STUDIES IN KAFFIRCORN MALTING AND BREWING*

XXII. THE ACUTE TOXICITY OF SOME FUSEL OILS FOUND IN BANTU BEER

I. F. H. PURCHASE, *Division of Toxicology, National Nutrition Research Institute, Council for Scientific and Industrial Research, Pretoria*

Bantu beer differs from ordinary beer in having a high fusel oil content. In commercially brewed beer on the Witwatersrand the fusel oil content is 227 mg./litre and the ratio of the fusel oil to the ethyl alcohol concentrations is higher than in ordinary beer.[1] The fusel oils occurring in the highest concentration are iso-amyl, iso-butyl, n-hexyl, β-phenyl-ethyl and n-propyl alcohols.

Richardson[2] found that the toxicity of the alcohols in the fatty acid series increased with increasing molecular weight. Subsequently this 'law' has been confirmed in general, with a few exceptions.

The purpose of this study was to compare the oral LD_{50} values of the long-chain alcohols occurring in Bantu beer before studying their long-term toxicity in rats. Although from time to time these values have been determined, comparison may be complicated by the fact that different workers have determined these values on different species.

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METHODS

Groups of 4 rats weighing between 60 and 100 G were starved for 24 hrs and given a single dose of alcohol by stomach tube. Doses of less than 0·1 ml. were made up to 0·25 ml. with polyethylene glycol 200. The range of doses used for a particular alcohol were selected after a preliminary experiment had established the approximate

degree of toxicity. The log interval between doses was constant, allowing the LD_{50} values to be calculated from the tables described by Weil.[3]

Postmortem examination of all rats was performed at death or when the survivors were killed at 10 days. Portions of heart, liver, lung and kidney from representative rats in each group were fixed in buffered formalin and embedded in wax. Sections (7μ) were stained with haematoxylin and erythrosin.

RESULTS

The LD_{50} values obtained are set out in Table I. No significant changes were seen in the sections of the lungs and hearts in any of the rats.

n-Propyl Alcohol

The LD_{50} values for males and females were similar (0·56 - 0·66 G/kg. respectively) but lower than the values obtained by others. The intraperitoneal LD_{50} is given as 2 ml./kg. (1·6 G/kg.)[4] or 4 ml./kg. (3·2 G/kg.).[5]

TABLE I. THE ACUTE ORAL LD_{50} VALUES FOR CERTAIN FUSEL OILS

Alcohol	*Mol. wt*	*LD_{50} (G/kg.) with 95% limits in brackets*	
		Female	*Male*
n-propyl	60	0·66 (0·33–1·34)	0·56 (0·20–1·58)
n-butyl	74	2·02 (1·18–3·28)	0·79 (0·28–2·24)
Iso-butyl	74	3·10 (1·97–4·86)	2·65 (1·79–3·99)
Iso-amyl	88	4·00 (2·45–6·17)	1·30 (0·67–2·41)
n-hexyl	102	0·72 (0·36–1·42)	1·80 (0·27–10·8)
β-phenyl-ethyl	122	0·65 (0·31–1·36)	1·43 (0·65–2·94)

The doses used ranged from 0·15 to 3·0 G/kg. for both sexes. After receiving the higher doses rats died in 6 - 24 hrs, but those that died after receiving lower doses survived to 4 or 5 days. Postmortem examination of the rats dying within 24 hrs revealed congestion of all organs. Those dying at 4 - 5 days showed signs of degeneration in liver and kidneys.

Histological examination of sections stained with H & E showed the following changes: The liver of rats dying in 24 hrs showed marked hyperaemia with dilation of the sinusoids. Those dying at 4 - 5 days showed hyperaemia and in some cases centrilobular vacuolation which could be described as fatty infiltration. The changes found in the kidneys were of a more extensive nature. The kidneys of animals dying in 24 hrs showed a very marked hyperaemia. The glomeruli were unaffected, but the majority of the convoluted tubules showed signs of cloudy swelling which was accompanied in some tubules by cast formation. A variable number of other tubules showed cells with pyknotic nuclei with loss of staining of the cytoplasm indicating a tubular necrosis (Fig. 1(a)). The hyperaemia and tubular necrosis were most prominent in the outer zone of the medulla, where in some cases all tubules were affected (Fig. 1(b)). Animals dying at 4 - 5 days showed less prominent degenerative changes, and those killed at 10 days showed predominantly degenerative changes without the tubular necrosis so prominent in the more acute deaths.

