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# INTERACTION OF THE CONSTITUENTS OF ALCOHOLIC BEVERAGES IN THE PROMOTION OF LIVER DAMAGE

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**SUMMARY**

Little has been studied of the adverse effects of the exposure of the liver to the interaction of ethanol with its congeners and acetaldehyde, coexisting in the contents of alcoholic beverages. Twenty four male Wistar rats were divided into four groups. Two groups (SH/DA; SH/FA) were submitted to daily treatment with synthetic hydroalcoholic solutions containing ethanol, methanol, higher alcohols and acetaldehyde in the same proportions as those found in most common distilled and fermented alcoholic beverages; the third group (SH/EA) was treated with a hydroalcoholic solution of ethanol; the fourth group served as control and received an equivalent volume of an isocaloric solution of dextrose. All the animals were killed at the end of the 9th week of the experiment. The ratio between the liver weight and body weight was found to be lower in the treated animals than in the control group. The histology of the liver was altered in the three groups which were submitted to treatment with the hydroalcoholic solutions, with quantitative and qualitative differences between the groups. These results suggest that the hepatoxicity of ethanol in alcoholic beverages is enhanced by interaction with its congeners and acetaldehyde; they also suggest that alcoholic beverages are not equivalent in their potential to cause liver damage.

## INTRODUCTION

In critical areas of uncertainty in the pathogenesis of alcoholic hepatic disease it is necessary to circumscribe some problems to experiments on animals in order to promote research which may clarify various questions, specifically in the field of toxicology [1,2]. The alcoholic beverages which are more frequently consumed in Portugal contain different viticultural alcohols besides ethanol. These alcohols presumably promote hepatic vulnerability to them and to other composites, therefore requiring an assessment of their consequent interactions since they have not been studied in depth [3-7].

Persistent exposures, specifically to ethanol and its congeners – although the absolute relative concentrations of the latter are much lower to that of ethanol – may lead to toxico-metabolic effects which may influence the development of structural disease in the target organs [1,8-11].

With the knowledge of the origin of the alcohols similar to ethanol, and their concentrations in the alcoholic beverages habitually consumed, it is relevant to study, in the experimental model, their probable pathogenic contribution to the development of the alcoholic hepatic lesion.

## METHODS

Adult male Wistar rats from a stabilised colony in a vivarium at the Gulbenkian Science Institute (Oeiras), weighing 196 to 230 grams, were used. After a week of adaptation (light, temperature, humidity, food and water) at the vivarium of the Nutrition Laboratory of the National Health Institute (NHI), they were randomly divided into four groups of six animals each and isolated in standard single compartments in order to receive daily intraperitoneal (I.P.) treatment with different hydro-alcoholic solutions which were specifically prepared and controlled every week. The animals had access to solid food of the same composition for all four groups (SAPEC-Rodents/Setúbal; nutritional energy value - digestible - of 29Kcal/100g of food with the following composition: total proteins 22,0g/100g dry weight; total fat 4,6g/100g dry weight; starch 30,0g/100g dry weight; sugar 7,5g/100g dry weight; oligoelements and vitamins) and identical access to water kept in measuring bottles. One of these groups served as the control, the other three being the test groups. Each animal in the control group received a measure of dextrose isocaloric solution

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identical to the volume of ethanol injected into each animal of the remaining 3 groups.

The period of treatment lasted nine weeks. Solid food and water allocation, as well as body weight, were controlled every week.

The synthetic hydro-alcoholic solutions prepared reproduce the composition (proportional concentration of ethanol and congeners and acetaldehyde) of distilled alcoholic beverages (D) and fermented (F) habitually consumed by our population.

The solutions were made from raw material of a high level of purity, their control being made by gaseous chromatography according to the methodology practised at the Quality Control Laboratory of the Grapevine and Wine Institute.

In *Table 1* a discrimination of the exact concentrations is made (the means were taken from an extensive lot) of the various volatile constituents of fermented and distilled commercialized alcoholic beverages.

The volume of the prepared hydro-alcoholic solution, injected daily by intraperitoneal route (I.P.) in each animal of the 3 groups of treatment (Groups D,F, and E), contains 250mg of ethanol/100g body weight and the remaining products in the proportions described in *Table 2.* The I.P. route was chosen because it is effective in the experimental model, according to dynamic studies of ethanol concentration in the blood and liver [12], if the level of prolonged alcoholemia can be related to the structural alterations of the liver.

