Toxicity and Carcinogenicity of Some South African Cycad *(Encephalartos)* Species

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SUMMARY

The carcinogenicity of 4 species of South African cycads is reported for the first time. Different quantities of the various parts of the cones of *Encephalartos umbeluziensis*, *E. villosus*, *E. lebomboensis* and *E. laevifolius* were incorporated into the diet of rats for varying periods of time. Renal nephroblastomas were produced in some rats receiving mixtures of the kernel and outer flesh of the seeds of *E. umbeluziensis*, outer flesh only of *E. villosus*, *E. lebomboensis* and *E. laevifolius*, and the kernel of *E. laevifolius*. The latter also caused the development of a renal carcinoma in one rat. Hepatocellular carcinomas were present in some rats which had received mixtures of the kernel and outer flesh of *E. umbeluziensis* and *E. villosus*.

All 4 cycads tested caused acute intoxication in rats, and this resulted in centrilobular hepatocellular necrosis, while chronic intoxication produced nodular hyperplasia of the liver and hyperplastic and hypertrophic alterations in the kidney tubular epithelium. Chronic changes in the liver and kidney were seen in some of the rats fed the peduncles and/or scales of 2 of the cycad species.

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Members of the group of plants known as cycads fall into 3 families: Cycadaceae (containing 1 genus, *Cycas*), Stangeriaceae (1 genus and 1 species, *Stangeria eriopus*) and Zamiaceae (8 genera including *Encephalartos*). In Africa they are represented by the 45 or more species of *Encephalartos*, of which about 29 occur in Southern Africa, and *S. eriopus*. Only 6 species of *Encephalartos* and *S. eriopus* are common in the Transkei and Ciskei.

Although it has long been known that several cycad species are toxic when ingested by man and animals, some have been and still are used medicinally, and as food for human consumption in various countries of the world.[1] When used as a regular source of food the part consumed is first detoxified by a variety of processes which are all based on the fact that the toxin is water-soluble. Poisoning in man is invariably due to ignorance or incomplete detoxification, and the manifestations of toxicity range from a mild to a severe (sometimes fatal) gastro-enteritis, with evidence of liver injury and jaundice exhibited in some cases.[2] In addition, it has been suggested that the ingestion of a flour prepared from the seeds of *Cycas circinalis* might be responsible for the high incidence of amyotrophic lateral sclerosis on the island of Guam. The relationship, if any, however, remains unresolved.[3]

In certain tropical and subtropical countries various cycad species are responsible for serious outbreaks of poisoning in cattle and sheep.[4] Two different syndromes result: the acute disease is characterised by a gastro-enteritis with the development of liver lesions in some; in the other, which is encountered principally in cattle, a progressive paralysis occurs due to degenerative lesions in nerve fibres of various tracts of the spinal cord.[4]

Several toxic azoxyglycosides have been isolated biochemically from certain cycad species. These are macrozamin[5] (methylazoxymethanol-β-primeveroside), cycasin[6] (methylazoxymethanol-β-d-glucoside) and several neocycasins comprising the common aglycone, methylazoxymethanol, linked to different sugar moieties.[7] Methylazoxymethanol acetate has been artificially synthesised.[5] In addition, an amino acid, α-amino-β-methylaminopropionic acid, which has proved neurotoxic to chickens, has been isolated from *C. circinalis* and may also occur in other cycad species.[8]

When one of the glycosides is administered to animals, methylazoxymethanol is responsible for the deleterious effects; toxicity is thus dependent upon severance by enzymatic action of the carbohydrate component from the rest of the molecule. Methylazoxymethanol is not only a potent hepatotoxin and carcinogen,[5] but is also neurotoxic when administered to newborn experimental animals,[9] and it is teratogenic.[10] Cycasin occurs in *Cycas revoluta* and *C. circinalis*, which grow in Japan and Guam respectively,[6,11] while macrozamin has been isolated from 4 Australian species of cycads,[12] from *Encephalartos barkeri* and *E. hildebrandtii*,[13] which grow in East Africa, and recently from the kernels of the Southern African species *E. transvenosus*, the modjadji cycad of the northern Transvaal, and *E. lanatus* from the Middelburg (Transvaal) district (Altenkirk 1974: personal communication).

