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Studies on Toxicity of Ngaione in Rats

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Introduction

Ngaione is a toxic sesquiterpenoid essential oil occurring in several *Myoporum* spp. The plants are confined mainly to Australasia and the islands of South West Pacific, but they are also found in Japan, China and Mauritius. [5]

Brief descriptions of toxicity due to ngaione in the rat have been given by Cunningham and Hopkirk[2] and Denz and Hanger[3] The former reported that 600 mg/kg of the essential oil given by the oral route was non toxic but the latter authors reported the oral LD_{50} for the compound to be 480 mg/kg (95% confidence limits of 390 to 590 mg/kg body weight). Apart from brief mention of changes in the liver no detailed descriptions were given of the pathology present in affected animals. In the present experiment the effect of a single intraperitoneal dose of the oil in the rat is recorded.

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. They were fed on a proprietary cubed diet and given tap water *ad libitum*.

Ngaione isolated from leaves of *Myoporum deserti*[4] was freshly diluted 1:1 or 1:2 in arachis oil for dosing. Fifteen rats were divided into 5 groups of 3 animals. The groups were dosed with ngaione by the intraperitoneal route at 100, 140, 196, 275 and 385 mg/kg body weight respectively. Rats which were about to die were killed, and necropsies perfo-rmed immediately; surviving rats were killed and necropsied up to four days after dosing. Samples of liver, stomach wall, lungs, heart, spleen and kidney were fixed in buffered neutral form alin for histo-pathological examination. Paraffin sections 7μm in thickness were prepared and stained wth Mayer's haematoxylin and eosin. The LD_{50} for ngaione was determined by the method of Weil[11].

Results

All animals dosed appeared ill and depressed within 1/2 to one hour and those of the two highest dose rate groups died within 3 to 10 hours. Of the animals given the intermediate dose of 196 mg/kg, two died within 18 to 36 hours and one rat in the group given 140 mg/kg died in 38 hours. The con-dition of the remaining rats began to improve by 48 hours and all animals had appetite and appeared normal by 3 to 4 days. The LD_{50} was calculated to be 166 mg/kg with 95% confidence limits of 96 to 287 mg/kg.

Gross Pathology: Necropsies of the rats which died from the acute toxic effects of ngaione indicated that the liver was the only organ in which lesions were present consistently although diffuse haemorr-hages were often seen in the stomach wall, and free blood was found in the lumen of the stomach and in various other parts of the lower alimentary tract. The rectal contents were always firm and dry. The livers of all rats dying within 10 hours of dosing were markedly swollen, firm and uniformly dark red as though acutely congested. In rats dying between 18 and 24 hours after dosing, the livers were swollen and dark red and there was a distinct regular zonal mottling apparent on the capsular surface. This was more evident on the cut surface which was alter-nately dark red and pale pinkish gray. The liver of the rat dying at 36 hours was swollen, firm and pale yellow with irregular reddish zonal mottling which was most apparent on the cut surface. The surviving rats which were killed for pathological examination

at 4 days had pale swollen livers in which faint regular zonal mottling was still present. The livers of the latter rats which had been given 140 mg/kg of ngaione had large discrete greyish-yellow infarcts located in either the right or left central lobes and these lesions were most obvious on the diaphragmatic surface.

Histopathology: The histopathological features of the organs other than the liver and stomach wall were unremarkable.

In the livers of the rats dying between 3 and 10 hours after dosing, the portal areas were congested and dilated and the periportal sinusoids were distended and filled with blood. In most of the centrolobular and midzonal hepatocytes there were irregular or round vacuoles containing a pale pink homogeneous material (Fig. 1). In the parenchymal midzone many individual or groups of hepatocytes, together with associated sinusoidal lining cells had disintegrated and disappeared leaving small blood lakes containing some residual pyknotic nuclei or other parenchymal cell fragments. The cytoplasm of many of the hepatocytes of the midzonal areas was more eosinophilic than normal and the nuclei were pyknotic or karyorrhectic. The periportal parenchymal areas in some livers appeared much less affected than the adjacent midzonal parenchyma, while in others the degenerative changes affected all the parenchymal cells without any indication of a definite zonal distribution of injury. Generally, while the degree of degeneration was most severe in confluent midzonal areas, degenerative change was also present to a lesser extent in the periportal and centrolobular zone as well, but each in a differing degree.

There was entensive submucosal oedma and haemorrhages of various parts of the stomach wall, and small blood vessels in the centres of some diffuse haematoms appeared to be ruptured (Fig. 2). The mucosa of the glandular and cardiac regions appeared to be undamaged.

In animals dying from 18 to 24 hours there was a continuous band of coagulative necrosis of the midzonal hepatocytes, the affected zone having been converted to a discrete, bright, pink-staining amorphous mass consisting of red cell, hepatocyte fragments and Kupffer cell neclei(Fig. 3). Except for the presence of occasional more darkly staining hepatocytes, the parenchyma of the centrolobular zone appeared normal while in the periportal parenchyma the cytoplasm was paler pink staining, foamy and sometimes vacuolated.

In the rat dying at 36 hours after 196 mg/kg of ngaione, there was extensive midzonal and periportal coagulative necrosis of the liver, with marked invasion of the confluent necrotic zones by macrophages and polymorphonuclear leucocytes. The smallest portal tracts were more prominent than normal, owing to infiltration with increased numbers of mononuclear inflammatory cells, slight biliary ductular hyperplasia, and slight increased deposition of fibrous tissues (Fig. 4).

