INTRODUCTION

In Côte d’Ivoire, traditional brandy commonly known as "Koutoukou" (TK) comes from distillation of fermented palm oil sap (Elaeis guineensis Jacq.). It is popular drinking among middle- and low-income populations (Tehoua et al, 2011). The cost per liter is relatively lower compared to industrial liquors (Koffi et al, 2017). Several economically weak alcohol consumers (young and old) indulge in this artisanal drink, which sometimes causes many intoxications due to toxic effects (methanol, heavy metals) present in it (Koffi et al, 2019). Some authors have pointed out that Koutoukou toxic effects are linked to deficiencies due to production equipment, distillation techniques and the daily dose consumed. Therefore, the lack of training of traditional producers and the inadequacies highlighted would give Koutoukou high toxicity that could lead to death of consumer (Gnagne, 1990; AIP, 2015; Koffi et al, 2019).

According to work carried out by Camara et al (2002), the Koutoukou toxicity is due to high ethanol content. This traditional high alcoholic beverage could have harmful effects on health when consumed in high doses. Some authors have shown that acute Koutoukou consumption has very pronounced effects on human vigilance (Camara et al, 2002). However, a study on regular and moderate Koutoukou consumption showed a beneficial effect on liver function and lipid metabolism (Tehoua et al, 2011). However, previous studies by Koffi et al (2019) have produced improved Koutoukou (IK) in laboratory by controlling fermentation, distillation temperature and applying quality control standards during production.

The objective of this study is to determine impact of chronic Koutoukou alcoholization on hematological and biochemical parameters in Wistar rats compared to data from traditional Koutoukou (TK) consumption and industrial Koutoukou (InK) sold in supermarkets.

MATERIAL AND METHODS

Material

Biological material

The biological material is composed three types of Koutoukou, which are: traditional Koutoukou (TK), improved Koutoukou (IK), industrial Koutoukou (InK) and Wistar rats.
Three Koutoukou productions

Traditional Koutoukou (TK) comes from production collected from traditional producers. On the other hand, improved Koutoukou (IK) corresponds to control of fermentation time, temperature and distillation time. Industrial Koutoukou (InK) comes from local industries brands sold in supermarkets of Abidjan (Côte d'Ivoire) and considered as alcoholic witness.

Wistar rats

The Wistar rats used are 140, including 70 females and 70 males aged 8 to 10 weeks, weighing between 110 and 138 g. These rats came from Nangui Abrogoua University. They have been acclimatized to animal house of laboratory of animal physiology, pharmacology, and pharmacopeia. These animals were fed IVOGRAIN® granules and tap water continuously in bottles for six (6) months (July to December 2018).

Technical equipment

The technical equipment is essentially composed a GAY LUSSEAC centesimal alcoholometer, breeding cages, feeding bottles, a gastric tube suitable for feeding animals, pastoral pipettes, EDTA tubes, dry tubes, racks, cooler and gloves. The rats were weighed using a scale (METTLER TOLEDO, PB 153-L, Switzerland). Biology laboratory equipment was also used:

- PLC (ROPONIK®, India) and a centrifuge (HERAEUS SEPA TECH, Germany for biochemical tests,
- analyzer counter automaton counter (Sysmex XT-2000 I, Japan) for blood count (BC).

METHODS

Determination of Koutoukou doses to be administered

The Koutoukou doses administered to rats were determined based standard glasses and a Koutoukou consumers survey. Standard glasses are volume units of alcoholic beverages established by WHO to avoid exceeding the blood alcohol level (0.5 g/l). Thirty (30) ml corresponds to standard glass of drink in 40% vol. The survey of hundred consumers revealed that they drink between 2 and 4 "KTK rounds" on average per day. One "KTK round" corresponds to about two standard glasses. These approaches resulted in selection of equivalent about 30 ml of "half KTK round", about 125 ml of "two KTK rounds" and about 200 ml of "four KTK rounds" as the volume to be administered to rats. In these selected volumes, the pure alcohol (PA) quantities are 12, 50 and 80 g of pure ethanol respectively.

