Prevention of Experimental Dietary Hepatic Injury by Extracts of Some Tropical Plants.* (28320)

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One type of cirrhosis which occurs in young Jamaican children has been suspected of being of toxic origin and possibly caused by concoctions of tropical plants, called "bush tea" (1,2). The morphological picture of this type of cirrhosis is non portal and characterized by the presence of occlusive disease of medium size and smaller hepatic veins; hence the condition was named Veno-Occlusive disease(3, 4).

Since 1950, we have tested many Jamaican "medicinal" plants† on hundreds of rats fed either a normal or a cirrhosis producing diet. Among the plants tested, Senecio and Crotalaria were found to be profoundly toxic, confirming a previous report(5). Both these plants were known to be used by the Jamaican population and Senecio had come under suspicion because of literature reports(3), while Crotalaria had come under grave suspicion clinically because of observations made in the West Indies(6). Some extracts of *Eupatorium dalea* and of *Parthenium hysterophorus* have also been found to produce hepatotoxic effects. Extracts of a large number of medicinal plants used in Jamaica(7), which we have tested, were found neither harmful nor beneficial, in the amounts administered.

This report deals with extracts of *Picramnia antidesma* Sw. and of *Turnera ulmifolia* L., both of which exhibited distinctly beneficial effects in prevention of experimental dietary hepatic injury and of acute necrotizing nephrosis in white rats. The following "medicinal" plants used in Jamaica(7) were found neither harmful nor beneficial, in the amounts used: *Achyranthes indica* (Mill), *Aloe vulgaris* (Lam), *Andiographis paniculata* (Ns.), *Bidens reptans* (L.), G Don. *Cuscuta americana, Emilia sonchifolia, Eupatorium odoratum* (L.), *Eupatorium villosum* (Sw.), *Heliotropium angiospermum, Heliotropium indicum* (L.), *Heliotropium parviflorum* (L.), *Mikania microcantha, Momordica charantia* (L.), *Monodica thorontia, Pluchea odorata* (L. Cass), *Stachytarpheta jamaicensis* (indica) (Vahl.), *Tribulus cistoides* (L.).

These investigations, covering a period of over 10 years, were carried out on over 3400 rats. Extracts of Crotalaria were tested on 780, of Senecio on 880, of Picramnia on 740, of Turnera on 100 and of all other plants on 900 rats.

Methods. Male, Sprague-Dawley white rats were used. The average starting weight of the animals was about 60 to 65 g in all groups of any experiment, except Experiment 123 (Table I), in which average initial weight was 104 g. Rats were put on one of 2 cirrhosis-producing diets. Diet LF consisted of casein (Vit. test) 8, lard 6, cod liver oil 2, sucrose 80, salt mixture #2 (U.S.P. XIII) 4, and niacin 1 mg per 100 g. Diet L2 consisted of casein 10, lard 20, cod liver oil 2, sucrose 64, salt mixture #2 (U.S.P. XIII) 4, and niacin 1 mg per 100 g. A daily supplement of B Vitamins (thiamine 20 μg, riboflavin 25 μg, pyridoxin 20 μg, calcium pantothenate 100 μg and menadione 20 μg were given separately in a supplement dish. Duration of most of the experiments was 75-91 days, but one experiment, #123 (Table I), lasted 160 days. Fresh or dried leaves of Picramnia or of Turnera were extracted with hot water, as in the preparation of Jamaican bush tea. In one experiment (Ta-

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TABLE I. Effect of Extracts of Picramnia and Turnera on Experimental Cirrhosis.

Exp No.	Group	Days in exp	Avg food intake, g/day	Diet	Supplement	Ceroid 0	Ceroid ≥+	Cirrhosis 0	Cirrhosis ≥+	A.N.N. 0	A.N.N. +
123*	I	80	5.0 ± .2§	LF	None	3	7	3	7	3	7
	II	80	5.6 ± .2	"	Extr. Picramnia No. 8	9	1	9	1	2	8
	III	80	5.1 ± .2	"	Extr. Turnera No. 9	10	0	9	1	4	6
204†	I	160	5.4 ± .2	"	None	0	10	0	10	0	10
	II	160	6.2 ± .1	"	Extr. Picramnia No. 53	7	3	7	3	7	3
233‡	I	80	5.3 ± .3	"	None	1	9	2	8	2	8
	II	80	6.0 ± .05	"	Extr. Picramnia No. 62	10	0	10	0	9	1

* In Exp 123 the aqueous extract of Picramnia and of Turnera contained the equivalent of 1.0 g of fresh leaf weight per ml. Avg starting wt: 104 g.

† In Exp 204 7 of 10 rats in Group I, but only 2 in Group II died with severe cirrhosis before termination of experiment. Thus the figures for average food intake are not strictly comparable, in spite of the attempted paired group feeding as in Exp 123 and 272. Rats in Group II received a daily dose of extract with 49 mg of dry residue. Avg starting wt: 66 g.