In times of food shortage and famine, starch obtained from the stems and kernels of *E. hildebrandtii* is used after detoxification for human consumption.[14] Preparation of beer from the stems of *Encephalartos* species has also been described in Central, East and South Africa and in Mozambique.[15]

Dyer reported that there are many references to the edibility of the fleshy outer covering of the seeds of some species of *Encephalartos*, and that baboons, monkeys, rodents and some birds (louries) are said to be particularly fond of them.[16] He warned, however, that it would be most inadvisable for humans to sample them. He has recently stated that he has had reliable information that the outer flesh of the modjadji cycad is eaten by the

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local population where it grows in the northern Transvaal.

Probably the first account of the toxicity of cycads occurring in the Republic of South Africa was that of Reitz, who mentioned that several members of the Boer Commando, including its leader, General J. C. Smuts, ate the seeds of the 'Hottentots bread', *Encephalartos altensteinii*, while in the Suurberg of the eastern Cape Province during the South African War of 1899 - 1902.[17] Serious illness was experienced by many of those concerned and Smuts himself was severely incapacitated for at least 5 days. Dyer is of the opinion that the offending species was not *E. altensteinii* but *E. longifolius*.[16]

The outer flesh and/or kernels of the South African cycads *E. cycadifolius, E. eugene-maraisii, E. horridus, E. kosiensis, E. lehmanii, E. longifolius* and *E. villosus* have either been proved to be acutely toxic when administered to experimental rabbits or have been suspected, or are known to be poisonous when ingested by man.[18] Furthermore, *E. longifolius* has also been reported to have caused poisoning in cattle near Riebeeck-East in the Cape Province.[16]

The purpose of this article is to record the toxicity and/or carcinogenicity in rats of some species of cycads occurring in South Africa.

MATERIALS AND METHODS

Specimens of the seeds and/or entire cones of *Encephalartos umbeluziensis, E. villosus, E. lebomboensis* and *E. laevifolius* were obtained. In some species, the amount of plant material available was limited, but, when possible, the various parts of the cones were tested individually for toxicity and/or carcinogenicity. Thus, in some cases the kernels, outer flesh of the seed, cone scales and peduncles of the cones were separated from each other. The plant material was dried in an oven at 55°C before it was ground into a powder, to facilitate mixing in various concentrations by weight with the basic rat rations, which in all experiments except Experiment 1 (*E. umbeluziensis*) comprised the standard commercial rat ration used routinely by the National Research Institute for Nutritional Diseases of the South African Medical Research Council. In Experiment 1 the basic feed ration consisted of equal quantities by weight of the standard ration and maize meal.

Two control groups of rats served all 4 experiments. They received a diet which comprised the basic ration — in one group this was identical with that fed to the rats in Experiment 1, while in the other it was standard rat ration — to which 10% of dextrin was added by weight.

Young male and female Wistar rats weighing between 30 g and 50 g were used, and were weaned onto the different experimental feed rations, the details of which are given in Tables I - IV. These rations, with the exception of those given to the control groups, were fed for varying periods of time, after which surviving rats were given the standard commercial rat ration. The rats in the control groups received the experimental rations during the entire duration of the experiment. The feed rations and drinking water were available *ad libitum* to the animals.

Postmortem examinations were done on animals which died or were slaughtered when the presence of intra-abdominal neoplasms was detected or at the conclusion of the experiment, and material from all major organs was taken for histological examination.

RESULTS

The survival times of the animals and the principal lesions seen are presented for convenience in tabular form (Tables I - IV).