The Koutoukou dose to be administered to rats is calculated according to Widmark formula (Widmark, 1932). For example, the determination of 50 g PA (or 125 ml) equivalent in 50 % vol to be administered to 110 g rat is carried out according to following calculation and according to rats gender:

**Determination of KTK volume equivalent to be administered to male rats**

According to Widmark, (1932), the alcohol average volume consumed by 70 kg man is calculated as follows:

\[ \text{Dose} = 50 \text{ g} \rightarrow 70 \text{ Kg BW} \]

\[ X \rightarrow 1 \text{ Kg BW} \]

**Volume to be administered**

\[ \text{Volume} = \frac{\text{rat weight} \times \text{dose}}{\text{Kg BW}} \times \text{alcohol density} \]

\[ \text{Volume} = \frac{110 \times 0.71}{1000} \times 0.5 \times 0.8 = 0.195 \text{ ml} \]

Therefore, the volume equivalent of 125 ml of Koutoukou 50 % vol to be administered to 110 g male rat is 0.195 ml. Thus; the Koutoukou doses administered to male rats were calculated from 12, 50 and 80 g pure alcohol /Kg BW.

**Determination of KTK volume equivalent to be administered to female rats**

The alcohol average volume to be consumed by 60 kg woman is calculated as follows:

\[ \text{Dose} = 50 \text{ g} \rightarrow 60 \text{ Kg BW} \]

\[ X \rightarrow 1 \text{ Kg BW} \]

\[ \text{Volume} = \frac{\text{rat weight} \times \text{dose}}{\text{Kg BW}} \times \text{alcohol density} \]

\[ \text{Volume} = \frac{110 \times 0.83}{1000} \times 0.5 \times 0.8 = 0.228 \text{ ml} \]

Therefore, the volume equivalent of 125 ml of Koutoukou 50 % vol to be administered to 110 g female rat is 0.228 ml. Thus; the Koutoukou doses administered to female rats were calculated from 12, 50 and 80 g pure alcohol /Kg BW.

Rats conditioning

The animals are subjected to 22 ± 2 °C and 12 hours of light and 12 hours of darkness. The rats were fed daily with IVOGRAIN® granules and tap water collected in bottles. The experimental protocol and animal handling procedures were conducted according to good laboratory practice (OCDE, 1998).

Koutoukou administration

Before the Koutoukou (KTK) administration, the rats in each batch (male and female) were weighed individually. Their bodyweight are ranged from 110 to 138 g. They received by force-feeding with cannula, the different Koutoukou doses once day at same time (8 h 30 min) for 28 days. The different administrations were summarized into four (4) groups:

- In T group (Witness) (20 animals including 10 rats/lot), lots T and Tr (witness in reversibility study) received 2 ml/100g BW of distilled water;
- In IK group (40 animals including 10 rats/lot), lot IK30 received 30 ml (12g PA), lot IK125, received 125 ml (50 g PA) equivalent, and lots IK200 and IKr (IK200 in reversibility study) received 200 ml (80 g PA) equivalent of improved Koutoukou;
- In TK group (40 animals including 10 rats/lot), lots TK10, TK125, and TK200, IKr received 30, 125 and 200 ml equivalent of traditional Koutoukou respectively.
• In InK group (reference) (40 animals including 10 rats/lot), the same volumes equivalent was given to rats in lots InK30, InK125, InK200, and InKr respectively.

Reported effect persistence or reversibility
Following the 28th experimentation day, rats in Tr batches (receiving 2ml of distilled water/100g BW), IkKr (received 200 ml equivalent of improved Koutoukou), TkKr (received 200 ml equivalent of traditional Koutoukou) and InKr (received 200 ml equivalent of industrial Koutoukou), were no longer gave until day 42. This period was withdrawal period. They had access to tap water and granules. This phase made it possible to monitor the reversibility of Koutoukou effect induced in animals during alcoholization (OCDE, 2008).