The livers of the rats given the lowest dose of ngaione(100mg/kg) were almost normal at 4 days except the cytoplasm of the periportal hepatocytes stained more bluish than that of the centrolobular cells. The livers of the rats in the group dosed with 140 mg/kg contained a few residual foci of necrotic hepatocyte debris, surrounded by macrophages, in the midzonal areas of the parenchyma. Surviving parenchymal cells adjacent to these areas were larger and paler than normal and contained large nuclei with large prominent or multiple nucleoli. Mitotic figures were plentiful in hepatocytes in all areas of the parenchyma and Kupffer cell nuclei in the midzonal sinusoids were plump and oval. The periportal and centrolobular parenchyma were otherwise normal.

In summary, the liver lesions were mostly zonally distributed, involving chiefly the midzonal or the midzonal and adjacent periportal parenchyma. The centrolobular parenchymal region was usually least affected.

Disussion

The experiment confirmed the earlier finding by Cuningham and Hopkirk[2] that ngaione caused injury consistently only to the liver in the rat. However, the LD_{50} of 166 mg/kg was substantially lower than that of 480 mg/kg recorded in rats given ngaione by the oral route by Denz and Hanger[3]. As had been

found in mice[3,10] the live lesion seen in the rats was primarily a midzonal hepatic necrosis. The diffuse haemorrhage and oedema of the stomach wall, with haemorrhage into the gut lumen, which were seen in most of the more severely intoxicated animals, were similar to the alimentary lesions recorded in sheep or cattle acutely intoxicated with *Myoporum deserti*[1] or affected with other acute hepatotoxicities such as those caused by *Trema aspera*[7], *Xanthium pungens*[6] and carbon tetrachloride in which mucosal haemorrhages and extensive blood staining of the abomasal, small intestinal and caecal contents frequently occurred. These alimentary lesions are not haemorrhagic gastroenteritis as has been suggested in some of the earlier reports of intoxication in sheep or cattle but is probably secondary to the extensive and rapidly developing liver damage which can be expected to cause partial but sudden obstruction of the blood flow through the organ.

Conclusion

The LD_{50} for ngaione, administered by the intraperitoneal route, for young male rats, was 166 mg/kg with 95% confidence limits of 96 to 287 mg/kg. Pathological changes were consistently present only in the liver and histopathologically there was midzonal necrosis. Haemorrhages were present in the stomach wall, and other parts of the lower alimentary tract, at higher dose rates of the oil, and although blood was found in the gut contents the mucosa appeared normal.

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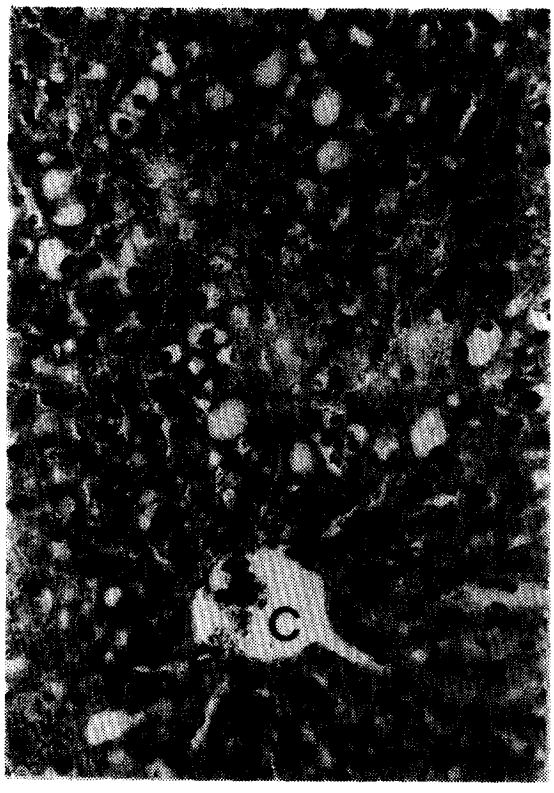
Legends for Figures

Fig. 1. Section of liver of a rat dying 4 hours after dosing with the compound at 385 mg/kg body weight. Irregularly round vacuoles are numerous in the damaged hepatocytes and congestion of midzonal area is apparent. C=Central vein. Haematoxylin and eosin (H & E.). ×250.

Fig. 2. Section of stomach wall of a rat dying 4 hours after dosing with ngaione at 385mg/kg body weight. The blood vessel (arrow) in the centre of a diffuse haematoma appears to be ruptured. H & E. ×100.

Fig. 3. Liver of a rat dying 22 hours after dosing with ngaione at 196 mg/kg body weight. A relatively broad band consisting of red cells, necrotic hepatocyte fragments and Kupffer cell neclei is seen in the midzonal region. C=Central vein, P=Portal vein. H & E. ×250.

Fig. 4. Liver of a rat surviving for 4 days after dosing with ngaione at 140 mg/kg body weight. There is infiltration of mononuclear inflammatory cells and macrophages in the midzonal area and slightly increased fibrous tissue in small portal tracts. H & E. ×250.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Ngaione의 흰쥐에 對한 毒性에 關한 硏究

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*Myoporum deserti*에서 얻어지는 有毒性植物油인 ngaione을 體重 90～110g의 Wistar系 雄性白鼠에 腹腔內로 注入한 다음 有毒性을 調査하여 다음과 같은 結果를 얻었다.

흰쥐에서의 ngaione의 致死量 (LD_{50})은 體重 kg當 166mg이었고 95% 信賴限界는 體重 kg當 96～287mg이었다.

Ngaione 注入後 肝臟에서 언제나 病變이 觀察되었으며 이들의 病理組織學的 所見은 小葉中間部壞死(midzonal necrosis)였다.

Ngaione의 注入量이 많은 動物에서는 자주 胃壁(粘膜下織)과 大腸의 一部에서 散漫性出血을 同伴하고 있었다.