Bull. Environ. Contam. Toxicol. (1992) 49:866–870

Environmental Contamination and Toxicology

Induction of Micronuclei in Mice Bone Marrow Cells by Home Made Aguardientes Collected in Southern Chile and Their Incidence in Gastric Cancer

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There is world wide concern about the mutagenic, teratogenic, and carcinogenic effects of alcohol (Obe and Ristow, 1979; Obe and Anderson, 1987). Chile is considered to be among the top three countries having the highest alcohol consumption per inhabitant, with mean ingestion of over 12 liters of absolute alcohol per inhabitant per year (Health Ministry, Alcohol and Drug).

Alcoholism among young people (age 15 and under) and women, specially in the 8th Region of Chile (Medina, 1986), adds to the already existing problem.

Chile has one of the highest frequencies of gastric cancer in the world (Doll et al., 1970) and a high incidence of esophageal cancer (Medina and Csendes, 1983). Home made and commercial "aguardientes" were assayed for micronuclei in order to demonstrate the possible association between the mentioned pathologies and the excessive consumption of these alcoholic beverages.

The micronucleus test developed by Schmid and coworkers (1975) screens for a chemical's ability to induce chromosomal breaks. This is measured by the frequency of erythrocytes with micronuclei derived from acentric chromosomal fragments or lagging chromosomes (Heddle and Carrano, 1977; Mavournin K, 1990). In the present study we report the *in vivo* induction of micronuclei in bone marrow polychromatic erythrocytes in mice by four "aguardientes" obtained by different processes and containing different additives. Home made "aguardientes" were tested and compared to ethanol and commercial "aguardiente". No quality control was used in the production of the home spirits to prevent the formation of toxic and/or mutagenic agents. For purposes of this study, we assumed that these agents are responsible for the mutagenic effects rather than alcohol *per se*.

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MATERIAL AND METHODS

The four home made "aguardientes" were collected in different towns of the 8th Region, Chile: Rere (R), Yumbel (Y), Coelemu (C) and Florida (F). The commercial sample (D) was purchased in Concepción. Copper and aluminium alembics were used and the additives were: *Ugni molinae* Turcz (Myrtaceae). *Juncus procerus* E. May (Juncaceae). *Eriobotrya japonica* (Thum) Luide (Rosaceae). There was no temperature nor quality control.

Five Balb/c mice 8 weeks old (Molecular Biology Department's Biotherium, University of Concepción) weighing about 20 g were assigned to each treatment group. All the samples were prepared at 25 v/v dilution in water and the animals were injected a single dose intraperitoneally at 0.02 ml per g of body weight. This dose was selected because it showed a teratogenic effect in mice (Walsh et al. 1983; Padmanabhan et al. 1986). The corresponding negative control was used.

The micronuclei test was performed as proposed by Schmid (1977), and modified according to Das and Kar (1980). Mice were killed 30 h. after treatment by cervical dislocation. Five bone marrow preparations per animal were made. Slides were stained with May-Grünwald and Giemsa according to the schedule outlined by Cole et al. (1979), which maximizes the staining differences between polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE), and prepared for microscopic analysis. An average of 5000 PCE and the corresponding NCE per treatment group were scored blindly. The frequency of cells with micronuclei (MN) per 1000 cells was calculated for each animal. The Mann Whitney U test was employed for statistical analysis. The significance was tested at $p < 0.05$ level.

RESULTS AND DISCUSSION

Table 1 shows the average frequency of micronucleated polychromatic erythrocytes (MNPCE) and the ratio between PCE/NCE of the home made "aguardientes" compared to its negative control, commercial "aguardientes" and ethanol. The results indicated that home made spirits had different clastogenic activity in the bone marrow of Balb/c mice.

Statistical analysis showed that two of these "aguardientes" samples R and C, significantly increased the frequency of micronucleated cells.

The R sample, which induced the highest frequency of MN, caused a significant reduction ($p < 0.01$) in the PCE/NCE ratio. This result could be due to a decrease in the turnover of these cells. The results provided evidence for a toxic effect by the R sample. This sample showed a high concentration of higher alcohols, which have been previously demonstrated to have cytotoxic effects (Koerke et al. 1976).

Both ethanol and commercial "aguardiente" failed to induced MN in Balb/c mice when administered at the same ethanolic concentration.

The results can be attributed to the alcohol congeners present in this kind of home made spirits. Several authors have demonstrated that many of the evaporated residues of some alcoholic beverages presented mutagenic activity (Loquet et al. 1981; Nagao et al. 1981; Hoeft and Obe, 1983; Bull et al. 1987). Nykänen and Suomalainen (1983) list some 1300 compounds, such as different types of alcohols, aldehydes like acetaldehyde and acrolein, amines like N-nitroso-dimethyl-nitrosoamine (NDMA) and N-trisododiethylamine (NDEA), phenolic compounds like tannins and hydrocarbons like toluene, benzene and benzopyrene, some of which are well known to be mutagenic. According to this, we think that our beverages, because of the manufacturing procedures could contain a high number of these compounds.

Table 1. The frequency of micronucleated polychromatic erythrocytes (PCE) in bone marrow cells in Balb/c mice, induced by "aguardientes" and ethanol.

Treatment		Nº of mice used	Polychromatic erythrocytes Number analyzed	micronucleated o/oo ± SEM	Ratio of PCE/NCE mean ± SEM
Control		5	6099	1.17 ± 1.08	0.74 ± 0.12
Ethanol		5	4673	1.48 ± 1.34	0.45 ± 0.03
Samples	D	5	5631	1.86 ± 1.22	0.63 ± 0.04
	R	5	5022	4.93 ± 2.19**	0.26 ± 0.03**
	C	5	5198	3.80 ± 1.74*	0.56 ± 0.04
	Y	5	5699	2.42 ± 0.35	0.60 ± 0.03
	F	5	5889	3.10 ± 1.64	0.46 ± 0.09

Values marked with asterisks are significantly different from negative control, ethanol and commercial "aguardiente" (sample D) by Mann Whitney U test. (*$P < 0.05$; **$P < 0.01$).

Ethanol under the mentioned conditions failed to induce MN in Balb/c mice, these results agree with those published by Tates et al. (1980) who used a 20% v/v dosage for 6 weeks and Cahubey et al. (1977) who used a 35% v/v dosage for 27 days. However Baraona et al. (1981) found an increase in the induction of MN in CD rats fed a 36% v/v of ethanol for 6 weeks.

Based on these data we cannot rule out ethanol as not being mutagenic, since an increase in dose and/or exposure time could improve our ability to detect mutagenicity caused by ethanol or by its metabolites.

Epidemiological studies should be conducted to establish the relationship between the excessive consumption of these home made "aguardientes" and the onset of gastric cancer in our Region.

Acknowledgements. Supported by Dirección de Investigación, Universidad de Concepción, Proyecto 20.31.04. We thank Mrs. Eugenia Spano for her excellent laboratory work.

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Received September 10, 1991; accepted May 28, 1992.