n-Butyl Alcohol

The LD_{50} obtained was 2·02 G/kg. in males and 0·79 G/kg. in females. In spite of the difference between these figures the 95% confidence limits overlapped, indicating that the difference was not significant. The intraperitoneal LD_{50} obtained by Lendle[5] was 1·2 ml. (0·97 G/kg.) which is similar to the value obtained here, but Macht[4] gives the intraperitoneal value as 0·3 ml. (0·24 G/kg.).

The dose range used was 0·15 - 3·0 G/kg. for males and 0·32 - 4·9 G/kg. for females. Rats dying after higher doses survived only 2 - 6 hrs, but those dying after lower doses survived to 5 - 7 days. The postmortem findings were similar to those observed with n-propyl alcohol, namely congestion predominating in the early deaths, with degenerative signs becoming visible in the animals dying after 5 days.

Histological examination of the sections showed changes similar to those observed with n-propyl alcohol, the only difference being in the severity of the lesions. There was again a marked hyperaemia of the liver in the early deaths, but in only one section from an animal dying after 5 days was there any sign of fatty infiltration. The lesions in the kidney were slightly less marked than in the n-propyl alcohol group. Animals receiving a low dose showed hyperaemia and cloudy swelling with cast formation in the cortex, but the only signs of necrosis were in the medulla. The sections from animals receiving a higher dose and dying in 24 hrs showed more extensive necrotic changes.

Iso-Butyl Alcohol

The LD_{50} values were similar in males and females (2·65 and 3·10 G/kg. respectively). Macht[4] found the intraperitoneal LD_{50} to be 0·9 ml. (0·72 G/kg.).

The dose range used was 0·8 - 12·0 G/kg. Most rats receiving a lethal dose died within 18 hrs, but 1 rat receiving a low dose died after 5 days.

The postmortem findings were similar to those in the n-butyl alcohol group. The changes observed histologically were indistinguishable from those observed after n-butyl alcohol dosing. In particular the degree of the changes was similar to that observed with n-butyl rather than n-propyl alcohol.

Iso-Amyl Alcohol

There was a considerable difference between the LD_{50} values obtained for males and females (1·30 compared with 4·00 G/kg.). These values are much higher than those reported by Macht[4] of 0·15 ml. (0·12 G/kg.) and by Lendle[5] of 0·48 ml. (0·39 G/kg.).

The dose ranges used were 0·325 - 4·95 G/kg. in males and 0·81 - 12·0 G/kg. in females. Deaths in female rats occurred in 4 hrs after high doses (4·95 and 12·0 G/kg.) and none died in the 10 days after the lower doses (0·81 and 2·0 G/kg.). In contrast, although the males receiving the highest dose (4·95 G/kg.) and a group receiving 12·0 G/kg. died in 4 hrs, several rats receiving lower doses

Fig. 1. Sections of kidneys of male rats dying 6 - 24 hours after a single dose of alcohol (haematoxylin and erythrosin × 160). *1(a)*: Cloudy swelling and cast formation in the cortex after a dose of 10 ml. (8·2 G) n-hexyl alcohol/kg. *1(b)*: Degenerative changes in the outer zone of the medulla of the same section as in Fig. 1(a). *1(c)*: Tubular necrosis in the cortex after a dose of 10 ml. (8·0 G) n-propyl alcohol/kg. *1(d)*: Tubular necrosis in the medulla of the same section as in Fig. 1(c).