Biological blood tests
Venous blood samples were taken from retroorbital sinus of rat eye before the drinks administration on days 28 and 42. After 10-hour fast, venous blood was collected from retroorbital sinus by sterile Pasteur pipette (Laroche et Rousselet, 1990). According to method described by Waynforth (1980), each animal was previously anesthetized in bell containing cotton soaked for two to three minutes. After disinfecting the eyelid with 70% alcohol, the pipette tip was introduced into lateral corner of eye, with slight rotation until it reached the venous plexus. Thus, 0.5 to 2 ml of blood was immediately collected in tubes containing EDTA (ethylene diamine tetraacetic acid anticoagulant) for hematological analyses and in dry tubes for biochemical analyses.

Hematological analysis
Blood count (BC) was performed directly to avoid cell autolysis and to obtain reliable results using automatic counter (Sysmex XT-2000 I, Japan). The CB provided information on leukocytes (granulocytes or polymonuclear cells, lymphocytes and monocytes), erythrocytes, reticulocytes, hemoglobin, hematocrit, mean globular volume (MGV), mean corpuscular hemoglobin rate (MCHR), mean corpuscular hemoglobin concentration (MCHC) and thrombocytes (platelets) (Langforth et al, 2003).

Blood biochemical analysis
The blood collected in dry tube was centrifuged 3000 rounds for 10 minutes in centrifuge (HERAEUS SEPATECH). The serum obtained was stored -20 °C until biochemical parameters were determined. Serum biochemical parameters were determined by ROPONIK® brand PLC and reagents from BioSystems Costa Brava Laboratory (Spain). The parameters measured were: urea, glucose, creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total and direct bilirubin, total cholesterol, HDL-cholesterol, triglycerides and total protein (Tehoua et al, 2011).

Statistical analysis
The statistical analysis of values was performed by GraphPad Prism version 5.01 for Windows (GraphPad Software Inc, San Diego, MO, California /USA, 2007). The two-factor analysis of variance test (ANOVA 2) associated with Bonferroni's posthoc test made it possible to determine the significance of results. The various statistical tests were considered significant for probability level p less than 0.05 (p < 0.05). In representation of results, asterisks (*, **, *** ) expressed significant differences from witness.

RESULTS
Koutoukou influence on hematological parameters
The distilled water and Koutoukou (IK, TK, and InK) effect on hematological parameters in rats are presented in Tables I and II. The results indicate that eleven (11) parameters measured during alcoholic period (28 days), eight (8) parameters varied significantly.

Koutoukou influence on hematological parameters in female rats
In female rats, significant variations are observed at leukocyte line level (leukocytes, lymphocytes, and granulocytes) and erythrocyte line level (hemoglobin, erythrocytes, and hematocrits) for three Koutoukou types.

Chronic alcoholization with improved Koutoukou (IK) for 28 days and after weaning
For improved Koutoukou, leukocyte values increases significantly (p < 0.001) from 7.02 ± 0.21 to 14.04 ± 1.45 10^3/μl only 80 g PA dose. The lymphocyte level increases (p < 0.05) from average dose of 50 g PA with values ranging from 2.50 ± 0.10 to 9.20 ± 1.35 10^3/μl on alcohol consumption days (Table I).

From weaning onwards, no significant differences were observed in leukocyte and erythrocyte parameters except for thrombocyte line which showed significant increase (p < 0.01) from 333.4 ± 13.7 to 477.0 ± 13.0 10^3/μl (Table III).

Chronic alcoholization with traditional Koutoukou (TK) for 28 days and after weaning
For traditional Koutoukou, leukocyte values increase significantly (p < 0.05) from 50 g PA dose. These values range from 7.48 ± 0.52 to 11.80 ± 0.90 10^3/μl respectively. Lymphocytes also increase (p < 0.05) from 12 g PA with values ranging from 2.40 ± 0.57 to 9.05 ± 3.05 10^3/μl. Granulocytes decreases significantly (p < 0.05) from 50 g PA dose, between 4.47 ± 0.02 and 1.20 ± 0.30 10^3/μl. For erythrocyte line, only hemoglobin level decreases significantly (p < 0.05) from 13.45 ± 1.55 to 10.35 ± 0.95 g/dl at 80 g PA dose during 28 alcoholization days (Table I).

