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Effect of Dietary Protein on Toxicity and Liver Lesion Caused by a Single Intraperitoneal Dose of Ngaione in Rats

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Introduction

Cattle and sheep become poisoned following consumption of leaves of some *Myoporum* spp. which grow in Australia and New Zealand. The plants contain furanosesquiterpenoid essential oils, the best known of which is ngaione[3,10].

It has been shown that, apart from the presence of non-nutrient potential inducers of the hepatic microsomal mixed function oxidases (HMFO) in sheep, the level of protein can affect the activity of this enzyme system[9], in that the HMFO are higher in sheep fed high protein rations than in sheep given chaffed hay of low protein content. This observation was made following the report of McLean and McLean[6] that diets low in protein, when fed to rats caused marked reduction in the HMFO and at same time rendered the animals less susceptible to carbon tetrachloride. Since ngaione, like carbon tetrachloride[6], appears to require HMFO metabolism for toxicity, and since HMFO metabolism in sheep can vary with dietary protein content in the paddock, an experiment was carried out to examine the effect of dietary protein level on the toxicity of ngaione for the rat.

Materials and Methods

The animals used in the experiment were young male Wistar rats weighing from 90 to 110g, bred by the Central Animal Breeding House of the University of Queensland.

Ngaione isolated from leaves of *Myoporum deserti*[3] was freshly diluted 1 part in 2 with arachis oil for dosing.

Eighty-three rats were divided into 3 groups of 27 and one group of two animals. The last group was fed on the standard cubed diet and given tap water *ad libitum*. Each of the larger groups was fed for 10 days on a synthetic diet prepared in accordance with McLean and McLean[6] using locally supplied purified ingredients. The diets contained 0, 15 and 30% protein as casein respectively. At the end of the period on the synthetic diets each group of 27 rats was subdivided into two groups of 6 and one of 15. Hexobarbitone sleeping time[11] and *p*-nitroanisole metabolism by liver homogenates[7] were determined using the two groups of 6 rats respectively. In sleeping time experiment rats were given a single intraperitoneal injection of hexobarbitone at 150 mg/kg body weight and the time recorded between loss and return of the righting reflex. The time of incubation for measurement of *p*-nitroanisole metabolism by the liver homogenates was 40 minutes and the results of the microsomal *p*-nitroanisole demethylation was expressed as micromoles product formed/g wet liver/40 minutes since, beyond this period, the relationship of the amount of reaction product formed with time was not linear. Each remaining group of 15 animals was further divided into 5 sub-groups of three and each sub group was given ngaione, intraperitoneally, at a different dose-rate. The five three-animal sub-groups from each of the 15% and 30% protein groups were given 100, 140, 196, 275 and 385mg ngaione/kg body weight respectively, and the five sub-groups from the non-protein group were dosed with 50, 75, 113, 169 and 254 mg of the oil/kg body weight respectively. The animals were necropsied for pathological study

when moribund, or at 5 days after dosing, at which time they were considered to have survived the acute effects of the intoxication. Samples of liver wers fixed in buffered neutral formalin for histopathological examination. Paraffin sections 7 μm in thickness were prepared and stained with Mayer's haematoxylin and eosin. The LD_{50} for ngaione was determined by the method of Weil[12].

Results

The two rats fed the cubed diet gained 47.0 and 48.9g respectively during the 10 day period of the experimet. During this period the rats fed the non-protein diet lost 23.5±2.9g body weight while those fed 15 and 30% protein gained 35.6±7.2 and 48.3 ±6.0 g body weight respectively. The effect of the three synthetic diets on hexobarbitone sleeping time and *p*-nitroanisole metabolism *in vitro* are set out in Table 1. The activity of the HMFO in the non-protein group was markedly less than in the groups fed 15 and 30% protein, which were similar. The hexobarbitone sleeping time in the non-protein group was increased about five-fold. The livers of the 15 and 30% protein groups were macroscopically and microscopically normal, while those of the non-protein group were yellowish owing to fatty infiltration. In other respects the organs of the rats on the synthetic diets, prior to dosing with ngaione, were unremarkable. The toxicity of ngaione and the type of zonal liver injury for the three groups are also set out in Tale 1. The LD_{50} of the oil was similar in each diet group.

The time of death at each dose rate were similar to those described in previous LD_{50} determinations[4]. Lesions were observed only in the liver and stomach and, as in previous studies, small amounts of free blood were often found in the intestinal contents.

Liver lesions caused by ngaione were consistently periportal in the 30% protein group and always centrolobular in the non-protein group. In the 15% protein group however, centrolobular, midzonal and periportal lesions were all observed, sometimes all three locations in different lobes of the same liver. In general however, in the 15% protein group, centrolobular lesions were present in the rats given the two higher dose rates of ngaione, and midzonal or periportal in the livers of the lower three dose rate sub-gronps.

