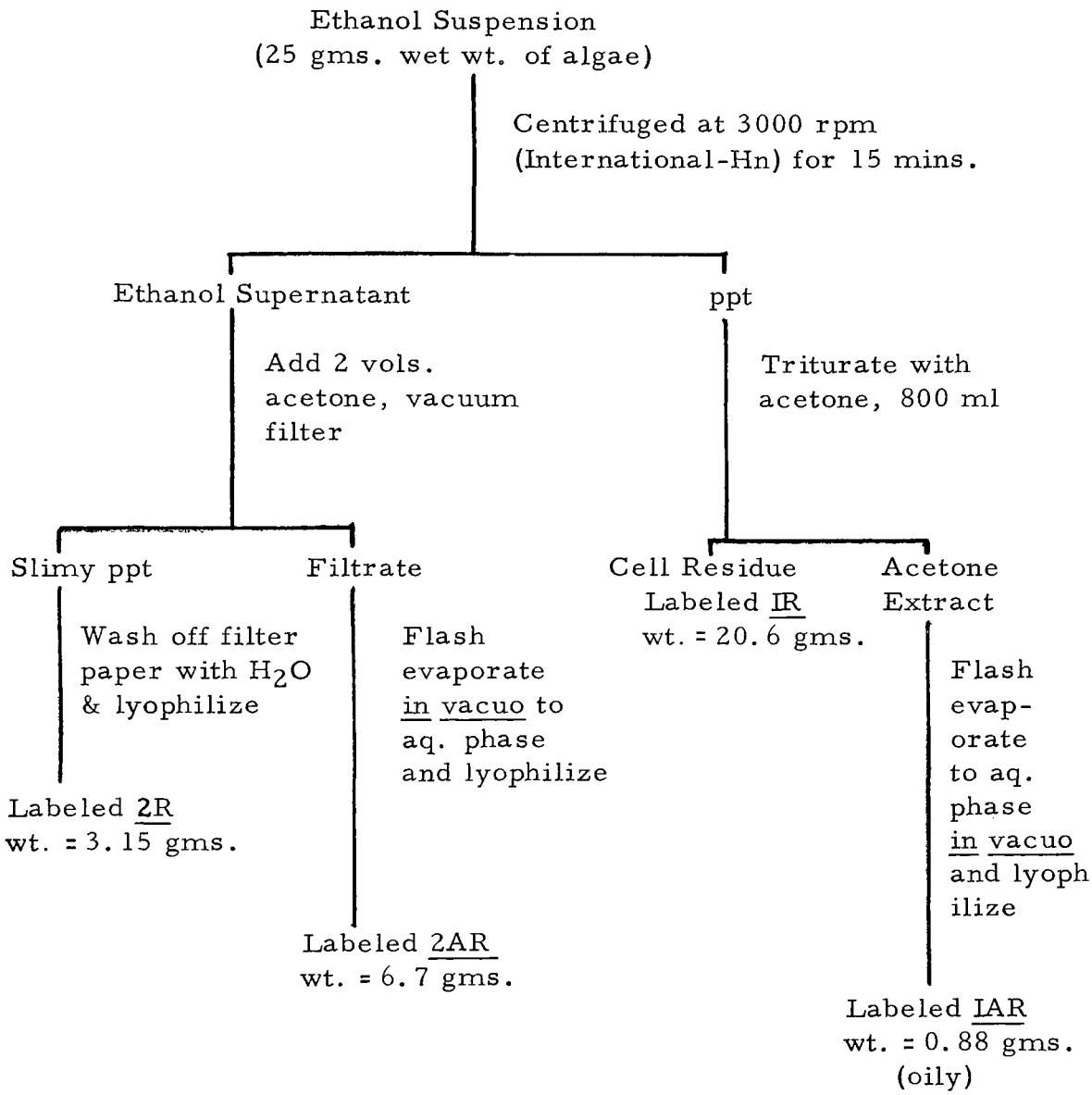
A description of the scope of each of the major technical areas follows:

A. Collection of algal samples: After consultation with Dr. L. P. Seyb, Deputy Director, Pacific Northwest Water Laboratory, FWQA, it was decided that the ideal time to collect the alga at Upper Klamath Lake, Oregon, was during the peak of its primary bloom in the middle of June. On 12 June 1969 Mr. and Mrs. Richard Marin of the World Life Research Institute accompanied Mr. Sanville of the Pacific Northwest Water Laboratory to Upper Klamath Lake to collect the algae. A small skiff from the Eutrophication Research Program of FWQA was used to collect the algae. All algal samples were collected with the use of dip nets. Samples of the algae were squeezed to remove the excess water, and the samples were immediately deposited in plastic iced chests. This material was later frozen. In addition samples were placed in concentrated isopropyl alcohol. All samples were taken in Upper Klamath Lake near Bare Island, along the south side of Buck Island, and at Pelican Marina. These were the areas where the algal growth was most profuse. Other areas of the lake were examined, but the growth was insufficient to warrant collecting. Approximately 8 gallons of algae were collected. The iced material was promptly frozen. A small sample of the algae was air dried, but it was found that the material broke down rapidly, and it was finally decided that this process was not suitable for the studies to be undertaken.