After weaning, significant increases (p < 0.001) are observed from 11.80±0.90 to 16.50 ± 1.5 10^3/μl for leukocytes, from 8.05 ± 0.35 to 11.45 ± 2.15 10^3/μl for lymphocytes and from 334.5 ± 19.5 to 584.0 ± 12.0 10^3/μl for blood platelets (Table III).

Chronic alcoholization with industrial Koutoukou (InK) for 28 days and after weaning
Industrial Koutoukou alcoholization showed that leukocyte lineage increases from 50 g PA dose. These values range from 7.03 ± 0.46 to 16.01 ± 1.30 10^3/μl for leukocytes (p < 0.001) and from 2.23 ± 1.26 to 8.80 ± 1.40 10^3/μl for lymphocytes (p < 0.05).
For erythrocyte line, a significant increase is also recorded at 80 g PA dose. The erythrocyte rate decreased (p < 0.01) from 4.92 ± 0.06 to 9.64 ± 0.43 μl/dl and hematocrit (p < 0.05) from 38.50 ± 3.1 to 50.15 ± 2.85 μl/dl during the feeding time of animals (Table I).

After weaning time, the white blood cell line decreases significantly (p < 0.01) from 16.01 ± 1.3 to 13.20 ± 1.2 μl/dl for leukocytes and from 8.80 ± 1.72 to 7.58 ± 2.14 μl/dl for lymphocytes (Table III).

Koutoukou Influence on hematological parameters in male rats

In male rats, the significant variations were observed in leukocyte line (lymphocytes, monocytes, and granulocytes), erythrocyte line (mean corpuscular hemoglobin concentration (MCHC)) and thrombocyte line (blood platelets) for three Koutoukou types.

Table I Evolution of hematological parameters of Koutoukou doses received by female rats

<table>
<thead>
<tr>
<th>Distilled water</th>
<th>Improved Koutoukou</th>
<th>Traditional Koutoukou (reference)</th>
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<tr>
<td><strong>Leukocytes</strong></td>
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<tr>
<td>0d</td>
<td>7.10 ± 0.69*</td>
<td>7.25 ± 0.13*</td>
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<tr>
<td>28d</td>
<td>7.37 ± 1.02*</td>
<td>7.25 ± 0.13*</td>
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<tr>
<td><strong>Monocytes</strong></td>
<td></td>
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<tr>
<td>0d</td>
<td>6.99 ± 0.29*</td>
<td>6.62 ± 0.22*</td>
</tr>
<tr>
<td>28d</td>
<td>7.06 ± 0.06*</td>
<td>6.65 ± 0.15*</td>
</tr>
<tr>
<td><strong>Granulocytes</strong></td>
<td></td>
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<tr>
<td>0d</td>
<td>3.45 ± 0.20*</td>
<td>3.80 ± 0.08*</td>
</tr>
<tr>
<td>28d</td>
<td>3.88 ± 0.15*</td>
<td>4.13 ± 0.10*</td>
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<tr>
<td><strong>Erythrocytes</strong></td>
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<tr>
<td>0d</td>
<td>4.24 ± 0.60*</td>
<td>4.20 ± 0.13*</td>
</tr>
<tr>
<td>28d</td>
<td>4.36 ± 0.43*</td>
<td>4.59 ± 0.03*</td>
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<tr>
<td><strong>Hematocrit</strong></td>
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<tr>
<td>0d</td>
<td>13.67 ± 0.84*</td>
<td>13.70 ± 1.10*</td>
</tr>
<tr>
<td>28d</td>
<td>13.87 ± 3.19*</td>
<td>13.50 ± 3.39*</td>
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<tr>
<td><strong>Platelets</strong></td>
<td></td>
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<tr>
<td>0d</td>
<td>373.4 ± 14.1*</td>
<td>380.0 ± 12.0*</td>
</tr>
<tr>
<td>28d</td>
<td>387.7 ± 12.5*</td>
<td>357.8 ± 11.8*</td>
</tr>
</tbody>
</table>

For improved Koutoukou (IK), the lymphocyte count increases significantly (p < 0.01) from 3.00 ± 1.30 to 8.13 ± 0.31 μl/dl in high doses (80 g PA). Granulocytes decrease significantly (p < 0.01) from 12 PA dose, these values are between 4.90 ± 0.30 and 1.82 ± 0.24 μl/dl. The platelets level in blood decreases significantly (p < 0.001) from 376.5 ± 13.7 to 288.7 ± 12.2 μl/dl (Table II).

