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Green tea as an antioxidant which protects against alcohol induced injury in rats — a histopathological examination

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Our study with animal models was designed to test the hypothesis that green tea protects against chronic (over 4 weeks) alcohol induced liver injury in rats. The research was conducted on Wistar male rats divided into 4 research groups: I — received the Libera-De Carli control diet (L-DC), II — received (L-DC) and green tea, III — received (L-DC) and ethanol and IV — received (L-DC), green tea and ethanol. When comparing groups I and II we saw less intensive steatosis in group II than in group I, which can suggest that green tea may affect the accumulation of fat in the hepatocytes and protect them against steatosis and disruption. In III, the ethanol group, the steatosis of the liver increased considerably and the green tea which was given with ethanol in group IV did not halt this, as in group IV we also observed intensive steatosis in the liver. From this data we conclude that green tea has an important, although not fully understood role in preventing liver injury.

Key words: rats, liver, ethanol, green tea, steatosis, histopathology

INTRODUCTION

Ethanol is metabolised in the liver in 90%. Its metabolism is accompanied by free radical products which stimulate lipid peroxidation and are responsible for hepatic injury. It seems that natural antioxidants from green tea may play an important role in liver protection. The aim of the present study was to investigate the effect of green tea on rat hepatocytes exposed to ethanol.

MATERIAL AND METHODS

Male Wistar rats of 2, 12 and 24 months old were used in the experiment. All the procedures were in accordance with the guidelines for the care and use of laboratory animals and the local Animal Care Committee approved the protocol. The rats were housed in individual cages and pair-fed with either the nutritionally adequate liquid Lieber DeCarli control diet (L-DC) containing 47% f total energy as carbohydrate, 18% as protein and 35% as lipids, or the identical diet with ethanol substituted isocalorically for carbohydrate (36% of total energy). A liquid diet (control and ethanol) containing 7 g green tea (*Camellia sinensis*) extract/l diet was also prepared. The rats were divided into the following groups: I — the control group, fed for 5 weeks on a control (L-DC) diet (n = 6), II — the green tea group, fed for 5 weeks on a control (L-DC) diet containing green tea (7 g/l) (n = 6), III — the ethanol group, fed for one week on a control (L-DC) diet and for the next 4 weeks on the ethanol (L-DC)

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diet (n = 6) and IV — the ethanol and green tea group, fed for one week on a control (L-DC) diet containing green tea (7 g/l) and then for 4 weeks with the ethanol (L-DC) diet also containing green tea (7 g/l) (n = 6).

The animals were sacrificed under an ether anaesthetic at 9 am after completing the experiment and samples were taken from two different external parts of the liver for morphological examination with a light microscope. Sections for light microscope examination were fixed in 10% formalin neutralised to pH 7.0, embedded in paraffin and stained with haematoxylin-eosin, Azan and Gomori's silver stain. One frozen section from each group was stained with Sudan III for the presence of plasma lipids in the hepatocytes. The microscopic structure of the liver, percentage of steatosed hepatic lobules and intensity of steatosis were evaluated. For the current study each lobulus was divided into two zones, the central and the peripheral. The degree of fatty degeneration was assessed using the semiquantitative score: + (0–25%), ++ (25–50%),+++ (50–75%), ++++ (75–100%) of steatosed hepatocytes. The data obtained in this study was analysed by means of Hi^2 or the Fisher exact test to determine the degree of significance between different groups. The values for $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Microscopic observations revealed various stages of steatosis in all the groups investigated. Figure 1 — group I — numerous small fatty vacuoles were observed in hepatocytes. Figure 2 — group II — there were fewer hepatocytes containing fatty vacuoles. Figure 3 — group III — we saw a very large number of fatty vacuoles in whole lobule. Figure 4 — group IV — the steatosis was more intense and occurred generally in the whole lobule. The range of steatosis along with its dominant localisation in the liver lobulus is shown in Table 1.

Our results suggest that green tea may affect the accumulation of fat in the hepatocytes. When comparing groups I and II we found a higher percentage of lobules without hepatocyte steatosis in group II, which might demonstrate that there is less fat deposition in the liver thanks to green tea and its cytoprotective effect. Our findings are similar to those reported by other authors, who also reported the inhibiting activity of green tea substances on fat metabolism [2, 5].