B. Pharmacological screening procedures: In view of the fact that the pharmacological evaluation of this alga was aimed at trying to develop a commercial use of this pest it was suggested that the pharmacological screening be done by a reputable pharmaceutical company. Samples of the alga were therefore submitted to Atlas Chemical Industries, Inc., at Wilmington, Delaware. This phase of the work was under the supervision of Dr. K.K. Kimura, Director of Medical Research, and Dr. D.H. McCurdy, Head of Pharmacology. The alga was then submitted through their routine natural products screening program according to the following scheme:

1. Chemical Extraction Procedure

Two liters of the ethanolic suspension of Aphanizomenon flos-aquae was processed for pharmacological screening by preparing extracts for testing according to the following schematic procedure:


Ethanol Suspension
(25 gms. wet wt. of algae)
Centrifuged at 3000 rpm
(International-Hn) for 15 mins.
Ethanol Supernatant
ppt
Add 2 vols.
acetone, vacuum
filter
Triturate with
acetone, 800 ml
Slimy ppt
Filtrate
Cell Residue
Labeled IR
wt. = 20.6 gms.
Acetone
Extract
Wash off filter
paper with H_2O
& lyophilize
Flash
evaporate
in vacuo to
aq. phase
and lyophilize
Flash
evap-
orate
to aq.
phase
in vacuo
and lyoph-
ilize
Labeled 2R
wt. = 3.15 gms.
Labeled 2AR
wt. = 6.7 gms.
Labeled IAR
wt. = 0.88 gms.
(oily)

2. Pharmacological Tests Performed

The fractions obtained from the chemical extractions were passed through a screening procedure which was designed by the pharmacology staff of Atlas Chemical Industries to elicit a broad spectrum of psychomotor, neurological, and cardiovascular responses. These techniques employ a rat screen in which the various fractions (2R, 2AR, 1R, and 1AR) were injected intraperitoneally into laboratory rats which were carefully observed over a period of 48 hours.

The signs observed and recorded included the following:

a. change in motor activity
b. ataxia
c. analgesia
d. loss of righting reflexes
e. loss of corneal reflexes
f. loss of pinna reflexes
g. paralysis of hind legs
h. screen grip hind leg loss
i. screen grip front leg loss
j. tremors
k. fasciculation
l. tonic or clonic convulsions.

Respiratory Changes:

a. change in rate of respiration.

Eyes:

a. enophthalmus
b. exophthalmus
c. palpebral ptosis
d. changes in pupillary size
e. nystagmus
f. lacrimation
g. chromodacryorrhea.

Ears and Mucosa:

a. blanching
b. hyperemia
c. cyanosis.

General:

a. salivation
b. tail erection

c. pilomotor erection
d. urination
e. diarrhea
f. Robichaud test - skin fold reaction
g. circling motions
h. tail-lashing
i. writhing
j. rectal temperature
k. body weight
l. body tone
m. head tap reaction
n. body touch reaction
o. catalepsy
p. excessive curiosity.

The responses of the test animal are plotted against an arbitrary standard which has been developed over the years in the Atlas Pharmacology Laboratory as a result of testing compounds having known pharmacological activity. These compounds include a broad range of psychomotor, neurological, and cardiovascular drugs. The degree of the response is assigned a number value. For example a good central nervous system depressant might have a Central Nervous System Depressant rating of 30 with a theoretical maximum rating of 100. Unless a response rating of a minimum of 10 is obtained the results are generally not considered as very significant.

Table I summarizes the results in terms of the pharmacological responses obtained from the algal extracts. It will be noted that the pharmacological responses were very low in every aspect, even when the extracts were administered in very high dosages (500 mg). The headings for the various general categories reflect the results affecting the various systems psycho-neurological (ANS= Autonomic nervous system; CNSS= Central nervous system stimulation; CNSD= Central nervous system depressant; Misc. = general signs not included within the other categories). Any evidence of cardiovascular activity would have been included in this category although there may be some overlap in the autonomic responses.

Comments on the results:

Fraction 2R

50 mg/kg: There was very little activity. A slight increase in rectal temperature was noted at 30 min. post inject-

ion, which slowly returned to normal by the 24-hour reading. At the experimental necropsy, 48-hour post injection, the intestines were immotile, the heart was not beating, the liver was engorged with blood and there was rhexis hemorrhage in the lungs.

500 mg/kg: There was very little activity. There was a decrease in body weight recorded from 1-48 hours post injection. Necropsy, after completion of the experiment, 48 hours post injection showed that the heart was not beating, the liver was engorged, and that there was rhexis in the lungs.