After weaning, the values of white blood cell line, particularly lymphocytes, decrease significantly (p < 0.01) from 8.13 ± 0.31 to 7.00 ± 0.30 μl/dl and granulocytes from (p < 0.001) to 2.60 ± 0.30 to 1.82 ± 0.24 μl/dl. On the other hand, blood platelets increase significantly (p < 0.001) from 288.7 ± 12.2 to 601.0 ± 15.0 μl/dl (Table III).

The values are expressed as means followed by the standard error on the mean (M ± SEM); n = 5 rats per batch, the means assigned to the same letter in a column are not significantly different (p > 0.05); **= highly significant (p < 0.01) and ***= very highly significant (p < 0.001).
Chronic alcoholization with traditional Koutoukou (TK) for 28 days and after weaning

For traditional Koutoukou, the lymphocyte count increases (p < 0.001) at high doses (80 g PA) with values ranging from 3.15 ± 0.55 to 10.33 ± 0.47 μl/μl. On the other hand, the granulocyte level decreases (p < 0.001) from low dose (12 g PA). These values range from 4.40 ± 0.20 to 1.9 ± 0.03 μl/μl at same dose (12 g AP), the MCHC increased significantly (p < 0.001) from 36.90±4.60 to 60.63 ± 3.44 g/dl. The platelet count decreases significantly (p < 0.001) from average dose (50 g PA) from 367.0 ± 11.0 to 298.3 ± 9.05 μl/μl after force-feeding days (Table II).

After weaning, the lymphocyte (p < 0.01) and granulocyte (p < 0.001) rates decrease from 10.33 ± 0.47 to 8.80 ± 0.60 μl/μl and from 1.9 ± 0.03 to 1.75 ± 0.45 μl/μl respectively. However, the monocyte level increases significantly (p < 0.001) from 1.47 ± 0.23 to 1.75 ± 0.15. The thrombocyte line also shows significant increase (p < 0.05) from 298.3 ± 9.05 to 452.0 ± 16.01 μl/μl (Table III).

Chronic alcoholization with industrial Koutoukou (InK) for 28 days and after weaning

For industrial Koutoukou (reference), the lymphocyte count increases significantly (p < 0.01) at high doses (80 g PA) with values ranging from 3.00±1.80 to 8.24 ± 1.48 μl/μl. The granulocyte level decreases significantly (p < 0.001) from low dose (12 g PA) with values between 4.54 ± 0.20 and 1.41 ± 0.45 μl/μl. The Platelet values decrease significantly (p < 0.001) from 357.0 ± 9.00 to 220.3 ± 15.0 μl/μl from mean dose (50 g PA) (Table II).
After weaning, significant increases (p < 0.01) are recorded in both enzyme markers and serum metabolites. The values range from 111.6 ± 6.95 to 167.2 ± 9.95 UI for alkaline phosphatases, 30.55 ± 1.58 to 34.59 ± 1.16 mg/dl for HDL cholesterol and 58.4 ± 8.06 to 88.1 ± 2.36 mg/dl for triglycerides (Table VI).

**Chronic alcoholization with traditional Koutoukou (TK) for 28 days and after weaning**

With traditional Koutoukou, transaminase values increase significantly (p < 0.001) from low dose (12 g PA). ASAT values range from 25.98 ± 3.95 to 197.8 ± 9.20 UI and ALAT values range from 22.14 ± 6.46 to 68.47 ± 4.67 UI. A significant increase (p < 0.001) is also recorded in low and medium-dose HDL cholesterol levels (12 and 50 g PA) with values ranging from 51.15 ± 3.85 to 99.1 ± 8.35 mg/dl, followed by a decrease (p < 0.001) in this parameter from 54.05 ± 3.50 to 39.0 ± 7.90 mg/dl at high dose (80 g PA). Triglyceride and total protein levels decrease (p < 0.001) respectively low and medium doses (12 and 50 g PA) with values between 119.57 ± 8.55 and 61.2±7.70 mg/dl for triglycerides and between 7.89 ± 0.35 and 4.70 ± 0.84 mg/dl for total proteins (Table IV).