The development of steatosis in rats receiving ethanol seems to be clear and well-founded. However, the increase in lipid accumulation in the liver of rats from group IV is somewhat surprising. Ethanol causes liver steatosis by releasing fatty acids from fatty tissue and stimulation of fatty acids synthesis in the liver [1]. Chronic consumption of ethanol causes a decrease in the hepatic antioxidant enzymes, which can lead to an increase in fat oxidation [3]. The significant increase in hepatic steatosis observed in group IV might be caused by an in-

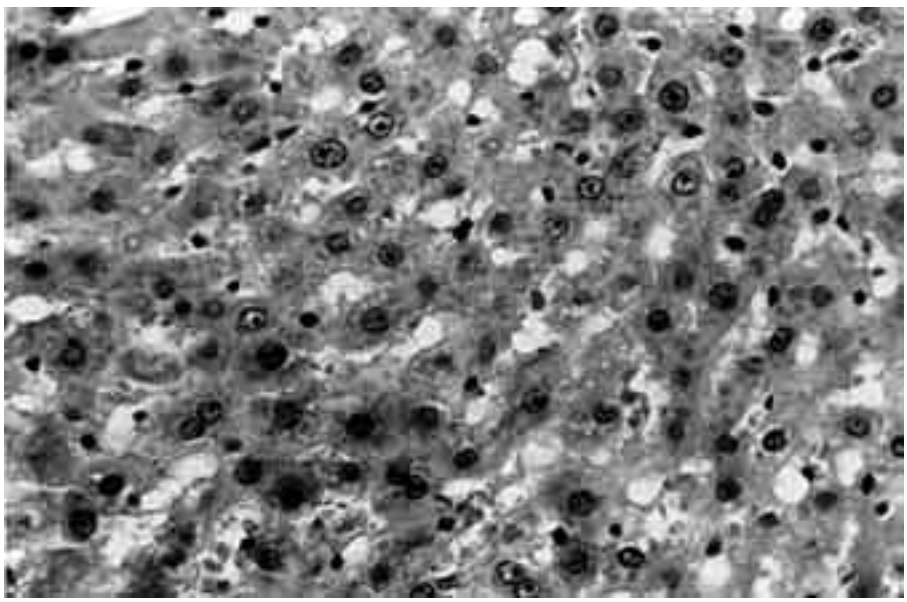

Figure 1. Group I. Hepatocytes with a large number of fatty vacuoles in the cytoplasm. Haematoxylin-Eosin staining of 200 ×.

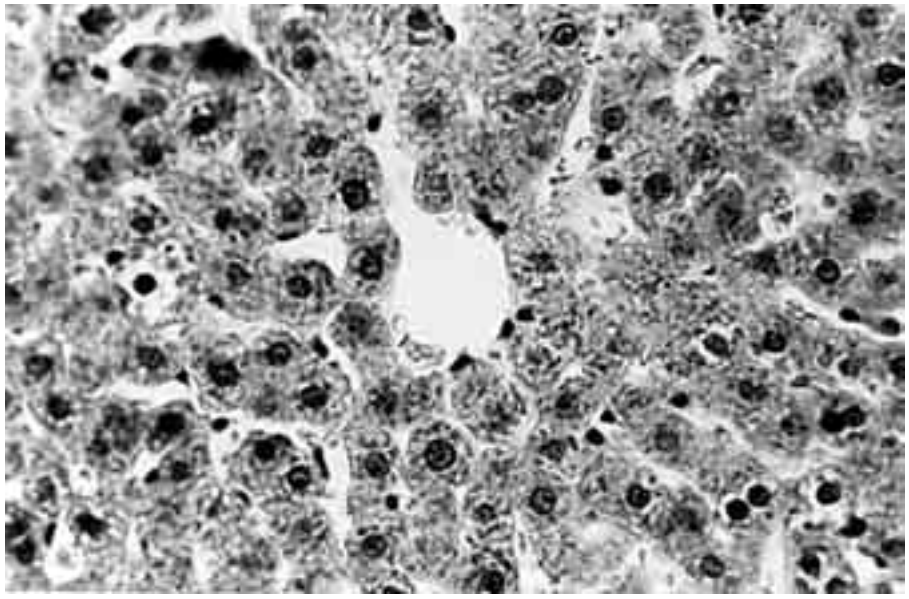

Figure 2. Group II. Hepatocytes with distinct cell-cell borders with nucleus in the centre of the cell and few fatty vacuoles in the cytoplasm. Haematoxylin-Eosin staining of 200 ×.

