KAFFIRCORN MALTING AND BREWING STUDIES*

XXI: THE EFFECT OF THE FUSEL OILS OF BANTU BEER ON RAT LIVER

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The growing consumption of Bantu beer brewed by the South African municipalities has increased by 700% since 1953/54, to reach a level of 140·58 million gallons in 1966/67.[1] This has prompted intensive research into the chemistry, biochemistry, microbiology and technology of Bantu beer-brewing.

Previous studies[2-18] indicate that the changes in the activity of many serum and liver enzymes and histopathological changes in the liver and other organs of mouse, rat and man result from the prolonged consumption of ethyl alcohol.

Bantu beer differs significantly from normal beer in having a high content of solids (on the average 4 - 6%) and a relatively low ethyl alcohol level (3%).[19] The fusel-oil content (227 p.p.m.), however, is much higher than that of European and British beer (averaging about 110 p.p.m.).[20]

In view of the high consumption of Bantu beer in South Africa, the potential toxicity of the fusel oils present in this beverage was investigated by means of biochemical and histological tests carried out on experimental animals.

METHODS

Wistar albino rats with an initial body-weight of 47 G ± 7 G (CSIR strain), were divided into groups as follows:

Group 1: 20 male and 20 female rats were given the following mixture of analytic-grade chemical compounds dissolved in tap water as their only drinking source:

Ethyl alcohol 6% ; ethyl acetate 0·004% ; iso-amyl alcohol 0·12% ; β-phenyl ethyl alcohol 0·12% ; iso-butyl alcohol 0·2% ; and acetic acid 0·2%. This solution contained all the components at twice the concentration of that normally found in Bantu beer.[21] This solution was freshly made up once a week.

Group 2: 20 male and 20 female rats were given a 2% solution of iso-amyl alcohol as their only drinking source. This solution was prepared every week.

Group 3: 20 male and 20 female rats, used as controls, were maintained on tap water as their only drinking source.

The temperature in the experimental rooms in which the rats were kept was 75°F ± 2°F, the humidity 50% ± 5% and the light was controlled to 12 hours/day.

All rats were fed a stock ration *ad libitum* and their weights were recorded weekly for 56 weeks.

The activity of alcohol dehydrogenase (ADH), glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and the protein content were determined at 2 - 4-week intervals in the livers of rats from each of the experimental groups. Because no significant differences were found during the first 28 - 29 weeks of the experiment, the rest of the animals were sacrificed after 53 - 56 weeks. In the course of the experiment 6 rats contracted pneumonia and were discarded. Pneumonia was common in the rats killed at 53 - 56 weeks.

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Enzyme Assay

Rats were weighed to the nearest gram and then killed by decapitation. Their livers were removed immediately and placed in de-ionized water at 0°C, and subsequently weighed to the nearest 0·01 G. The livers were homogenized with a glass homogenizer in de-ionized water at 0°C, diluted to 50 mg. of liver/ml., and the homogenate was used for all enzymatic and protein assays.[4]

ADH assay reaction mixture consisted of 0·1 ml. aqueous supernatant from liver homogenate, centrifuged at 3,000 r.p.m. for 30 min. at 0°C, 3·0 ml. glycine-semicarbazide buffer,[22] 0·1 ml. DPN (10 mg./ml.), and 0·05 ml. 20% ethanol in water.[23] The reaction mixture was prewarmed for 10 min. in the spectrophotometer. The increase in absorption at 340 mμ, i.e. the rate of reduction of DPN as a function of ADH in liver homogenates, was measured with a Beckman DK-2A ratio recording spectrophotometer at 37°C for 3 - 10 min.[24,25]

The measurements of GOT and GPT activity were carried out according to the method of Wróblewski and Cabaud[26] on the uncentrifuged liver homogenate containing 5 mg. of tissue/ml.

The protein content of diluted uncentrifuged liver homogenates (1 mg./ml.) was measured with the Folin-Ciocalteau phenol reagent.[27]

Histopathology

Specimens of liver, kidney, heart, spleen and lung were placed in 5% buffered formalin and embedded in wax. Sections (5 μ) were cut and stained with haematoxylin and erythrosin for histological examination.

RESULTS

Body and Liver Weight

Although there was a slight difference in the mean body-weight between groups at 28 - 29 weeks and at 53 - 56 weeks (Table I), the difference was not statistically significant when tested with the Kruskal-Wallis test.[28]

TABLE I. MEAN BODY-WEIGHT (IN GRAMS) OF RATS IN THE 3 GROUPS

Weeks	Males			Females		
	I	*II*	*III*	*I*	*II*	*III*
28 - 29	346·8	331·3	354·8	210·4	212·1	225·7
53 - 56	395·2	372·6	422·4	242·7	249·3	273·8

There was no difference between the liver weight of the 3 groups (Table II) or the liver/body-weight ratios.

TABLE II. MEAN LIVER WEIGHT (IN GRAMS) OF RATS IN THE 3 GROUPS

Weeks	Males			Females		
	I	*II*	*III*	*I*	*II*	*III*
28 - 29	11·444	11·331	11·793	7·226	7·872	7·772
53 - 56	12·849	12·942	13·289	7·411	7·736	7·968

ADH

The results of the ADH determinations (Table III) show

that the activity of this enzyme was the same in control as in experimental rats, whether expressed as μmoles/min./G, μmoles/min./whole liver, or μmoles/min./G of liver protein.