Fraction 2AR

50 mg/kg: There was very little activity. Some slight fluctuation in pupil size occurred throughout the observation period. There was a decrease in body weight noted from 0.5 to 48 hours post injection. At 24 and 48 hours post injection the animal had lost an apparent 12 gms. The experimental necropsy, 48 hours post injection, revealed no gross abnormalities.

500 mg/kg: There was very little activity. There was some pupillary size fluctuation throughout the observation period. Body weight was decreased and by 24-48 hours post injection the animal had lost 10-11 gms. The experimental necropsy, 48 hours post injection, revealed no outstanding gross abnormalities.

Fraction 1R

50 mg/kg: There was a slightly decreased pupil size from 1-48 hours post injection. The symptomatology seemed to be delayed in onset. Slight piloerection, slight rectal temperature decrease, and slight

decreased body tone were noted at approximately 24-48 hours post injection. The animal lost approximately 15 gms. in body weight by 24-48 hours post injection. At the experimental necropsy, 48 hours post injection, the right kidney was enlarged, whereas the left kidney was normal. There was petechial hemorrhaging in the liver and lungs. The intestines, although motile, were empty.

500 mg/kg: Symptomatology was delayed in onset. At 4-24 hours post injection, the animal was slightly to moderately depressed with a slight loss of screen grip and a marked loss in its ability to function on the rotorod. There was slight to marked enophthalmus, slight decreased pupil size, slight piloerection, slight decreased body weight, and slight decrease in body tone. The animal was found dead at 48 hours post injection. A necropsy was not performed.

Fraction 1AR

50 mg/kg: There was very little activity. A slight decrease in rectal temperature was noted from 10 minutes to four hours post injection. At the post experiment necropsy, 48 hours post injection, the heart was not beating, the liver was engorged with blood, there was rhexis hemorrhaging in the lungs and kidney. Some particles of injected material were found in the peritoneal cavity.

500 mg/kg: There was only slight activity which seemed to be delayed in onset. At 24-48 hours post injection, a marked Robichaud test, 12 gms. loss in body weight, and slight decrease in body tone were noted. At the experimental necropsy, 48 hours post injection, the peritoneal wall was irritated. There was rhexis hemorrhage in the lungs and kidney. Particles of injected material were found in the abdominal cavity.

3. Antimicrobial Tests Performed

The various fractions utilized in the pharmacological screen were also assayed for antimicrobial activity. These fractions were tested against Escherichia coli, Beta streptococcus, Pseudomonas aeruginosa, Mycobacterium fortuitum, Staphylococcus aureus, and Candida albicans. The extracts were tested in concentrations of 1.0, 0.1, and 0.01 percent and incubated at 37°C for 48 hours. There was no evidence of inhibition observed at any dose level, and in fact, may have promoted bacterial growth. In view of the complete lack of activity, no further testing was done for antiviral activity.

4. Antitumor Tests Performed

A sample of the alga preserved in concentrated isopropyl alcohol was submitted to the Natural Products Section, Development Branch, Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Maryland, for evaluation for antitumor activity. The sample was then placed through their routine screen and tested in Leukemia L-1210 and Leukemia P388 in mice. It was inactive in all and was low in toxicity. It is noteworthy that the CCNSC had previously tested four other samples of this same alga species which had been cultured in the laboratory. These samples were tested in Leukemia L-1210, Leukemia P388, and in Walker 256 carcinosarcoma in rats, but none in KB cell culture. In none of the tests were the samples found to have any significant antitumor activity.

TABLE I

Fraction	Dose (mg/kg)	Weighted Score Expressed as the Percent of the Maximum Possible Score Per Category			
		ANS	CNSS	CNSD	Misc.
2R	50	0	0.30	0	2.06
	500	0	0	0	5.50
2AR	50	1.89	0	2.30	0
	500	1.57	0	0.15	6.42
1R	50	4.09	0.41	1.15	4.81
	500	4.51	0	2.84	1.37
1AR	50	0	0	0.76	0.91
	500	0	0	1.92	4.12

SECTION V

ACKNOWLEDGMENTS

We are indebted to Doctors K.K. Kimura, D.H. McCurdy, R.C. Landis, and their associates at Atlas Chemical Industries, and Dr. J.L. Hartwell of the National Cancer Institute for his assistance in testing the algal extracts. We wish to acknowledge the cooperation of Dr. L.P. Seyb and his associates of the Pacific Northwest Water Laboratory, Federal Water Quality Administration, for their assistance in collecting the algal samples.

SECTION VI

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27 Abstract

Samples of the freshwater alga Aphanizomenon flos-aquae (Linnaeus) Ralfs were collected at Klamath Lake, Oregon, during the peak of its primary bloom on 12 June 1969. This alga infests many freshwater ponds and lakes of North America and has been incriminated in fatalities in fish, fowl, and cattle.

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