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By

Lazare Tehoua
André B. Konan
Eric K. Kwadjo
Marcel K.G. Bouafou
Jacques Y. Datté
Michel A. Offoumou

Research Article

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¹Lazare Tehoua, ²André B. Konan*, ³Eric K. Kwadjo, ⁴Marcel K.G. Bouafou, ⁵Jacques Y. Datté and ⁶Michel A. Offoumou

^{1,2*,5,6}Laboratory of Nutrition and Pharmacology, UFR Biosciences, Félix Houphouët-Boigny University, 22 BP 582 Abidjan 22, Cote d'Ivoire.

³Laboratory of Animal Biology and Cytology, UFR Natural Sciences, University Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.

⁴Division of Life Sciences and Earth, Department of Sciences and Technology, Ecole Normale Supérieure (ENS), 25 BP 663 Abidjan 25, Côte d'Ivoire.

^{2*}Corresponding Author's Email: akonanb@yahoo.fr, Tel: (+225) 05 40 97 50 / 66 10 62 53

ABSTRACT

Objective: Previous studies have shown that regular and moderate consumption of koutoukou (KTK, a traditional spirit) is associated with a negative body mass index in female rats. In addition, the consumption of this drink in the latter also has an effect on the liver. Therefore, this work is carried out in order to assess the impact of chronic use of koutoukou (KTK) on the structure of rat liver tissue. In fact, atrophy or hypertrophy of an organ, if it is not of physiological origin, may indicate pathology of the latter.

Methods: The rats were divided into 4 groups of 8 individuals (4 males and 4 females). These animals received distilled water as drinking water (Lot 1 or control) and KTK at 5 % (Lot 2), 10 % (Lot 3) and 12 % (Lot 4) in their drinking water. After 90 days of experiment, histological sections of liver were examined under an optical microscope.

Results: The chronic alcohol KTK has no impact on the structure of male rat liver. However, in females, significant changes (inclusions and lesions) were observed depending on the alcohol content of this traditional spirit.

Conclusion: Koutoukou caused in female rats changes of liver tissue, which could partly justify liver hypertrophy observed in female rats subjected to similar treatment.

Keywords: Koutoukou, Liver, Rats, Alcoholization.

INTRODUCTION

Since the dawn of time, alcohol is found to be at the center of the celebrations or human funeral. Nowadays, its consumption has increased and affects all segments of the population including the most vulnerable (young and disadvantaged people). These people turned to the consumption of inexpensive alcoholic beverages and often adulterated (Bangué, Tchapalo and Koutoukou). Koutoukou (KTK), which is the subject of this study, is a traditional spirit. In Côte d'Ivoire, it is essential to have alcohol being served along with meals at funerals, birthday party, child naming ceremony etc. However, like any alcohol, this drink could have negative repercussions on the weight and condition of the organs involved in metabolism in drinkers.

Previous work has shown that the consumption of KTK has provoked in male rats, no significant variation in the weight of various organs such as the liver. It does not affect the morphology of the latter. On the other hand, in female rats, it caused a significant increase in the mass of the liver. It has affected the morphology of this organ, leading to its enlargement (Téhoua et al., 2013). This hypertrophy of the liver, observed in other organs, could be an indication of a disease or the revelation of a nutritional disturbance of metabolism in the latter (Bouafou et al., 2011).

Indeed, the weight changes of some organs involved in the phenomena of digestion and absorption of nutrition is an indirect way of exploring organs called regulators in nutrition studies (Adrian et al., 1991). Atrophy or hypertrophy of an organ, if it is not of physiological origin, may be an indication of pathology of the latter. This reveals a disorder of the nutritional metabolic in that organ.

Therefore, in this study, histology and histopathology of the liver of rats subjected to moderate alcoholization to KTK are made. They will assess the damage to the structure of the liver, an organ playing a main role in nutritional homeostasis (Leverve, 1999).

MATERIELS AND METHODS

Koutoukou

Koutoukou used is the only one produced in the South Comoé, precisely Aboisso. He was taken directly from the producer to avoid manipulation by resellers that may affect product quality.

Animals

The experiment was carried out on 32 male and female rats, with body weight, respectively, between (164-185) g and (115-133) g. Rats *Rattus norvegicus* (Wistar strain) were grouped in batches of four in the animal house of the Faculty of Biosciences, maintained at ambient room temperature (27 ° C) with a stable humidity, and a system adjusting the photoperiod.

Experimental Protocol

Experimental procedures and protocols used in this study were approved by Ethics Committee of Félix Houphouët-Boigny University. These guidelines were in accordance with the internationally accepted principles for laboratory use and care (National Research Council, 1996; Mosihuzzaman and Choudhary, 2008). The animals were divided into four (4) batches of eight (8) animals (4 males and 4 females). The weight of each animal was evaluated. The control group (group 1) received distilled water while groups 2, 3 and 4 received respectively the concentrations of 5 %, 10 % and 12 % (v/v) of KTK according to the works of Tehoua et al. (2011). All treatments were done orally for 90 days. Each animal was fed *ad libitum*.

Organs Sampling (liver)

At the end of 90 days experiment, the rats were sacrificed and organs (liver) of each animal were sampled and weighed. Organs (liver) collected were preserved in 10% formalin to prevent tissue destruction and then taken to the pathology laboratory of the Hospitalier University Hospital Centre of Treichville, where histological sections of livers collected were made.

Sections Analysis and Results Expression

It is done by microscopic examination of sections of sampled organs (liver) using an optical microscope LEICA GZ 6 -5169, according to the method described by Lafond (2006). This examination is done in two steps:

The first step has to check and assess the quality of histological slides and staining. Only slides with sufficient quality were kept for interpretation. The quality of these has been evaluated based on three criteria: the wealth of material of interest, respect for tissue structure and cell morphology.

