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## EFFECT OF CHRONIC ALCOHOLIZATION BY KOUTOUKOU ON THE WEIGHT AND ORGANS (LIVER, HEART AND KIDNEY) OF TREATED RATS (*RATTUS NORVEGICUS*)

L. Tehoua<sup>1</sup>, B.A. Konan\*<sup>1</sup>, M.K.G. Bouafou<sup>2</sup> and J.Y. Datté<sup>1</sup>

Laboratory of Nutrition and Pharmacology, UFR-Biosciences, Félix Houphouët Boigny University<sup>1</sup>, 22 BP 582 Abidjan 22, Côte d'Ivoire

Division of Life Sciences and Earth, Department of Sciences and Technology, Ecole Normale Supérieure (ENS)<sup>2</sup>, 25 BP 663 Abidjan 25, Côte d'Ivoire

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### Correspondence to Author:

Dr. Brou A. Konan

Laboratory of Nutrition and Pharmacology, UFR-Biosciences, Félix Houphouët Boigny University, 22 BP 582 Abidjan 22, Côte d'Ivoire

E-mail: akonanb@yahoo.fr

### ABSTRACT

**Purpose:** We evaluate the chronic consumption of koutoukou (KTK) on the weight and organs (liver, heart and kidney) of treated rats.

**Methods:** This study was focused on animals divided into four (4) and by sex. One of the groups being used as control receives distilled water as drinking water; the three other groups receive KTK in their drinking water at a rate of 5 %, 10 % and 12 % for 90 days. At the end of the experiment the rats were sacrificed and organs (liver, heart and kidney) were collected and weighted.

**Results:** This study showed that the chronic and moderate consumption of koutoukou caused weight loss in female rats and an increase of organs (liver, heart and kidney) weight in proportion to alcoholometric.

**Conclusion:** The results of our study showed that a chronic and moderate consumption of koutoukou affects organs (liver, heart and kidney) in female rats. The consumption of this drink for the latter is associated to body mass index negative in female rats.

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**INTRODUCTION:** For time immemorial, alcohol is used for rejoicing and funeral. Nowadays, its consumption has increased and affects every part of the population including the most vulnerable (young men and people living in squalor). This population used to consume non-expensive alcoholic beverages which are often adulterated (Bangui, tchapalo, koutoukou). Koutoukou (KTK) which is the focus of this study is in Côte d'Ivoire the most alcoholic beverage use for funerals, rejoicings and meals with friends, however, this drink could have adverse effect on the weight and state of organs involve in its metabolism in the consumer bodies. The metabolism of alcohol in the human body is complex, and several studies on the one hand showed that alcohol was the cause<sup>1, 2</sup> or not<sup>3-6</sup> of obesity and on the other hand was associated with severe physical, mental and social troubles<sup>7-9</sup>.

In France, Obépi-Roche study showed that The prevalence of obesity (BMI  $\geq 30$  kg/m<sup>2</sup>) in French adults aged 18 years old and over was 14.5 % and that of the overweight (25  $\leq$  BMI  $\leq$  30 kg/m<sup>2</sup>) 31.9 %<sup>10</sup>. Overweight and obesity are mainly a problem of public health.

Though, we do not yet have statistic data on the prevalence of obesity and overweight in Côte d'Ivoire, however the chronic and excessive consumption of Koutoukou by the population deserves special attention.

For this reason, this study is designed to assess the impact of a chronic consumption of Koutoukou on the weight and organs of treated rats (liver, heart and kidneys).

## MATERIALS AND METHODS:

**Ethics:** Experimental procedures and protocols used in this study were approved by Ethical Committee of Health Sciences, Félix Houphouët Boigny (Abidjan, Côte d'Ivoire). These guide line were in accordance with the internationally accepted principles for laboratory use and care<sup>11</sup>.

**Animals:** The experiment was carried out on 32 male and female Wistar rats, body weight range, respectively, between (164-185) g and (115-133) g. The animals were kept and maintained under conventional laboratory conditions of temperature, humidity and light/dark cycle of 14/10 hours and allowed free access to food (standard pallet diet) and drinking tap water *ad libitum*.

**Koutoukou:** The Koutoukou used for this study was procured from the south Comoe region in Aboisso (Côte d'Ivoire). It was directly bought from the producer to avoid manipulation by resellers that may affect product quality.

**Experimental protocol:** The animals are distributed, in four (4) batches of eight (8) animals (4 males and 4 females by group). The weight of each animal was evaluated. The control group receives distilled water while groups 2, 3 and 4 respectively receive concentrations of 5, 10 and 12% (v/v) of Koutoukou. The selected doses come from the work of Tehoua *et al.*<sup>12</sup>. All treatments are done orally for 90 days. Each animal has access to food *ad libitum*.