‡ Avg starting wt: 62 g.

§ Standard error of mean.

ble III, Exp. 125) the aqueous decoction was subjected to extraction with ether, and in another experiment (Table III, Exp. 244) with ethylacetate, and the dry residue was then dissolved in 50% ethanol.‡

The daily dose of plant extract, mixed with a small amount of the experimental diet, was first served to the animals. After these were ingested, more diet was offered to the animals and, in most experiments, on a paired group feeding schedule.

Results. The results clearly indicate that crude aqueous extracts of *Picramnia antidesma* and of *Turnera ulmifolia*, when mixed into the diet in amounts representing about 100 mg of dry residue per day, exert a preventive effect on development of dietary cirrhosis, on deposition of ceroid and on occurrence of acute necrotizing nephrosis (A.N.N.) which frequently develops in cirrhotic rats. These effects were demonstrated both in rats receiving a low casein-low lard diet (LF), and those fed a low casein-high lard diet (L2), which is more conducive to the development of ceroid and of cirrhosis(8). Experiment 204 (Table I), in which the observation period was extended to 160 days, is especially convincing. Seven of the 10 rats in the control group died with severe cirrhosis before the end of the experimental period, whereas in the group receiving extract of Picramnia only 2 died before termination of the experiment and 7 of the 8 surviving rats showed no pathological changes in either liver or kidneys.

In most experiments, paired group feeding was attempted. Exceptionally, group feeding was given part of the time (Exp. (272, Table II) or *ad lib* feeding during the entire experimental period (Exp. IV, Table II). In both experiments, a statistically significant increase in average gain of weight was observed in the groups of rats fed active extracts of Picramnia. In Exp. 272 (Table II), the extract made from fresh leaves of Picramnia was found to have a greater protective effect than one prepared from dried leaves. This became especially apparent in the occurrence of acute necrotizing nephrosis.

Some variation in the potency of different extracts of Picramnia was noticed, but only as an exception. In Table III such an observation is recorded with extract 215. The slight (statistically insignificant), beneficial effect of the aqueous extract became greatly potentiated after extraction with ether. The ether extract itself appeared to aggravate the hepatic and renal injury, probably due to its

‡ These first attempts at purification were made by Dr. F. Zilliken, Philadelphia and by Professor Haynes, University of the West Indies.

TABLE II. Effect of Extract of Picramnia on Cirrhosis of Rats Kept on High Fat Diet.

Exp No.	Group	Supplement	Amt (mg/day)	Wt (g) Start	Wt (g) Final	Ceroid <+	Ceroid ≥+	Cirrhosis <+	Cirrhosis ≥+	A.N.N. 0	A.N.N. +
IV*	I	None		66	106 ± 4.4	0	9	1	8	1	8
	II	Picramnia Extr. III	99.2	65	137 ± 3.3	9	1	9	1	10	0
	III	Picramnia Extr. V	101.0	66	132 ± 8.8	7	1	7	1	8	0
272†	I	None		71	104 ± 3.7	0	10	0	10	0	10
	II	Picramnia Extr. 100	101	71	121 ± 4.6	7	1	6	2	1	7
	III	Picramnia Extr. 101	101	71	128 ± 1.7	9	1	9	1	9	1

Diet in both Experiments: L2.

* Exp IV. Duration 91 days. Group feeding up to 70 days. Last 3 weeks daily avg food intake Group I: 5.4; II: 7.2; III: 6.4 g. Weight difference between I as control and II or III is statistically significant ($t = 5.6$ and 2.5 respectively).

† Exp 272. Duration 80 days. Group II received extract made from dried leaves, Group III one from fresh leaves of the same batch. Weight difference between I as control and II or III is statistically significant ($t = 2.7$ and 7.0 respectively).

TABLE III. Effect of Purified Extracts of Picramnia on Experimental Cirrhosis.

Exp No.	Group	Diet	Avg food intake (g)	Supplement	Amt (mg/day)	Ceroid 0	Ceroid ≤+	Ceroid ≥++	Cirrhosis 0	Cirrhosis ≤+	Cirrhosis ≥++	A.N.N. 0	A.N.N. +
215	I	LF	5.1 ± .2	None		0	4	6	0	4	6	2	8
	II	"	5.5 ± .1	Picram. Extr. 53	100	0	9	1	4	1	5	8	2
	III	"	5.9 ± .0	Z1*	90	5	4	1	9	0	1	9	1
	IV	"	4.5 ± .2	Z2†	7.7	0	0	10	0	0	10	2	8
244	I	LF	4.6 ± .15	None		0	2	6	0	0	8	0	8
	II	"	4.9 ± .1	Extr. 68	64	5	3	2	5	3	2	9	1
	III	"	4.8 ± .1	Extr. 69	41	0	5	4	1	2	6	3	6
	IV	"	5.1 ± .1	Extr. 70	12	4	2	4	6	1	3	6	4

Duration for Exp 215: 75 days. Avg starting wt: 61 g. For Exp 244: 80 days, avg starting wt: 54 g.