*Table 1* – Referential alcoholic composition for synthetic hydro-alcoholic preparations*.

| | Fermented | Distilled |
|---|---|---|
| Vinic ethanol | 10% volume | 50% volume |
| Acetaldehyde | 40,24g/h.aa | 321,92g/h.aa |
| Methanol | 193,08g/h.aa | 1.544,64g/h.aa |
| Higher alc. | 225,74g/h.aa | 290,24g/h.aa |

* Legislated values: Council Regulation (EEC) 1576/89, 29 May 1989, Decree 697/86, 21 November, Legislative Publication Issue 1 N°269, 86-11-21.

*Table 2* – Qualitative composition of the synthetic hydro-alcoholic preparations and amounts co-administered in each animal (I.P./day) of the respective group (D;F;E).

| | H.S.A.D. | H.S.A.F. | H.S.A.E. |
|---|---|---|---|
| Vinic ethanol | 250 | 250 | 250 |
| Acetaldehyde | 1,7 | 1,0 | - |
| Methanol | 7,7 | 4,3 | - |
| *Higher alc. | 1,4 | 5,4 | |

mg/100g body weight; D = distilled; F = Fermented; E = ethanol
*butanol 2; n-propanol; isobutanol; allylic alcohol; n-butanol and isopentanols

The animals were sacrificed by decapitation, after 9 weeks of the experiment, and the organs were immediately retrieved (liver, heart, pancreas and skeletal muscle), weighed(Sartorius U.3600 electronic scales). In this paper we will only analyse the changes in weight of the liver and its structural alterations at light microscopy.

Four fragments of the liver were taken from each animal; they were placed in a 10% formalin solution for 24 hours, routinely processed and embedded in paraffin; 5mm sections were stained with hematoxin-eosin, PAS and Perls methods, and Gordon-Sweet for reticulin. Frozen sections of some fragments were stained with oil red to enhance the lipids. In the histologic study the following parameters were assessed: vacuolisation of the nuclei of the hepatocytes, mitoses and increase in the number of binucleated cells; in what concerns the cytoplasm of hepatocytes, vacuolisation, steatosis, eosinophilic degeneration and focal or confluent necrosis were recorded; inflammatory infiltration and hyperplasia and hypertrophy of the Kupffer cells were also assessed. These parameters were recorded in terms of present or absent; steatosis, eosinophilic degeneration, necrosis and inflammatory infiltration were semi-quantitatively assessed.

## RESULTS

Knowing the variations of body and organ weight in the animal model as well as in man, attributed to toxico-metabolic effects induced by ethanol, it is important to assess the behaviour of both in the different groups - *Table 3.*

*Table 3* – Allocation of food, liver weight and liver weight/ /body weight relationship.

| Groups | animals n | liver weight (g) | allocation of food (g) | liver weight/100g body weight |
|---|---|---|---|---|
| Control | 6 | 17,6+2,4 | 193,9+32,9 | 4,14 |
| D.H.A.S.** | 6 | 17,0+2,4 | 190,9+34,4 | 4,03 |
| F.H.A.S.** | 6 | 15,5+1,8 | 196,7+32,2 | 3,55 |
| E.H.A.S.** | 6 | 14,8+2,9 | 210,8+41,9 | 3,88 |

weekly average; H.A.S.- hydro-alcoholic solution (D - distilled; F - fermented; E - ethanol)

The different quantitative and qualitative compositions of the hydro-alcoholic solutions introduced do not seem to influence the *ad libitum* allocation of solid food in the groups under treatment and as their value is similar to that of the control group, it is accepted that all the groups have a similar caloric acquisition with an average final body weight gain of about 150 grams. The study of the liver weight/body weight relation shows different tendencies in inter-group comparison, the liver contributing with greater percentage in final body weight in the animals of the control group than in the groups under alcoholic treatment; this fact is more evident in group F.

In a comparative study, the macroscopic aspect of the livers of the animals of the groups treated with alcohol (D,F,E) could not be differentiated by any consistent particularity.

The alterations observed in the microscopic study of the liver are summarised in Table 4. All the animals of the three groups submitted to alcoholic treatment had focal necrosis and regeneration of the hepatocytes, steatosis and inflammation (Fig. 1, 2, 3, 4), while the animals in the control group had no lesions of the hepatic tissue. The alterations were always of a slight or moderate degree. In group D necrosis and inflammation of the hepatic tissue reached more severe grades than in groups F and E, in two animals there were some areas of confluent hepatic necrosis in zone 3; steatosis was more intense in groups F and E than in group D; the vacuoles of lipidic origin were of small size and were sometimes located in the sinusoidal poles of the hepatocytes.