TABLE III. MEAN VALUES OF ADH ACTIVITY EXPRESSED AS μM OF ETHANOL UTILIZED/MIN./G OF LIVER HOMOGENATE AT 37°C

Weeks	Males			Females		
	I	*II*	*III*	*I*	*II*	*III*
28 - 29	3·445	3·680	3·785	3·823	3·780	3·520
53 - 56	3·844	3·717	3·770	3·723	3·810	3·466

Transaminases

Similar results were obtained with both the transaminases, although there was a slight increase in GOT activity in all 3 groups between 28 and 56 weeks. The detailed results are shown in Tables IV and V.

TABLE IV. MEAN VALUES OF GOT ACTIVITY IN WRÓBLEWSKI-CABAUD UNITS[26]

Weeks	Males			Females		
	I	*II*	*III*	*I*	*II*	*III*
28 - 29	40·0	47·1	45·3	42·2	41·3	38·7
53 - 56	64·4	66·2	68·0	64·3	61·0	58·0

TABLE V. MEAN VALUES OF GPT ACTIVITY IN WRÓBLEWSKI-CABAUD UNITS[26]

Weeks	Males			Females		
	I	*II*	*III*	*I*	*II*	*III*
28 - 29	37·0	43·0	38·0	37·0	39·0	38·0
53 - 56	37·0	39·7	37·5	32·7	34·3	34·6

No significant difference could be found in the liver protein content (Table VI).

TABLE VI. PROTEIN IN LIVER (%)*

Weeks	Males			Females		
	I	*II*	*III*	*I*	*II*	*III*
28 - 29	21·5	20·4	20·2	17·8	18·9	17·8
53 - 56	20·0	18·9	18·5	18·5	19·0	18·05

*The percentage of liver protein is based on the analyses of the uncentrifuged homogenates.

Histopathology

No significant abnormalities were observed in any of the organs examined from normal rats. Those animals which were sacrificed showed purulent pneumonia and slight degenerative changes in the liver and kidney. The rats suffering from pneumonia were equally distributed in all groups.

DISCUSSION

The combination of ethanol and fusel oils found in Bantu beer was essentially non-toxic to experimental rats, even though the concentration in their drinking water was twice that found in beer. Iso-amyl alcohol, a fusel oil occurring in large quantities, was also non-toxic to rats when added to drinking water as a 2% solution.

There appears to be very little published work on the chronic toxic effects of fusel oils. Richardson[29] considered that the acute toxicity of fusel oils increased with increasing chain length, and this has subsequently become known as Richardson's law. Most of the publications on alcohols deal with ethanol and these form the only basis for comparison with the results of this experiment.

A solution of 15% ethanol in drinking water produced no effect on body-weight,[30] although a smaller quantity given by stomach tube as a 25% solution retarded the growth of rats.[31,32] The low concentration of alcohols fed continuously in our experiment was thus not likely to affect the body-weight.

High concentrations of ethanol (20%) produce an increase in ADH activity[4-7,23] in experimental animals. Patients with a chronic alcoholic history have considerably lower ADH activities in their cirrhotic liver tissues than control livers.[33] The lower concentration of the alcohols used in our experiment did not increase the ADH activity although no cirrhosis was present. Similarly, the GOT and GPT activities, which are considered as a reliable index of liver damage, were not altered by the administration of the mixture of alcohols, this fact confirming the observations on the effect of ethanol.[34]

Schlesinger *et al.*[4] found an increase in liver protein content in rats maintained for 90 - 104 days on 10% ethanol, an observation which was not confirmed in this experiment.

Histological examination of the liver also indicated that the mixture of alcohols was not hepatotoxic, and again confirmed the observations made with ethanol. The absence of any significant toxic effect of the mixture of alcohols on rats is therefore in agreement with the observations on the effect of ethanol. The mixture of alcohols used in this experiment provided less than 30% of the total calories, which indicates that no significant dietary imbalances were produced by the addition of alcohols to the diet. Without such dietary imbalances, even high concentrations of ethanol must be regarded as non-toxic.[31] The same considerations appear to apply to the mixture of fusel oils in Bantu beer.

One other possible explanation of the negative results of this experiment is that the CSIR strain of Wistar rats (which is not inbred) is resistant to the effects of alcohol. McClean and Rodgers[35] as well as Bennett and Hebert[23] observed marked individual and genetic differences in susceptibility to ethanol among different strains of mice. Acute toxicity studies on the CSIR strain, however, have not shown any marked differences in the LD_{50} values for various alcohols.[36]

Although the mixture of fusel oils did not produce any signs of toxicity, future studies on the effect of dietary factors, such as low protein consumption + vitamin-B deficiency, may reveal that fusel oils are toxic under certain circumstances.

SUMMARY

One hundred and twenty Wistar rats were divided into 3 groups. The first group of rats was given a solution of fusel oils (at a concentration twice that normally found in Bantu beer) as their only drinking source, the second group a 2% solution of iso-amyl alcohol, and the third (control) group was maintained on tap water.

No significant differences were found between the experimental and control groups after 56 weeks in respect of body-weight, liver weight, ADH, GOT and GPT activity and protein content of the liver. Histological examinations of the livers, kidneys, hearts, spleens and lungs did not show any significant abnormalities.

Thus, neither the combination of ethanol and fusel oils found in Bantu beer nor 2% solution of iso-amyl alcohol was toxic to experimental animals.

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