After weaning, significant decreases (p < 0.001) in serum enzyme marker levels are observed except for alkaline phosphatases (ALP) which increase (p < 0.001) from 115.6 ± 3.20 to 288.7 ± 4.45 UI. At serum metabolites level, triglycerides increase significantly (p < 0.001) from 61.2 ± 7.70 to 70.03 ± 4.58 mg/dl (Table VI).

**Chronic alcoholization with industrial Koutoukou (InK) for 28 days and after weaning**

Chronic alcoholization in industrial Koutoukou has shown that alkaline phosphatases (ALP) decreased significantly (p < 0.01) at high doses (80 g AP) with values ranging from 129.2 ± 4.50 to 101.2 ± 6.10 UI. However, transaminase level increases (p < 0.001) from low dose (12 g PA) and varies from 23.32 ± 3.10 to 91.7 ± 5.90 UI for ASAT and from 23.97±2.23 to 84.4 ± 14.4 UI for ALAT. As for serum metabolites, significant decreases are mainly observed from 12 g PA dose. The values range from 118.34 ± 3.57 to 43.90 ± 6.40 mg/dl for triglycerides (p < 0.001) and from 7.38 ± 0.08 to 5.04 ± 0.72 g/dl for total protein (p < 0.01). For HDL cholesterol, an increase (p < 0.001) from 50.40 ± 4.30 to 88.6 ± 1.35 mg/dl is recorded low and medium doses (12 and 50 g PA) followed by significant decrease (p < 0.01) from 51.62 ± 2.10 to 28.80 ± 0.41 mg/dl at high dose (80 g PA) (Table IV).

After weaning, the level of transaminases decreases significantly (p < 0.05) while that of serum metabolites increases significantly (p < 0.001) compared to the control (Table VI).

**Koutoukou influence on biochemical parameters in male rats**

In male rats, significant differences were observed both in serum markers (PAL, ASAT, ALAT, and Total Bilirubin) and serum metabolites (Total cholesterol, HDL cholesterol, triglycerides, and total proteins) for the three (3) types of Koutoukou (improved, traditional and industrial).

**Chronic alcoholization with improved Koutoukou for 28 days and after weaning**

For improved Koutoukou, only ASAT level increases significantly (p < 0.001) from 32.0 ± 2.92 to 91.5 ± 5.5 U/I from 12 g PA dose, while decreases (p < 0.001) in serum metabolites are observed from 12 g PA dose. The values range from 53.57±1.04 to 34.40 ± 0.25 mg/dl for HDL cholesterol and 136.3 ± 5.63 to 23.77 ± 7.18 mg/dl for triglycerides (Table V).

After weaning, alkaline phosphatases (ALP) increase significantly (p < 0.001) from 123.5 ± 4.38 to 194.9 ± 3.14 UI. Triglycerides increase from 23.77 ± 7.18 to 74.09 ± 4.17 mg/dl while HDL cholesterol decreases significantly (p < 0.001) from 34.40 ± 0.25 to 27.03 ± 0.42 mg/dl (Table VI).

**Chronic alcoholization with traditional Koutoukou for 28 days and after weaning**

For traditional Koutoukou, alkaline phosphatases (ALP) decreased significantly (p < 0.001) from 12 g PA dose with values ranging from 146.3 ± 4.00 to 116.7 ± 7.06 UI. However, the transaminase level increases (p < 0.001) from 12 g PA dose and varies from 33.9 ± 3.55 to 98.0 ± 4.20 UI for ASAT and from 35.40 ± 3.70 to 62.64 ± 1.76 UI for ALAT. The total bilirubin level increases (p < 0.001) at high doses (80 g PA), with values ranging from 0.98 ± 0.40 to 2.33 ± 0.17 mg/dl. For serum metabolites, significant decreases (p < 0.001) are recorded from low dose (12 g PA). Values range from 22.1±2.82 to 11.73 ± 3.92 mg/dl for total cholesterol, 52.69 ± 2.86 to 21.8 ± 3.85 mg/dl for HDL- cholesterol, 135.3 ± 2.12 to 38.50±9.11 mg/dl for triglycerides and 7.02 ± 0.74 to 4.97 ± 0.74 g/dl for total protein (Table V).