## DISCUSSION

Rodents have been the most commonly used laboratory animals in the field of experimental toxicology of alcoholic disease[13]. In this field of human pathology, the wide scale recourse to experimental study shows the desire of its better understanding in the multiple domains of organometabolic physiopathology, as well as to give support to potential actions of a preventive nature. However, in spite of the possibility of reproducing some of the structural lesions observed in man, the linear extension of the results obtained in the animal model must be prudently pondered, due to the specific differences in the dynamic behaviour of the metabolism of alcohol according to the type of animal [14,16].

*Table 4* – Histologic alterations of the liver of animals subjected to prolonged treatment with ethanol and congeners and acetaldehyde.

| Hepatic Lesion | Control | D.H.A.S. | E.H.A.S. | F.H.A.S. |
|---|---|---|---|---|
| Nuclear Vacuolisation | 0 | + | + | + |
| Binucleated cells | + | + | + | + |
| Mitoses | 0 | + | + | + |
| Cytoplasm Vacuolisation | 0 | + | + | + |
| Eosinophilic Degeneration | 0 | ++ | ++ | ++ |
| Steatosis | 0 | + | ++ | ++ |
| Focal necrosis | 0 | ++ | + | + |
| Confluent necrosis* | 0 | + | 0 | 0 |
| Inflammation | 0 | ++ | + | + |
| Activation of Kupffer cells | 0 | ++ | + | ++ |
| Haemosid. deposits | 0 | 0 | + | 0 |

0 scale at ++++ : + (>25%); ++ (>25%); +++ (>50%); * 2/6 cases

We have previously demonstrated, in experimental work, the acute hepatotoxicity of the ethanol, congeners and acetaldehyde, which are usual constituents of alcoholic beverages consumed by the Portuguese population [17].

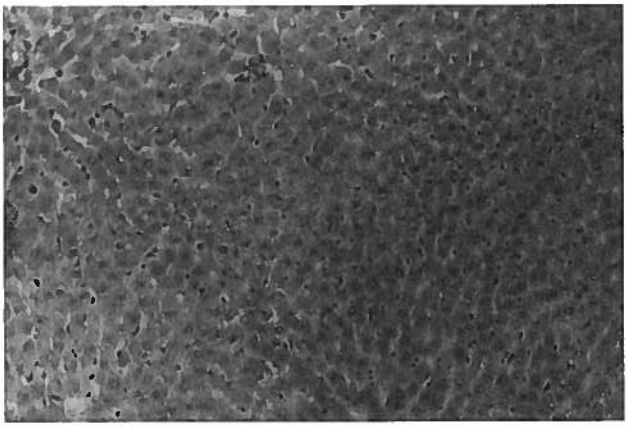

*Fig. 1* – Focal necrosis of hepatocytes (+); activation of Kupffer cells (++) SH/AF. H.E.x 160.

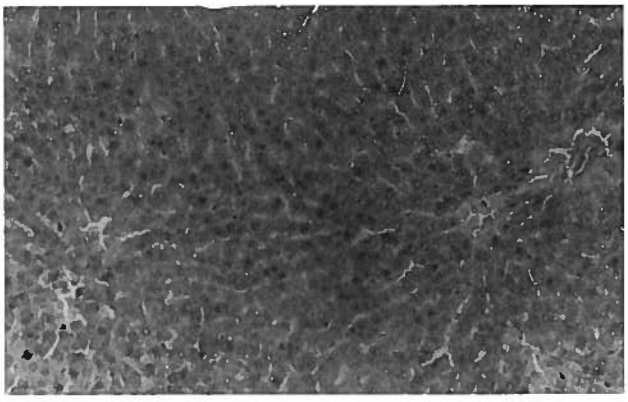

*Fig. 2* – Microvacuolar steatosis (+); nuclear vacuolisation (+); binucleated hepatocytes (+) H. E. x 160.

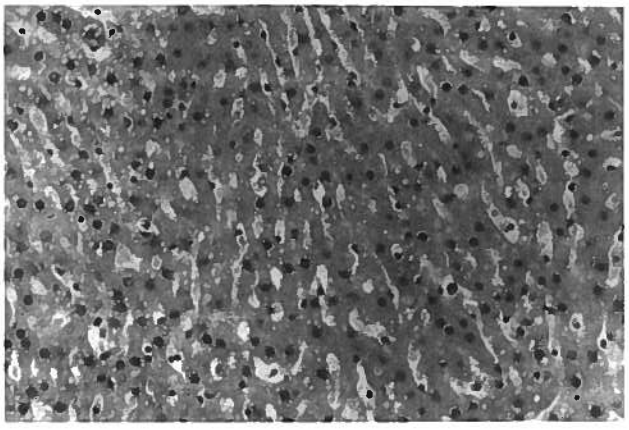

*Fig. 3* – Micro and Macrovacuolar steatosis (++); SH/AE H.E. x 160

In this study we assess the influence, under prolonged treatment, of the co-administration of ethanol, methanol and higher alcohols and acetaldehyde on the liver of male Wistar rats.