Discussion

As shown by McLean[6] feeding a non-protein diet for 10 days caused marked loss of body weight, and a reduction in the HMFO as compared with the animals fed 15 and 30% protein. The HMFO activities of the latter groups were similar. This also is consistent with the observations of McLean and McLean[6], that the activity of the enzyme system was maximal at dietary crude protein levels of 10%. In contrast to these author's observations that feeding rats a non-protein diet protected the animals against the acute toxic effects of carbon tetrachloride, it was found that this diet conferred no such protection on the animals to ngaione. Some 4 to 20 days of the protein-free diet caused a 90% drop in the HMFO *in vitro* of the rats used by the above authors, while in our experiment there was only

Table 1. Effect of Dietary Protein Level on Hexobarbitone Sleeping Time, Hepatic *p*-Nitroanisole Demethylation *in vitro*, LD_{50} and Liver Lesions Caused by Ngaione in Male Rats

*Dietary Groups	Hexobarbitone Sleeping Time (min.) (Mean±SD)	*p*-Nitroanisole Demethylation (μmoles product formed/g/40 min.)	LD_{50} (mg/kg body weight) (95% confidence limits)	Liver Lesions
Non-protein	112.0±21.1	0.75±0.16	124(82-189)	CL Necrosis
15% Protein	24.2±4.7	1.39±0.23	185(126-273)	MZ Necrosis CL Necrosis PP Necrosis
30% Protein	19.2±4.1	1.20±0.23	166(96-287)	PP Necrosis

* All diets consisted of purified ingredients as described by McLean & McLean (1966) and were fed for 10 days. CL=Centrolobular. MZ=Midzonal. PP=Periportal.

about 50% reduction in the microsomal enzyme activity. In drug-induced reduction of the HMFO in previous experiments, moderate protection from ngaione toxicity however was observed. It is possible then that, in the present diet-induced reduction in the HMFO, the degree of inhibition was not sufficient to be effective in enhancing the tolerance of the animals to the oil. However, in accordance with drug-induced inhibition of the HMFO, the reduction in the enzyme caused by the non-protein diet did result in a transplacement of the liver lesion to the centrolobular zone. At the 15% protein level of the synthetic diet, the zone of injury was varied extensively, from centrolobular to periportal, which suggested that the system determining this feature of the hepatic histopathology was very labile. This particular aspect of the hepatopathology caused by ngaione was further investigated in the mouse and is reported already[5]. It is of interest to note however that size of dose appeared to play a part in liver lesion placement in the 15% protein group, since with large doses the liver lesion became centrolobular as opposed to midzonal and periportal at low doses. This result was similar to that originally produced by Cunningham and Hopkirk[2] in sheep, in which it was reported that with high doses of ngaione or *Myoporum laetum* leaves the liver lesion was always centrolobular while at lower doses it was periportal. High protein diets nevertheless appeared to predispose to periportal lesions in this study, whatever the dose rate of the oil.

It was proposed by Seawright and Hrdlicka[8] that both toxic and nontoxic conversion of ngaione by HMFO occurred in the liver. The degree to which one or the other of these reactions was dominant in a particular liver cell might determine whether or not injury occurred owing to the presence of ngaione in the cell. The relative proportions of the particular enzyme reactions possible for ngaione might well be modified by various chemical pretreatment agents, as already demonstrated in the recent studies[4] and in investigations in various other animal species[1]. It appears also that the amount, and possibly the type, of protein in the diet may play a part here as well, in determining perhaps, not so much the overall toxicity of the oil for the animal as the placement of liver lesions that occur in poisoning.

Conclusion

The toxicity of ngaione for rats fed 0, 15 and 30% protein diets respectively for 10 days was similar, although HMFO *in vitro* of the non-protein rats was reduced to about 50% of that of the protein supplemented groups and the hexobarbitone sleeping time increased five-fold. The placement of the liver lesion in the non-protein group was always centrolobular while in the 30% protein rats it was periportal. In the 15% protein group the liver lesion was periportal, midzonal or centrolobular, depending apparently on the dose rate of the ngaione.

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蛋白質飼料가 Ngaione에 中毒된 흰쥐의 毒性 및 肝臟病變에 미치는 影響

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抄 錄

實驗動物 飼料중의 蛋白質含量이 ngaione에 中毒된 흰쥐의 毒性 및 肝臟病變에 미치는 影響을 觀察하였던 바 그 結果는 다음과 같다.

飼料中의 蛋白質含量을 0, 15 및 30%로 맞추어서 3個의 實驗群에 各各 10日間씩 給與한 다음 이들 動物에 ngaione을 腹腔內로 投與하였으나 이에 依한 毒性의 變化는 없었다.

蛋白質 飼料를 10日間 給與하지 않은 動物의 hepatic microsomal mixed function oxidases (HMFO)의 機能은 蛋白質飼料(15 및 30%)를 給與한 動物에서보다 約 50% 減少되었고, 睡眠時間은 約 5倍로 延長되었다.

ngaione에 依해서 惹起된 肝臟病變部位는 0 및 30% 蛋白質 給與群에서 各各 小葉中心部(centrolobular region)와 脈管周圍部(periportal region)에서 觀察되었으나 15% 蛋白質 給與群에서는 ngaione의 投與量에 따라서 小葉中心部, 脈管周圍部 및 小葉中間部(midzonal region)中에서 어느 部位에서나 觀察되었다.