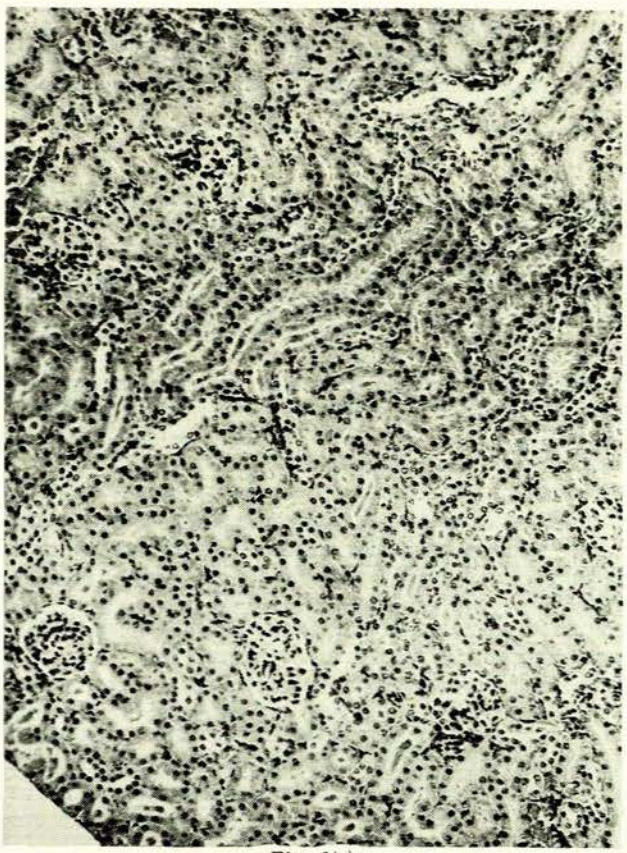

Fig. 1(a)

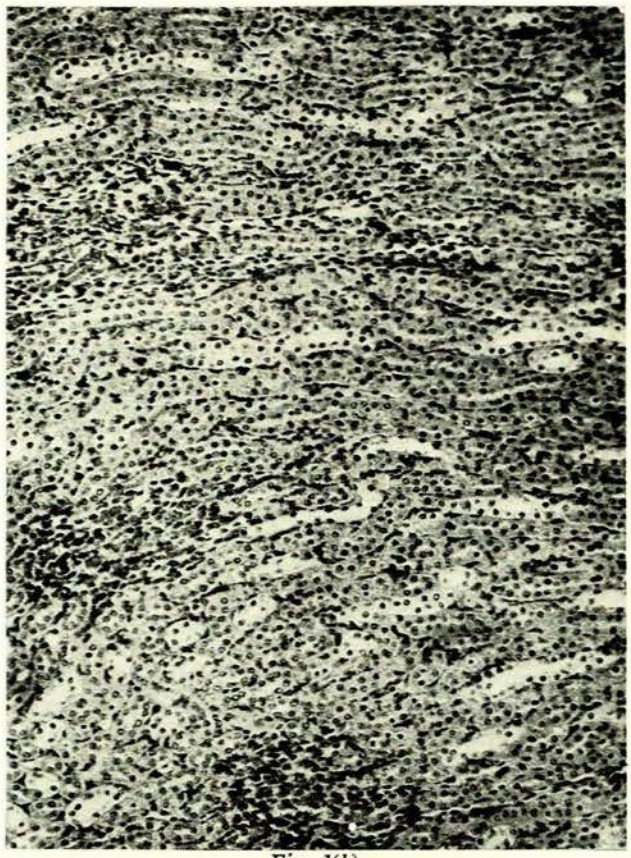

Fig. 1(b)