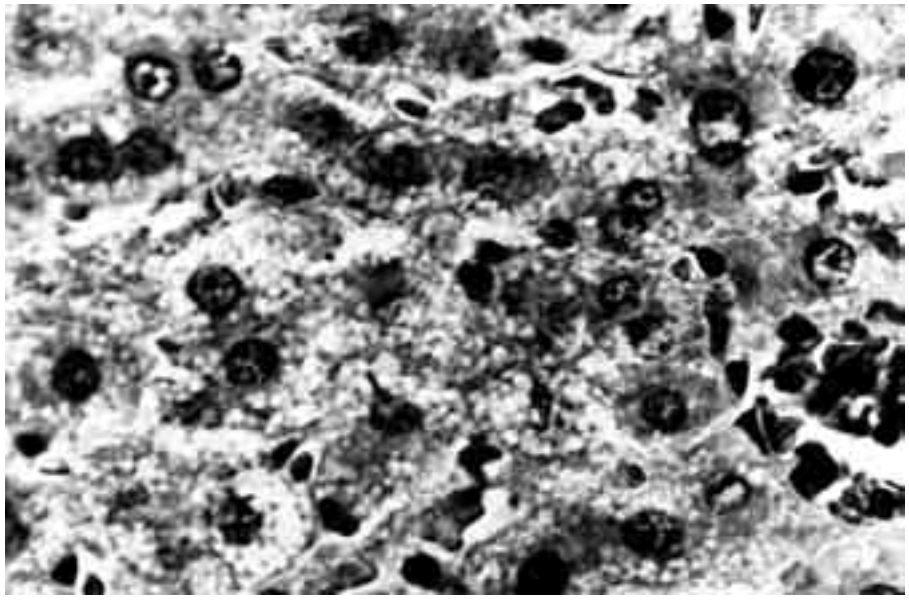

Figure 3. Group III. Hepatocytes with a very large number of fatty drops in the cytoplasm. Haematoxylin--Eosin staining of 400 ×.

sufficient concentration of antioxidants in green tea. This thesis is consistent with the results of other authors, who failed to prove any effect of the consumption of green tea on the increase of fatty compound oxidation [4, 6]. To sum up, it can be claimed that the results of our research confirm the essential role of green tea in liver oxidoreduction reactions and demonstrate its cytoprotective effect. In addition, this effect can be substantially dependent upon the degree of hepatocyte damage. Further study is necessary to evaluate and document the steatosis of liver observed in the group with ethanol and green tea.

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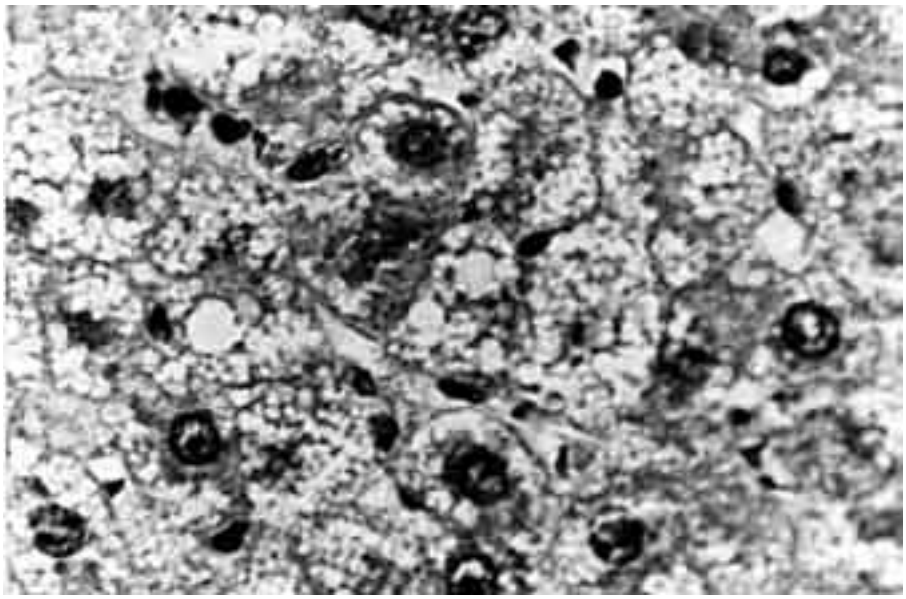

Figure 4. Group IV. Hepatocytes with a very large number of fatty drops in the cytoplasm shifting the nucleus. Haematoxylin-Eosin staining of 400 ×.

Table 1. Steatosed hepatocytes in 100 liver lobules in individual groups (SH) and degree of steatosis in individual parts of liver lobules (DS)

Predominant localisation of steatosis	Group I		Group II		Group III		Group IV	
	SH	DS	SH	DS	SH	DS	SH	DS
Central part	32	+	36[a]	+	19[a]	+	2[a,b,c]	+
Periferal part	5	+	1[a]	+	3	+	28[a,b,c]	++++
Whole lobule	41	+++	1	+	68[a,b]	+++	69[a,c]	++++
No steatosis	22	–	62	–	10[a,b]		1[a,b,c]	

Data points represent; (a: $p < 0.05$ in comparison with control group; b: $p < 0.05$ in comparison with alcohol group; c: $p < 0.05$ in comparison with green tea group)

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