* Z1 = Picramnia Extract 53 after ether extractions.

† Z2 = Ether Extract.

Extr. 68 = Aqueous, protein-free Picramnia Extract.

Extr. 69 = Protein residue.

Extr. 70 = Ethylacetate extract, soluble in 50% ethanol.

content of lipids, with a high concentration of unsaturated fatty acids(8).

No methodical attempts at further purification of effective protective substance in extracts of Picramnia have been undertaken. Exp. 244 seems to indicate that the active substance is not present in the protein fraction and appears to be extractable by ethylacetate.

Extract #53 (Exp. 204, Table I) has been analyzed for choline, methionine, total S and Vit. B_{12}.§ No free choline, or methionine or Vit. B_{12} was demonstrable. 14.6 mg of S was found per ml of extract containing 485 mg dry residue. Choline was present in the form of a glyceride (lecithin?) in the amount of 1.16%. Even for minimal, partial effect, 10-15 mg choline are required per day as supplement to the cirrhosis producing diet used. Since 1 cc of Extract #53 was given daily, the supplement of choline was only 1.0 mg/day. These data indicate that the activity of Picramnia may not be attributable to any of these dietary factors known to be beneficial for prevention of dietary hepatic injury(9). Since the salt mixture U.S.P. XIII used in these experiments is lacking several trace elements (manganese, zinc, iodine, copper, selenium), the possibility cannot be excluded that a trace element may play a part in the beneficial effect of Picramnia. Such effect has not been reported for trace elements in experimental cirrhosis in rats.

§ The data obtained with routine analytical methods were kindly furnished by Wyeth Laboratories, Philadelphia.

Summary. In a search for hepatotoxic plants native to Jamaica, it was found that the aqueous extracts of *Picramnia antidesma*

Sw. and of *Turnera ulmifolia* L. had a distinct protective effect on the development of dietary hepatic injury and of the accompanying acute necrotizing nephrosis, in rats fed a cirrhosis producing diet. The protective factor appears to be different from choline, methionine and Vit. B_{12}. It is not a protein or lipid.

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Kinetics of Plasma Hemoglobin Catabolism in the Rat. II. Cr^{51}-Tagged Hemoglobin.* (28321)

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Previous studies in this laboratory using I^{131}-tagged hemoglobin(1) revealed that the rat can clear and catabolize large amounts of intravenously injected hemoglobin with no indication of saturation of the plasma hemoglobin clearing mechanism. However, when Cr^{51} was used as a tag in place of I^{131}, marked discrepancies were noted. It soon became evident that Cr^{51}-labeling itself created specific conditions which altered the apparent plasma hemoglobin clearance. Furthermore, the metabolic fate of the Cr^{51} differed from that of I^{131}. This study was made to elucidate some peculiarities of Cr^{51} as a tag for hemoglobin.

Materials and methods. Fresh rat blood, collected by cardiac puncture, was added to half a volume of ACD† and approximately 30 μc $Na_2Cr^{51}O_4$‡ for each ml of blood. After this mixture was incubated for 45 minutes at 37°C, it was centrifuged, the supernatant was removed and the red cells washed twice with cold physiological saline. Then the red cells were lyzed with twice the volume of distilled water. Sodium bicarbonate was added, in substance, to give a final concentration of 1.5 g per 100 ml of fluid(1). The hemolysate was centrifuged for 25 minutes at 4500 rpm to remove all particulate matter. All hemoglobin solutions were prepared on the day they were used. For fractionation, the lysate of Cr^{51}-labeled rat erythrocytes was passed through a Sephadex§ column.

The Sephadex column was prepared in the following manner: "grade G-25 medium" Sephadex was suspended in physiological saline and the fine particles were removed by decantation before packing the column; elution was achieved with saline. For preparative fractionation, a column about 32 cm high and 2.5 cm in diameter was used; for qualitative studies, a smaller column (10 to 15 × 0.6 cm) was found practical.

For injection, the hemolysates were diluted to a hemoglobin concentration of 4 to

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† Trisodium citrate: 1.32 g, citric acid: 0.48 g, Dextrose: 1.47 g per 100 ml water.

‡ Rachrcmate, Abbott Laboratories, North Chicago, Ill. The specific activity of the different preparations varied from 130 to 400 mc Cr^{51}/mg Cr. This represents an average of 0.5 μg chromium per ml blood.

§ Pharmacia Fine Chemicals, Rochester, Minn.