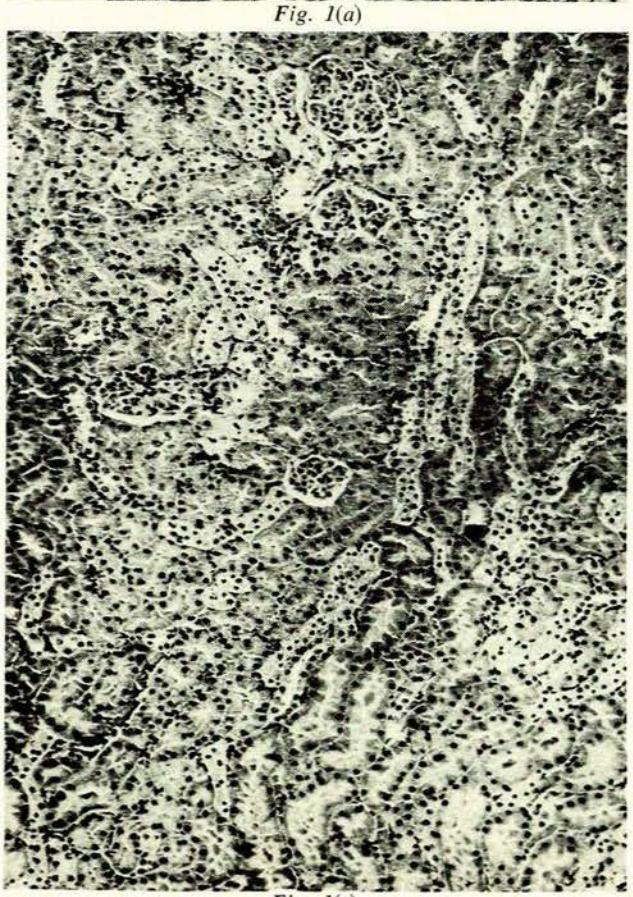

Fig. 1(c)

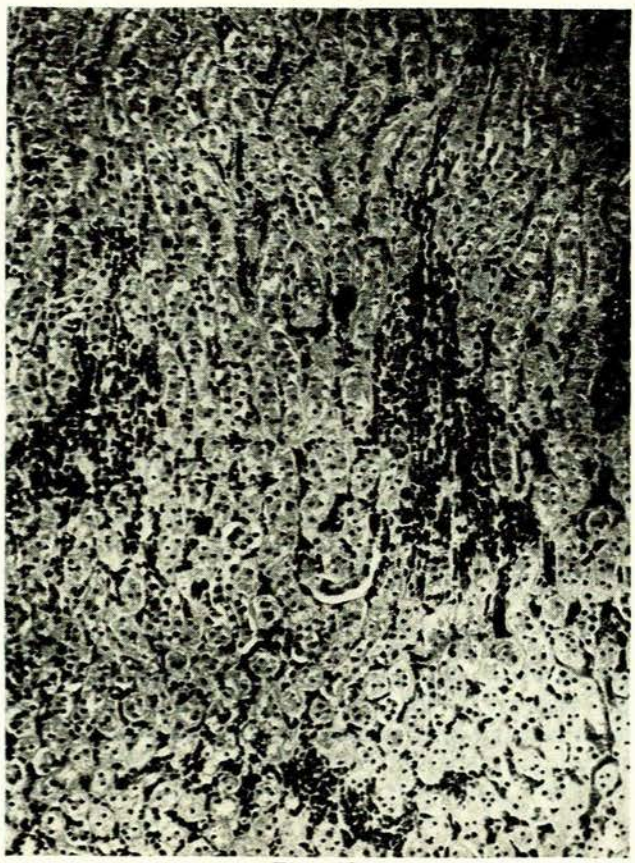

Fig. 1(d)

(0·81 and 2·0 G/kg.) died 1 - 5 days later. This may account for the different LD_{50} values obtained in males and females.

The postmortem findings were again similar to those from the n-butyl and n-propyl groups (hyperaemia predominating in the higher doses and degenerative changes visible in animals dying after 24 hrs).

Histological examination of the liver revealed hyperaemia but very few degenerative changes. The changes observed in the kidney sections were less pronounced than with the propyl or the butyl alcohols. The females killed at 10 days which had received lower doses (0·81 and 2·0 G/kg.) showed no significant changes in the kidney sections. Doses of 4·95 G/kg. produced cloudy swelling and cast formation in the cortex and a few pyknotic tubular cells in the medulla. At 12·0 G/kg. the cortex had more definite signs of necrosis, with some tubular cells showing pyknosis. In the males the changes were similar but more pronounced. Thus pyknosis and hyperaemia were seen in the outer zone of the medulla in all groups, and tubular necrosis was seen in the cortex after the two highest doses (2·0 and 4·95 G/kg.) which corresponded to the 2nd and 3rd doses in the females.

n-Hexyl Alcohol

The LD_{50} values were similar in the males and females (1·8 and 0·72 G/kg.). The dose ranges used were 0·44 - 8·8 G/kg. in males and 0·09 - 4·4 G/kg. in females.

