Toxicity Studies on Dehydroabietic Acid

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Dehydroabietic acid (DHA, podocarpa-8, 11, 13-tien-15-oic acid, isopropyl) is a monocarboxylic acid that occurs as a constituent or rosin isolated from pine and other coniferous trees. Rosin is the most important group of products obtained from the oleoresin of pine which is used in the naval stores industry throughout the world. The production of rosin from 1960-1970 was over 2 billion lb/year and approximately half this amount originated in the U.S. (Enos et al, 1970). Rosin and rosin derivatives also have application in the food industry where they are used in the manufacture of packaging materials.

The toxicity of resin acids to acquatic organisms has been known for some time (Marier, 1973). These compounds occur in pulp and paper wastes and have been shown to be toxic to fish at levels as low as 0.44 ppm (Marier, 1973). This degree of toxicity is comparable to that obtained for both pentachlorophenate and zinc (Marier, 1973) and as a result the resin acids are to be considered highly toxic to acquatic organisms. However, there is only limited published data available on the toxicity of resin acids to animals. Since these compounds can occur as environmental contaminants in our water supply it is therefore important to have information on the acute and sub-acute toxicity of the individual resin acids. Dehydroabietic acid (DHA) was selected for the present study because of its stability and commercial availability as well as the fact that it has been identified as a toxic component in pulp mill effluents (Marier, 1973; Rogers and Mahood, 1974). The present investigation therefore will deal with the acute and sub-acute toxicity of DHA in the rat.

MATERIALS AND METHODS

Dehydroabietic acid (78.3% pure) was obtained from K & K Laboratories, ICN Pharmaceuticals, Montreal, Canada, and was dissolved in corn oil for oral intubation in the acute toxicity study and for addition to ground stock cubes (Maple Leaf Mills, Toronto, Canada).

Acute Toxicity Study

Male (233-286 g) and female (171-206 g) Sprague-Dawley rats (Biobreeding Laboratories, Ottawa, Canada) were given single oral doses of DHA dissolved in corn oil (0.5 ml/100 g b.w.). There

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were four dose groups each with 5 animals and each LD_{50} determination was carried out in duplicate. The LD_{50} values and ranges were calculated according to the method of Horn (1956) based on an observation period of 14 days.

Subacute Toxicity Study

Male Sprague-Dawley rats weighing 150-225 g were obtained from Biobreeding Laboratories, Ottawa, and fed diets containing 0, 50, 500 and 5000 ppm DHA ad libitum. Ten animals from each group were killed after 14 and 28 days on diet. Food and water intake was measured weekly. Body weight was measured daily. Twenty-four hour urine samples were collected prior to each kill and analyzed for protein, glucose, blood and pH. Hematological investigations were made after each kill. The blood was examined for hemoglobin concentration, hematocrit value, erythrocyte count, total and differential counts of leucocytes, mean corpuscular volume (MCV) and mean corpuscular hemoglobin content (MCHC). A complete autopsy was performed on all animals. Tissues were fixed in 10% buffered neutral formalin.

Paraffin sections of larynx, thyroid, heart, lungs, liver, spleen, kidneys and brain were stained with H.P.S. (haematoxyline, phloxine, saffron) for histological examination. The brain, liver, kidneys and heart were weighed before sectioning. Portions of liver and serum were collected from each animal for biochemical tests. Protein content of liver and serum was measured by the biuret procedure (Gornall et al, 1948). Drug metabolism in vitro was studied by measuring liver aniline hydroxylase activity as outlined by Becking (1973) using a 20 minute incubation time. Other biochemical tests carried out on serum included Alkaline phosphatase (Azurechrome reagent, American Monitor Corporation, Indianapolis, Indiana, adopted for use on the Autoanalyzer II system); calcium (Rapid-Stat Method, Pierce Chemicals, Rockford, Illinois and adapted for use on the Autoanalyser II system); Sodium and potassium (flame photometric procedure); chloride (Autoanalyzer II method No. SE4-0003 FD4, Technicon Instruments, Tarrytown, N.Y.): cholesterol (Autoanalyzer II method No. SE4-0026FC4); total bilirubin (Autoanalyzer method No. SE4-0018FK4); urea nitrogen (Autoanalyzer II method No. SE4-0001FD4); electrophoresis (blood sera fractionated on the Beckman Micozone System, Beckman Instruments Fullerton, California); glucose (protein free filtrates were analyzed using the GOD-Perid method, Boehringer Mannheim Corp. St. Laurent, P.Q., Canada).