Hypertrophy of the organ and steatosis are the most common initial structural alterations, induced by the ethanol. The results show that for a similar caloric level, identical quantity of ethanol and the same duration of the

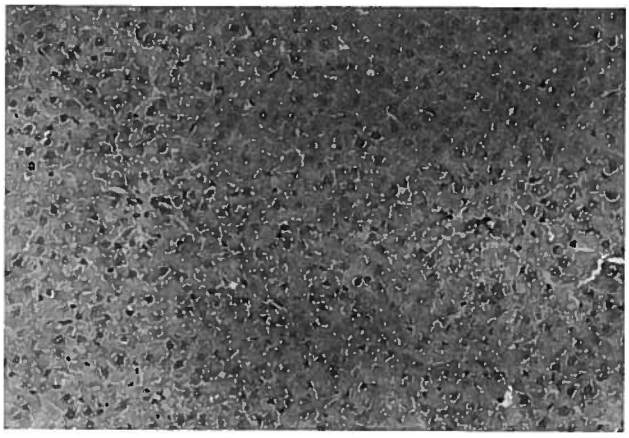

*Fig. 4* – Eosinophilic Degeneration of the hepatocytes with Councillman bodies (++); Microvacuolar steatosis (+)SH/AD H.E.x160

experiment, the animals treated with alcohol develop evident hepatic steatosis, in spite of a relatively low content of ingested fat[18,19], although the liver is proportionally smaller (liver weight/body weight relationship) than the liver of the control group and there is no fatty deposit in this group.

In a simultaneous parallel experiment (not published), we subjected a lot of 6 animals from the same colony to treatment with a commercialized fermented alcoholic beverage administered orally ad libitum; the average daily consumption approximately 2 grams of ethanol; after 6 weeks the percentage of the weight relationship (liver weight/body weight) was $4.6 \pm 0.4$ and $4.0 \pm 0.3$ for the respective control group. The difference between the results of the two experimental methods is curious, suggesting that in this context there is also a determining influence of the digestive tract on what occurs in the liver.

The interaction of the nutritional status-ethanol consumption in the development of the primary alcoholic hepatic lesion has once more been admitted [20]. However, in the experimental conditions we used, the results allow us to hold ethanol, which is the alcohol common to the three groups, as independently responsible for the development of hepatic steatosis; our studies are not in favour of the influence of the other volatile constituents introduced; we also found that in group D the fatty deposit is less pronounced than in the other two groups; it is curious to note that necrosis and inflammation are more intense in this group.

In the last decades, various authors have admitted the existence of other factors apart from the ethanol in the development of alcoholic disease of the liver, a preferential target organ in direct or indirect prolonged subjection to many toxins due to its topographic position in the splanenic vascular axis and due to its metabolic activity; which may also justify why some epidemiological paradoxes escape the linear relationship determining alcohol consumption/organ disease.

The experimental co-administration of ethanol and other constituents of alcoholic beverages supports the reason for the different qualitative and quantitative structural alterations of the liver in the different groups, which are more severe in group D.

It is known that the MEOS form of oxidative metabolisation suffers specific enzymatic induction (P 450 2E1 cytochrome) and is common to ethanol and other congeners, MEOS may potentially lead to the metabolic activation of various components which become hepatotoxic[8,9] being more prejudicial than adaptive.

Although this study does not reproduce the essential characteristics of chronic alcoholism, it allows the assessment of the effect of potential chemical cofactors, besides the ethanol, which may contribute to the promotion of alcoholic hepatic lesion. In fact, our results suggest a reinforcement of ethanol toxicity by the volatile components which exist in certain proportions in the alcoholic beverages frequently consumed. On this basis, a non conservative interpretation admitting the inequality between different types of alcoholic beverages is permissible, since the influence of methanol, of higher alcohols and acetaldehyde coexisting in them contribute to the development of structural lesions in the liver exposed to alcohol. These results substantiate the continuation of research in the important field of alcohology.

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