Experiment 1 (*E. umbeluziensis*—Table I)

The animals in the 2 groups which had received rations containing 2,5% or 10% kernel and outer flesh respectively, all died acutely between the 9th and 23rd days. The principal lesion observed was that of an acute necrosis of hepatocytes, which was mainly centrilobular in distribution, with some leucocytic infiltration. In those which survived the longest, collapse to the central portions of lobules was accompanied by connective tissue increase and some hepatocellular pleomorphism.

It is interesting to note that a large hyperplastic nodule of hepatocytes was seen in the liver of 1 of the 2 rats which had received a mixture of peduncle and cone scales when it was slaughtered 373 days after commencement of the experiment.

In group IV the 3 male rats and 2 of the 3 females which had received a ration containing 1% kernel and outer flesh, showed the presence of neoplasms. The first of these was encountered in rat 18 which was slaughtered on day 231, when bilateral nephroblastomas were encountered in the kidneys. In the other 4 rats, 3 exhibited bilateral kidney nephroblastomas and 1 a hepatocellular carcinoma. The livers of the 6 animals in this group also showed severe histological changes which consisted basically of a disturbance of normal architecture, foci or nodules of hepatocellular hyperplasia, bile duct hyperplasia — some of these were cystic while others were lined by hyperplastic epithelium — and dilatation of sinusoids in parts.

None of the 10 control animals showed any macro- or microscopic lesions of significance when they were slaughtered and then examined.

Experiment 2 (*E. villosus*—Table II)

Hepatocellular carcinomas were detected in group 1 in 1 male and 1 female rat, which had received a 1% mixture of kernel together with its outer flesh in the ration. One of these carcinomas exhibited transcoelomic metastasis to the peritoneum and omentum. The livers of all the 6 animals in this group showed an altered architecture and hyperplastic nodules histologically, and in some there was also bile duct hyperplasia. In the kidneys of 3 animals hyperplasia, pleomorphism, hypertrophy and hyperchromasia were present in many epithelial cells of the cortical tubules.

TABLE I. EXPERIMENT 1 — *E. UMBELUZIENSIS*

Group and ration	Rat No. and sex	Duration of feeding exptl ration (days)	Duration in expt (days)	Principal lesions
I Basic ration* + 10% kernel and outer flesh	1 M	15	D 15	Acute hepatopathy
	2 M	9	D 9	do.
	3 M	9	D 9	do.
	4 M	14	D 14	do.
	5 M	13	D 13	do.
	6 M	9	D 9	do.
II Basic ration + 10% peduncle and scale	7 M	66	S 373	NS
	8 M	66	S 373	Nodular hyperplasia
III Basic ration + 2½% kernel and outer flesh	9 M	19	D 19	Acute hepatopathy
	10 M	20	D 20	do.
	11 M	19	D 19	do.
	12 M	21	D 21	do.
	13 M	21	D 21	do.
	14 M	23	D 23	do.
IV Basic ration + 1% kernel and outer flesh	15 M	48	S 300	Nephroblastoma + hepatopathy
	16 M	48	S 300	do.
	17 M	48	S 300	Nephroblastoma + hepatocellular Ca
	18 F	48	S 231	Nephroblastoma + hepatopathy
	19 F	48	S 300	do.
	20 F	48	D 135	Hepatopathy
V (Control) Basic ration + 10% dextrin	21 M	245	S 245	NS
	22 M	245	S 245	NS
	23 M	245	S 245	NS
	24 M	245	S 245	NS
VI (Control) Commerial rat ration + 10% dextrin	25 M	300	S 300	NS
	26 M	300	S 300	NS
	27 M	300	S 300	NS
	28 F	300	S 300	NS
	29 F	300	S 300	NS
	30 F	300	S 300	NS

* Basic ration comprised equal quantities commercial rat ration and maize meal by weight.
D = died; S = slaughtered by decapitation; NS = nothing significant.