RESULTS

Acute Toxicity Study

The LD_{50} value obtained for males was 4,000 mg/kg (no confidence limits calculated) in the first experiment and 3,690 mg/kg (2270-5990) in the second. The LD_{50} value for females was 1,710 mg/kg (1260-2330) in the first experiment and 3690 (2710-5010) in the second. All animals

TABLE I

Tissue weights (% body weight) of rats treated with DHA[1]

Level of DHA in diet (ppm)	Liver Weight		Kidney Weight		Spleen Weight	
	14 days	28 days	14 days	28 days	14 days	28 days
0	3.08 ± 0.07	2.91 ± 0.04	0.81 ± 0.02	0.80 ± 0.01	0.23 ± 0.01	0.18 ± 0.01
50	2.95 ± 0.07	2.97 ± 0.06	0.78 ± 0.02	0.76 ± 0.02	0.22 ± 0.01	0.19 ± 0.01
500	2.86* ± 0.05	2.92 ± 0.06	0.77 ± 0.02	0.75* ± 0.02	0.20* ± 0.01	0.19 ± 0.01
5000	2.88* ± 0.05	2.94 ± 0.07	0.80 ± 0.02	0.76 ± 0.01	0.19* ± 0.01	0.19 ± 0.01

[1] Figures represent the mean ± S.E. of 10 animals.

* Denotes significant difference from control group P = 0.05.

which died after dosing exhibited signs of discoordination, diartria and paralysis of the hind legs culminating in death.

Subacute Toxicity Study

DHA did not have any effect on body weight gain, food consumption or water intake at any dietary level for the 14 and 28 day periods. The tissue weights, expressed as a percentage of the body weights are shown in Table I. DHA caused a reduction in the liver and spleen of animals fed 500 and 5000 ppm for 14 days. However, similar effects were not observed at the 28 day interval. All hematology was negative at both the 14 and 28 day time intervals. This included hemoglobin concentration, hematocrit value, erythrocyte count, total and differential counts of leucocytes, mean corpuscular volume and mean corpuscular hemoglobin content. Table II shows the results obtained for liver protein and aniline hydroxylase values. No effect was observed on liver protein

TABLE II

The effect of DHA on liver protein and aniline hydroxylase activity.[1]

Level of DHA in diet (ppm)	Liver protein (mg/g liver)		Aniline hydroxylase activity.[2]	
	14 days	28 days	14 days	28 days
0	126.3 ± 2.1	126.8 ± 0.8	15.4 ± 1.7	17.0 ± 0.7
50	119.6 ± 1.8	122.8 ± 1.5	14.7 ± 1.0	18.6 ± 0.2
500	124.0 ± 4.5	127.4 ± 1.1	15.7 ± 1.2	18.4 ± 1.5
5000	126.0 ± 4.2	125.5 ± 1.4	19.0 ± 0.9	20.6*± 1.1

[1] Figures represent the mean ± S.E. of 10 animals.

[2] Activity expressed as μMoles PAP/hr/mg protein.

* Denotes significant difference from controls at P = 0.05.

but aniline hydroxylase activity was increased after 28 days on 5000 ppm DHA. Of the other biochemical parameters studied (serum cholesterol, bilirubin, BUN, glucose, sodium, potassium and alkaline phosphatase) only serum protein and alkaline phosphatase were affected (Table III). Serum protein values were decreased at all

TABLE III

The effect of DHA on serum protein and alkaline phosphatase activity.[1]

Level of DHA in diet (ppm	Serum protein[2]		Alkaline phosphatase activity[3]	
	14 days	28 days	14 days	28 days
0	6.3 ± 0.1	6.7 ± 0.1	147 ± 21	71 ± 6
50	5.6*± 0.1	6.6 ± 0.1	173 ± 19	69 ± 4
500	5.9*± 0.2	6.7 ± 0.1	152 ± 14	67 ± 4
5000	5.9*± 0.2	6.6 ± 0.1	217 ± 27	91*± 5

[1] Expressed as mean ± S.E. of 10 animals.

[2] Serum protein expressed as g/100 ml serum.

[3] Alkaline phosphatase activity expressed in terms of IU/liter serum.

* Denotes significant difference from controls at P = 0.05.

dose levels after 14 days while no effect on this parameter was observed after 28 days. Alkaline phosphatase activity was not altered after 14 days and was increased only in the 5000 ppm group after 28 days. The reason for the differences in the control values in the 14 and 28 day groups is not clear. Urinalysis showed no differences in the control and treated groups for protein, glucose, blood, or pH.

DISCUSSION

Previous studies have identified the resin acids as environmental contaminants that possess a relatively high degree of toxicity to aquatic organisms (Marier, 1973). However, very little information is available on the toxicity of individual resin acids to animals. Studies have been reported on the hemolytic properties of various non-ionic hemolysins including the resin acids abietic, neoabietic and levopimaric (Segal et al., 1972), which showed that of all the compounds tested only the resin acids did not possess hemolytic properties. Radina et al (1972) have reported on the toxicity and antitumorgenic activity of some resin acids and their derivatives.

The results presented here indicate that DHA exhibits a low acute toxicity in the rat. The LD_{50} values for both male and female is in the 3-4 g/kg range with no delayed toxicity up to 14 days after the compound was administered.

There was no pathological or histological changes associated with exposure of up to 5000 ppm DHA in the diet for 28 days. The reason why liver and spleen weights and serum protein were altered at 14 days but not at 28 days might be due to an adaptive response which disappears after more prolonged exposure. The only other biochemical parameters affected were liver aniline hydroxylase activity and serum alkaline phosphatase activity which were both elevated, but only at the highest dose level and after 28 days on test. These data support the conclusion that DHA is only moderately toxic to the rat.

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