Most rats receiving a lethal dose died within 24 hrs, only 2 surviving to 4 and 5 days. The postmortem findings were similar to those in rats dosed with butyl or propyl alcohol.

Histological examination of the liver sections showed hyperaemia in the animals dying within 24 hrs, and in a few rats a slight fatty infiltration. The sections from other rats were essentially normal. The changes observed in the kidney sections were less marked than in the n-propyl or n-butyl alcohol groups but similar to the females in the iso-amyl group. There was no difference between the males and females in this respect. At the lowest dose, hyperaemia and cloudy swellings were present in the cortex and hyperaemia in the medulla. At the next dose, pyknosis was present in the tubular cells of the medulla. These changes were more advanced in the higher doses, with marked medullary necrosis and hyperaemia and cloudy swelling, cast formation and hyperaemia in the cortex in the highest dose range (Figs. 1(c) and 1(d)).

β-Phenyl-Ethyl Alcohol

The LD_{50} values were similar in males and females (1·43 and 0·65 G/kg.). The dose range used was 0·16 - 2·5 G/kg. in females and 0·10 - 5·0 G/kg. in males.

Most animals receiving a lethal dose died in 4 - 24 hrs, with only 1 dying at 4 days. The postmortem findings were similar to those observed in the n-propyl and n-butyl alcohol groups.

The liver sections showed hyperaemia and occasionally slight fatty infiltration. The changes in the kidney sections were more pronounced in males than females and generally less marked than after n-propyl alcohol dosing. In the females the most pronounced change was cloudy swelling and cast formation in the cortex and marked tubular necrosis in the medulla. In the males cortical changes were more pronounced, with tubular necrosis present at the higher doses. At the lowest doses, in each case the cortex showed cloudy swelling and the medulla a slight tubular necrosis.

DISCUSSION

The LD_{50} values obtained of the primary alcohols found in Bantu beer did not conform to Richardson's law. This law states that the toxicity of the alcohols in the fatty acid series increases with an increase in molecular weight. Although there is no significant difference between the values for n-propyl and n-butyl alcohol, the values for iso-butyl and iso-amyl alcohol are greater than those for n-propyl, which is in direct conflict with Richardson's law. The reason for this is not obvious but may be because previous workers have used the intravenous route (e.g. Baer)[6] or the intraperitoneal route (e.g. Macht)[4] or have used narcosis as the end-point (e.g. Rost and Braun).[7]

Although there was a considerable degree of variation in the histological lesions produced by an alcohol, those produced by n-propyl alcohol were clearly the most severe. Iso-amyl and n-hexyl alcohol produced the least prominent lesions, and thus it appears that the higher the molecular weight, the less the severity of the lesions produced. The reason for this may be that the alcohols with a high molecular weight have a slightly different mechanism of action, i.e. that narcosis was more prominent in the higher alcohols. This is not borne out by the results from Lendle's work.[5] He found that the 'breadth of narcosis' (i.e. the ratio between a narcotic and a lethal dose) was similar in ethyl, butyl and amyl alcohols, propyl alcohol being the exception with a higher ratio. The average time for recovery from narcosis given by him decreases with increasing molecular weight, which may indicate that an alcohol with a high molecular weight is excreted more rapidly than one with a low molecular weight, thus having less time to produce tissue damage. This is merely another way of saying that the higher alcohols produce their effects in this case due to a pronounced narcotic effect, whereas the lower alcohols remain in the body longer and exert other effects besides the narcotic effect.

SUMMARY

The acute oral toxicities of the fusel oils found in Bantu beer (iso-amyl, iso-butyl, n-butyl, n-hexyl, β-phenyl-ethyl and n-propyl alcohols) have been determined in rats. The values obtained did not conform to 'Richardson's law' which states that the toxicity of the alcohols in the fatty acid series increases with increasing molecular weight. The lesions produced by these alcohols in the livers and kidneys of rats decreased with increasing molecular weight.

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