TABLE II. EXPERIMENT 2 — *E. VILLOSUS*

Group and ration	Rat No. and sex	Duration of feeding exptl ration (days)	Duration in expt (days)	Principal lesions
I Ration* + 1% kernel and outer flesh	1 M	300	S 300	Hepato- + nephropathy
	2 M	300	S 300	Hepatocellular Ca + nephropathy
	3 M	300	S 300	Hepato- + nephropathy
	4 F	300	S 300	Hepatopathy
	5 F	300	S 300	do.
	6 F	300	S 300	Hepatocellular Ca
II Ration* + 10% outer flesh	7 M	7	S 300	Hepatopathy
	8 F	7	D 267	Hepatocell. Ca + nephroblastoma
III Ration* + 1% kernel	9 M	7	S 300	Mild hepato- + nephropathy
	10 F	7	S 300	do.
IV Ration* + 10% peduncle	11 M	13	S 300	Mild nephropathy
	12 F	13	S 300	do.
V Control (as for group VI Expt 1)				

* Ration comprised commercial rat ration.

The 2 rats in group II which had received a ration containing 10% outer flesh, showed only nodular hepatocytic hyperplasia. In addition, both a hepatocellular carcinoma, which exhibited pulmonary and renal metastasis, and bilateral renal nephroblastomas were encountered in 1 of them.

While no neoplasms were observed in the 4 rats in groups III and IV, those that had received 1% kernel for 7 days showed the presence of indistinct nodules of hepatocytic hyperplasia when they were killed on the 300th day of the experiment, while several groups of tubules in the kidney cortex of all 4 animals in these groups showed epithelial hyperplasia and hyalinised basement membranes.

Experiment 3 (*E. lebomboensis*—Table III)

In this experiment lesions were seen only in the animals that had received outer flesh in the ration. Three of the 4 rats died in the first 24 days, and the principal change noticed was in the liver, where a severe centrilobular necrosis of hepatocytes with haemorrhage and congestion was observed. The 1 animal which survived these acute toxic effects showed the presence of one large nephroblastoma which had entirely replaced the left kidney and one smaller one in the other kidney. Numerous nodules of hyperplasia of hepatocytes were noticed in its liver.

No lesions were detected in the organs of the animals which had consumed 1% kernel in their ration for 56 days.

Experiment 4 (*E. laevifolius*—Table IV)

In group I the only change considered to be of any significance was that of nodular hepatocytic hyperplasia, which was seen in 2 of the 6 animals which had received 1% kernel in their ration for 82 days. However, 3 of the rats in group III which had been given a ration containing 10% kernel for 22 days, developed renal neoplasms: in 2 these were nephroblastomas (unilateral in the one, and bilateral in the other) and in the third the neoplasm was a unilateral carcinoma. In the kidneys of 5 of the 6 animals epithelial hyperplasia of several tubules in the cortex was noticed. Furthermore, several foci of nodular hepatocytic hyperplasia, bile duct hyperplasia and sinusoidal dilatation were seen in the kidneys of several of them.

Only 1 animal in group II survived for any length of time after the 40th day, when the ration containing 10% outer flesh was no longer fed. Bilateral nephroblastomas and nodular hyperplasia of the liver were noticed when this rat was slaughtered on day 226. However, the livers of the other 5 animals in this group all showed histologically a partial collapse of lobules, connective tissue proliferation, pleomorphic hepatocytes and some bile duct proliferation.

DISCUSSION

Notwithstanding the fact that the scope of these experiments was hampered by limited amounts of plant material of the 4 South African species of cycads tested, interesting results were obtained, the most noteworthy being their carcinogenicity which is described for the first time. In this respect nephroblastomas were produced in the rats by incorporating into their rations various concentrations of mixtures of the kernel and outer flesh of the seed of *E. umbeluziensis,* outer flesh only of *E. villosus, E. lebomboensis* and *E. laevifolius,* and the kernel of *E. laevifolius.* Feeding the latter also caused the development of a renal carcinoma in 1 rat. In addition, hepatocellular carcinomas were seen in rats which had received mixtures of kernel and outer flesh of *E. umbeluziensis* and *E. villosus* in their diets for varying periods.