**Organs collection:** At the end of 90 days experiment, the rats were sacrificed and organs (Liver, Kidneys Heart) were collected and weighted.

**Data analysis:** Data were statistically analyzed using STATISTICA 7.1 software by the analysis of variance (ANOVA). Each time a significant difference ( $P < 0.05$ ) is

revealed, the ANOVA test is supplemented by the test post ANOVA of Tukey, in order to identify the variable (s) presenting significant differences compared to the control groups.

## RESULTS:

**Influence of a moderate consumption of Koutoukou on the body weight of treated rats:** As shown in **Table 1**, the increase in body weight of females was not significantly different between the control and treated groups by KTK at a rate of 5 %, 10% and 12% up to the 28th day (control =  $161 \pm 18.91$  g, KTK 5% =  $165.75 \pm 18.93$  g, KTK 10 % =  $152.25 \pm 18.77$  g, KTK 12 % =  $157.5 \pm 18.79$  g). This increase is significant the 58th and 72nd day of experimentation (control =  $162 \pm 19.77$  g; KTK 5 % =  $192 \pm 34.89$  g, KTK 10 % =  $184.5 \pm 33.68$  g; KTK 12 % =  $217.5 \pm 35.21$  g).

At the end of the experimentation, female rats treated with KTK 12%, non-significant decrease in weight is observed (from  $217.5 \pm 35.21$  to  $181.25 \pm 34.68$  g). Weight gain in female rats submitted to chronic alcoholization by koutoukou (CAK) seems to be influenced. Overall, the growth of female rats appears homogeneous, but female rats submitted to CAK showed a delay of growth, compared to the control group. And, this delay of growth is inversely proportional to the dose of Koutoukou received by female rats.

**Table 2** shows the evolution of body weight in male rats. The increase in weight is significantly different at day 42, and only with rats treated by KTK at a rate of 12 %. The effect of koutoukou on the weight gain of male rats submitted to chronic alcoholization to koutoukou (CAK) is not noticeable. At this level, only the rats treated by KTK at the rate of 12 % as drinking water, have a higher weight gain compared to control rats.

**TABLE 1: EFFECT OF KOUTOUKOU ON THE WEIGHT OF TREATED FEMALE RATS**

	Groups				
	Control	KTK (5 %)	KTK (10 %)	KTK (12 %)	
Day 0	$132.5 \pm 10.59$ a	$117 \pm 8.22$ a	$146.5 \pm 11.38$ a	$115 \pm 8.01$ a	
Day 14	$141.5 \pm 12.45$ a	$140 \pm 12.94$ a	$151.5 \pm 12.99$ a	$137.25 \pm 12.34$ a	
Day 28	$161 \pm 18,91$ a	$165.75 \pm 18.93$ a	$152.25 \pm 18.77$ a	$157.5 \pm 18.79$ a	
Weight of female rats (g)	Day 42	$176.25 \pm 19.75$ a	$176.75 \pm 19.71$ a	$168 \pm 19.56$ a	$177 \pm 19.77$ a
	Day 58	$162.5 \pm 19.77$ b	$193 \pm 34.89$ a	$180.5 \pm 33.67$ a	$203 \pm 35.02$ a
	Day 72	$162 \pm 19.77$ b	$192 \pm 34.89$ a	$184.5 \pm 33.68$ a	$217.5 \pm 35.21$ a
	Day 90	$194.5 \pm 34.82$ a	$191.75 \pm 34.77$ a	$191.25 \pm 34.77$ a	$181.25 \pm 34.68$ a

Means followed by the same letter on the same line are not significantly different at  $< \alpha$  0.05 by Tukey test. ns, not significant; \*, significant; \*\*, highly significant, Cal, calculate; theor, theoretical. KTK, Koutoukou (%).

**TABLE 2: INFLUENCE OF KOUTOUKOU ON THE WEIGHT OF TREATED MALE RATS**

		Groups			
		Control	KTK (5 %)	KTK (10 %)	KTK (12 %)
Weight of male rats (g)	Day 0	184.75±9.51 a	164.25±9.23 a	178±9.29 a	184.5±9.45 a
	Day 14	177±10.67 a	154.75±9.02 a	191.75±11.34 a	191.75±11.45 a
	Day 28	207.25±12.56 a	188.25±11.11 a	177±11.04 a	213.25±13.65 a
	Day 42	214.75±13.89 b	192.25±11.78 b	208.25±13.97 b	238±23.96 a
	Day 58	222±14.01 a	223.5±14.02 a	215.75±13.91 a	244.5±14.33 a
	Day 72	221.75±13.97 a	225.75±14.07 a	215±13.87 a	249.25±14.37 a
	Day 90	230.5±14.01 a	210.75±13.82 a	201.25±13.34 a	256.75±14.25 a

Means followed by the same letter on the same line are not significantly different at  $< \alpha$  0.05 by Tukey test. ns, not significant; \*, significant; \*\*, highly significant, Cal, calculate; theor, theoretical. KTK, Koutoukou (%).