The second step was the analytical reading of slides through the assessment of the bottom of the smear (presence of cellular fragments, well defined structures...) and the recognition of the different tissues and different structures.

Statistical Analysis of Results

The data obtained were processed statistically using the software STATISTICA 7.1 by analysis of variance (ANOVA). Whenever a significant difference ($P < 0.05$) was revealed, the ANOVA test is complemented by the Tukey post ANOVA, to identify the variable (s) with very significant differences compared to controls.

RESULTS

The macroscopic examination of the liver of control rats showed a normal appearance: light brown, soft and firm to the touch. Unlike control and male rats, usually in females, some organs have few homogeneous appearance with inclusions. They are a little larger than that of controls.

According to observations, compared to control rats subjected to distilled water, the consumption of KTK had no effect on the liver tissue of male rats. The parenchyma is made up of cells forming some span hepatocytes separated by sinusoids visible. These sinusoids converge towards the space door. The detail of a space door shows the following three components: the branch of the portal vein, the branch of the hepatic artery

and the bile canaliculus (Figure 1). Sections from the tissues of female rats submitted to KTK have a slightly different structure (Figures 2, 3 and 4) than the liver parenchyma of control rats (distilled water). Micrograph of liver tissue from female rats submitted to KTK (5%, 10% and 12%) shows basically three (3) types of anomaly. On the micrograph in Figure 2, the branch of the portal vein is distended and ovoid with a cluster of red blood cells inside. It is a hepatic congestion. The second micrograph (Figure 3) suggests large and small vacuoles in the cytoplasm of hepatocytes. These are lipid inclusions. It is steatosis. The third micrograph (Figure 4) shows the abnormal accumulation of extracellular matrix components in the liver parenchyma. It is fibrosis. The severity of damage is proportional to the alcohol content of the KTK consumed.

Figure 1: Liver section from control rats and male rats treated with KTK. This photograph shows the presence of hepatocyte racks (**Tr**), a branch of the portal vein (**Vp**) well differentiated, one bile canaliculus (**Cb**) and a branch of hepatic artery (**Ah**).

Figure 2: Liver section from female rats treated with 5 % KTK. This photograph shows the presence of a branch of the portal vein (**Vp**) well differentiated with Hepatic congestion (clusters of erythrocytes (**Ch**)), one bile canaliculus (**Cb**) and a branch of the hepatic artery (**Ah**).

Figure 3: Liver section from female rats treated with 10 % KTK. This photograph shows the presence of hepatocyte racks (Tr), a branch of the portal vein (Vp) well differentiated, one bile canaliculus (Cb) and a branch of the hepatic artery (Ah).

Figure 4: Liver section from female rats treated with 12 % KTK. This photograph shows the presence of microscopic lesions (fibrosis) (Fb) at the space door, the portal vein (Vp) well differentiated and a branch of the hepatic artery (Ah).

DISCUSSION

Moderate consumption of KTK for 90 days in female rats led to an increase of the liver relative weight. Histological examination of the liver of control rats showed a normal appearance: light brown, soft and firm to the touch. Unlike male rats, usually in females, some organs have a little homogeneous appearance with inclusions. They are a little larger than that of controls.

This hypertrophy could be synonymous with diseases caused by alcohol such as steatosis (excessive accumulation of fat in liver cells), alcoholic hepatitis (acute inflammation of the liver, accompanied by destruction of liver cells and frequently followed by permanent scarring) and alcoholic cirrhosis (destruction of normal liver tissue, leaving non-functioning scar tissue). The post-mortem examination of the animals revealed a significant increase in liver weight, under conditions of moderate consumption of KTK. This increase can be explained by the presence of blood congestion of central veins, inclusions and lipid accumulation of extracellular matrix components in the parenchyma, on histological examination of the liver of female rats treated KTK.

Changes in liver weight in female rats subjected KTK, characterized by their hypertrophy, can suggest the following hypotheses: KTK would contain some components metabolized with difficulty, imposing to these organs an increased activity (Adrian et al., 1991). These observations highlight the susceptibility of female rats in relation to the koutoukou. It also shows that male and female rats respond differently to koutoukou. In fact, the works of Tehoua et al. (2011) showed that exposure of rats to KTK after 30 days has led to modification of lipid, carbohydrate and protein metabolism.

The liver plays a dominant role in nutritional homeostasis (Leverve, 1999). Endowed with considerable metabolic properties and often unique, it is involved in the synthesis, degradation and metabolism of all macronutrients (alcohol) as well as the storage of glycogen as most micronutrients: vitamins and minerals. It represents 2.5 % of body weight and uses 20% of the total energy expenditure of rest. The metabolism of alcohol provides no storage form and therefore must be totally eliminated. In fact, 90-95 % of the ingested dose is oxidized in the liver, whereas 5-10% is oxidized in extrahepatic tissues.

The metabolism of alcohol in the human body is complex and several studies have shown that alcohol caused obesity (Jacobsen and Thelle 1987; Mannisto et al., 1996) or not (Hellerstedt et al., 1990; Colditz et al., 1991; Liu, 1996; Brunner et al., 2001) and severe physical, mental and social disorders (Pöschl and Seitz, 2004; Paljarvi et al., 2005; Stranges et al., 2006).

CONCLUSION

Chronic and moderate koutoukou consumption in rats for 90 days has led to an increase of the liver weight in female rats. This liver hypertrophy is the result of damage caused by the use of that traditional brandy on the liver tissue.

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Authors' Contributions

All authors contributed equally in the study. They made substantial contributions to the design of the study, the collection of the data as well as the preparation and analysis of the data. They also drafted the manuscript and gave final approval for its submission to the journal for consideration of publication.

Declaration of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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