Depending upon the concentrations and nature of the plant material of all the cycad species fed, lesions of an acute or chronic nature besides neoplasia were also noted in the liver and kidney. The most outstanding of these

TABLE III. EXPERIMENT 3 — *E. LEBOMBOENSIS*

Group and ration	Rat No. and sex	Duration of feeding exptl ration (days)	Duration in expt (days)	Principal lesions
I Ration* + 10% outer flesh	1 M	13	D 24	Acute hepatopathy
	2 M	13	D 12	do.
	3 F	13	D 395	Nephroblastoma + hepatopathy
	4 F	13	D 13	Acute hepatopathy
II Ration* + 1% kernel	5 M	56	S 300	NS
	6 M	56	S 300	NS
	7 M	56	D 14	NS
	8 F	56	S 300	NS
	9 F	56	S 300	NS
	10 F	56	S 300	NS
III Control (as for group VI Expt 1)				

* Ration comprised commercial rat ration.

TABLE IV. EXPERIMENT 4 — *E. LAEVIFOLIUS*

Group and ration	Rat No. and sex	Duration of feeding exptl ration (days)	Duration in expt (days)	Principal lesions
I Ration* + 1% kernel	1 M	82	S 300	NS
	2 M	82	S 300	NS
	3 M	82	S 300	NS
	4 F	82	S 300	Hyperplastic nodules
	5 F	82	S 300	NS
	6 F	82	S 300	Hyperplastic nodules
II Ration* + 10% outer flesh	7 M	40	D 47	Hepatopathy
	8 M	40	D 54	do.
	9 M	40	D 61	do.
	10 F	40	D 36	do.
	11 F	40	S 226	Nephroblastoma + hepatopathy
	12 F	40	D 33	Hepatopathy
III Ration* + 10% kernel	13 M	22	S 286	Mild hepatopathy
	14 M	22	S 286	Mild hepatopathy + nephropathy
	15 M	22	S 286	Renal Ca + hepatopathy
	16 F	22	S 286	Nephroblastoma + hepatopathy
	17 F	22	S 286	NS
	18 F	22	S 286	Nephroblastoma + hepatopathy
IV Control (as for group VI Expt 1)				

* Ration comprised commercial rat ration.

were the hepatocellular necrosis in rats dying acutely, the nodular hyperplasia of the liver and hyperplastic and hypertrophic alterations in the kidney tubular epithelium. It is well documented that some hepatocellular carcinomas develop in some animal species with hyperplastic liver nodules.[19] Not only were the kernel, outer flesh or mixtures of these responsible for some of these changes, but also the peduncle of *E. villosus,* which caused alterations of a chronic nature in the kidneys of the rats which had received it, and mixtures of the peduncle and cone scale in the case of *E. umbeluziensis* where nodular hyperplasia of the liver was seen in 1 of the animals which was given it. There seems no doubt, therefore, that the majority, if not all, parts of the cone of the latter 2 species of cycads tested contain the toxic fraction. It is probable that the ingestion of any part of plants of the *Encephalartos* genus is potentially fraught with danger and should be strenuously discouraged. Cycasin occurs in the seeds, roots and leaves of plants of the Cycadaceae and a single administration to rats of this substance will produce neoplasms—the kidney, liver and intestinal tract being the most susceptible to its carcinogenic action.[20] Renal nephroblastomas and adenocarcinomas, and hepatocellular carcinomas are among the common neoplasms induced by cycasin. Experimental animals other than rats are also susceptible to the carcinogenic action of cycasin and its active principle, methylazoxymethanol. The development of tumours in the liver (hepatocellular carcinomas, fibrosarcomas, cystadenomas and bile duct adenomas), kidneys (fibrosarcomas, adenomas, nephroblastomas) and lungs (adenomas) of rats fed the kernels of *E. hildebrandtii* has been reported.[2,14] This species contains macrozamin.[13]

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