**Effect of Chronic consumption of Koutoukou on treated rats organs:** The chronic alcoholization by koutoukou (CAK) gave no significant variation in weight of male rats different organs compared to controls (Table 3). In female rats, chronic Alcoholization by koutoukou caused a significant increase in weight of

various organs such as the liver, the heart and kidneys. Liver weight increased from 4.55±0.96 g for controls to 6.05±1.1 g, 6.84±1.23 g, 6.84±1.06 g, respectively, for rats treated with KTK at the rate of 5 %, 10 % and 12 % (Table 4).

**TABLE 3: EVOLUTION OF THE WEIGHT OF DIFFERENT ORGANS IN MALE RATS ACCORDING TO THE RATE OF KOUTOUKOU CONSUMPTION**

Weight of organs (g)	Control	KTK 5 %	KTK 10 %	KTK 12 %
Liver	4.55 ± 0.96 b	6.05 ± 1.1 a	6.84 ± 1.23 a	6.84 ± 1.06 a
Heart	0.69 ± 0.19 b	0.67 ± 0.18 b	0.91 ± 0.26 a	0.91 ± 0.26 a
Kidney	0.99 ± 0.36 b	1.26 ± 0.96 a	1.46 ± 0.98 a	1.46 ± 0.98 a

Means followed by the same letter on the same line are not significantly different at  $< \alpha$  0.05 by Tukey test. ns, not significant; \*, significant; \*\*, highly significant, Cal, calculate; theor, theoretical. KTK, Koutoukou (%).

**TABLE 4: EVOLUTION OF THE WEIGHT OF DIFFERENT ORGANS IN FEMALE RATS ACCORDING TO THE RATE OF KOUTOUKOU CONSUMPTION**

Weight of organs (g)	Control	KTK 5 %	KTK 10 %	KTK 12 %
Liver	4.55 ± 0.96 b	6.05 ± 1.1 a	6.84 ± 1.23 a	6.84 ± 1.06 a
Heart	0.69 ± 0.19 b	0.67 ± 0.18 b	0.91 ± 0.26 a	0.91 ± 0.26 a
Kidney	0.99 ± 0.36 b	1.26 ± 0.96 a	1.46 ± 0.98 a	1.46 ± 0.98 a

Means followed by the same letter on the same line are not significantly different at  $< \alpha$  0.05 by Tukey test. ns, not significant; \*, significant; \*\*, highly significant, Cal, calculate; theor, theoretical. KTK, Koutoukou (%).

As far as the kidney is concerned we had as well notice a variation of weight of the rats organs, from 0.99±0.36 g for control rats to 1.26±0.96 g for the rats treated with KTK at the rate of 5 %, 1.46±0.98 g for those treated with KTK at the rate of 10 %, and finally, 1.46±0.98 g for those treated with KTK at the rate of 12 %.

The CAK increased the weight of the heart of rats treated with KTK at the rate of 10 % (0.91±0.26 g) and at the rate of 12% (0.91±0.26 g) compared to control rats (0.69±0.19 g) and those treated with KTK at the rate of 5 % (0.67±0.18 g).

**DISCUSSION:** The Consumption of koutoukou causes an increase in weight of male rats. However, this increase of weight is observed at a dose of 12%. These observations match with the literature that combines alcohol consumption with an increase in BMI in humans <sup>6, 13</sup>. This increase is also related to the dose consumed.

In fact, the more the alcoholometric is higher the more weight gain is higher. These results corroborate those of Istvan *et al* <sup>14</sup>, who argued that a higher intake of alcohol is associated with body mass index (BMI) increased.

Thus, Jacobsen and Thelle <sup>1</sup> and Mannisto *et al* <sup>2</sup>, showed that the consumption of spirits was more beneficial to the body mass index than other drinks.

Basically, the consumption of koutoukou has a positive effect on male rats weight, whereas it is the contrary with female rats. Indeed, chronic Alcoholization by koutoukou (CAK) did not cause significant weight gain in female rats compared to controls. It has even caused, in female rats submitted to the dose of 12 %, a decreased of weight on day 90; these results match with those of previous studies <sup>3-6</sup>. The Koutoukou, in female rats is negatively correlated with body mass index (BMI). This result clearly shows that men and women react differently to koutoukou which is a beverage rich in ethanol. These observations match with those of INSERM <sup>15</sup>. This gender difference could be explained on three areas: the difference of body size between the woman and the man <sup>16-22</sup>, the deficiency of the activity of aldehyde dehydrogenase class III (ADH-v) in women <sup>23</sup>, on the hormonal status of Women <sup>24</sup>.

Body size plays an important role in alcohol tolerance. Alcohol is distributed throughout the body, but is rather uneven, it dissolves more easily in water than in fat body tissue. Differences of tolerance among individuals are due to the volume of water in the body. For equal weights, the body of a person who has less fat contains more water than that of a person who has more. The alcohol in the blood of a person with less body fat will be lower than that of an individual who has more.

Body fat is scientifically more important in women than in men we obviously notice that the volume of alcohol ingested it is distributed in a reduce volume of free water than in men, causing a higher ethanolaemia for the same quantity ingested, you must know that this alcohol will be less quickly eliminated in women than in men. These observations are similar to those of Jean-louis and Francoise <sup>25</sup>, carried out on smoking and alcohol abuse. Class III enzymes (ADH-v), relatively abundant in all tissues examined, including brain, leukocytes and stomach are the "ancestors" of ADH. They metabolize long-chain of alcohols and fatty acids x-hydroxylated <sup>26</sup> and are also known as glutathione-dependent formaldehyde dehydrogenase <sup>27</sup>.

They have high Km for ethanol, matching with concentrations that may be present in the stomach. They could therefore participate in the "first pass metabolism" of ethanol and explain the differences between men / women at this level, the latter having an activity of ADH-v lower than men <sup>23</sup>. According to Knight *et al* <sup>28</sup>, this difference in male / female is mainly due to a longer delay gastric emptying greater in women than in men, primarily due to a greater decrease of antral contractions in women. High concentrations of estradiol and progesterone corresponding to the luteal phase of the menstrual cycle and pregnancy slow gastric emptying and intestinal transit, and could (although acting in reverse) alter the absorption of koutoukou.

Hormonal modifications in women during the menstrual cycle and menopause, as well as oral contraceptives and hormone replacement therapy may affect the metabolism of koutoukou, and increase its toxicity. The weight changes of some organs involved in the phenomena of digestion and absorption of nutriments is an indirect way of exploration organs called regulators in nutrition studies <sup>29</sup>. Atrophy or hypertrophy of a body, if it is not physiological, may be an indication of pathology. It is, in this case an indication of a metabolic nutritional disorder in this organ.

The present study reveals that the consumption of koutoukou did not increase in male rats, the weight of the different organs such as the liver, the heart and kidneys. It does not affect their morphology. Whereas in female rats, we notice a significant increase of organs weight quoted above, it has also affected the morphology of these organs, causing their hypertrophy.

This hypertrophy of organs could be an indication of a disease or the revelation of a nutritional metabolic disorder. Thus, the consumption of koutoukou by female rats, affect directly the structure of the heart muscle and caused heart hypertrophy increasing the risk for a heart attack. These observations match with those of Mahmud who showed that, in women, a thickening of the left ventricular septum and posterior wall, and an increase of the left ventricular mass was at least observed in women who consume alcohol excessively <sup>30</sup>.

In a recent study, Tehoua *et al*<sup>12</sup>, showed after 30 days, that a moderate consumption of koutoukou by rats had a cardioprotective effect.

But, after 90 days, the results show that, even a moderate consumption of koutoukou by women could have adverse effects on the muscle structure. A moderate consumption of koutoukou for 90 days has increased the liver of female rats. An increase of the liver could mean diseases caused by alcohol such as: steatosis (excessive accumulation of fat in liver cells), alcoholic hepatitis (acute inflammation of the liver, followed by destruction of individual liver cells and often followed by permanent scarring) and alcoholic cirrhosis (destruction of normal liver tissue, leaving non-functioning scar tissue). Kidney hypertrophy observed in female rats appears to be a consequence of increased organ weights (heart and liver) caused by the consumption of koutoukou.

Modification of organ weight, characterized by the hypertrophy of the heart, liver and kidney observed in female rats submitted to CAK, can allow us to suggest the following hypotheses: Koutoukou could encompass components which cannot easily be metabolized. Coercing these organs to an increase of activity<sup>29</sup>. These observations highlight the susceptibility of female rats to koutoukou. It also shows that male and female rats respond differently to the consumption of koutoukou.

**CONCLUSION:** Chronic and moderate Alcoholization in rats by koutoukou for 90 days was used to assess the effect of this traditional drink on body mass index and organ weights (heart, liver and kidney) involved in the metabolism of koutoukou. This study showed that the use of this drink causes a decrease of body weight in female rats but increase the body weight in male rats. Koutoukou causes hypertrophy of the main organs involved in the metabolism of female rats. This study shows that consumption of koutoukou, even in a moderate proportion, is a risk of cardiac attack for women.

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