After weaning with traditional Koutoukou, several significant increases (p<0.001) are recorded with values ranging from 0.60 ± 0.02 to 1.08 ± 0.02 g/l for urea, 139.5 ± 3.70 to 255.9 ± 5.75 UI for ALP, 11.73±3.92 to 15.12±1.21 mg/dl for total cholesterol and 38.50 ± 9.11 mg/dl for triglycerides. However, decreases (p < 0.001) were observed in direct bilirubins (0.25 ± 0.01 to 0.07 ± 0.02 mg/dl) and HDL cholesterol (211±8.65 to 145.5 ± 3.42 mg/dl) (Table VI).

**Chronic alcoholization with industrial Koutoukou for 28 days and after weaning**

For industrial Koutoukou, significant increases (p < 0.001) are recorded for ASAT and total bilirubin from 12 g PA dose. The values range from 32.7 ± 4.22 to 93.6 ± 2.80 UI and from 0.99 ± 0.18 to 1.70 ± 0.21 mg/dl respectively. Significant decreases (p < 0.001) are mainly observed in serum metabolites. These are total cholesterol (22.1 ± 3.33 to 12.32 ± 3.57 mg/dl), HDL cholesterol (56.47 ± 4.59 to 24.89 ± 4.67 mg/dl), triglycerides (138.2±3.10 to 11.64±6.74 mg/dl) and total protein (7.11 ± 0.48 to 5.05 ± 0.11 g/l) (Table V).

After weaning with industrial Koutoukou, significant increases (p < 0.001) are recorded in both enzyme markers and serum metabolites. These values range from 0.79 ± 0.03 to 0.84 ± 0.25 g/l for urea, 143.7 ± 2.80 to 226 ± 7.51 UI for ALP and 11.64 ± 6.74 to 83.9 ± 2.45 mg/dl for triglycerides (Table VI).
<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (μM)</th>
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<tr>
<td>urea (g/l)</td>
<td>0.07 ± 0.03</td>
<td>0.16 ± 0.04</td>
<td>0.40 ± 0.10</td>
<td>0.45 ± 0.03</td>
<td>0.40 ± 0.07</td>
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<td>0.43 ± 0.12</td>
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<td>urea (g/l)</td>
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<td>0.38 ± 0.05</td>
<td>0.32 ± 0.03</td>
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<td>glucose</td>
<td>0.82 ± 0.11</td>
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<tr>
<td>creatinine (mg/dl)</td>
<td>0.81 ± 0.16</td>
<td>0.93 ± 0.14</td>
<td>0.88 ± 0.11</td>
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<td>creatinine (mg/dl)</td>
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<tr>
<td>creatinine (mg/dl)</td>
<td>0.96 ± 0.06</td>
<td>0.72 ± 0.10</td>
<td>0.67 ± 0.12</td>
<td>0.71 ± 0.06</td>
<td>0.74 ± 0.13</td>
<td>0.88 ± 0.07</td>
<td>0.89 ± 0.07</td>
<td>0.92 ± 0.04</td>
<td>0.81 ± 0.10</td>
</tr>
<tr>
<td>creatinine (mg/dl)</td>
<td>0.86 ± 0.06</td>
<td>0.86 ± 0.06</td>
<td>0.86 ± 0.06</td>
<td>0.86 ± 0.06</td>
<td>0.86 ± 0.06</td>
<td>0.86 ± 0.06</td>
<td>0.86 ± 0.06</td>
<td>0.86 ± 0.06</td>
<td>0.86 ± 0.06</td>
</tr>
<tr>
<td>triglyceride (mg/dl)</td>
<td>1.06 ± 0.06</td>
<td>0.96 ± 0.06</td>
<td>0.92 ± 0.15</td>
<td>0.91 ± 0.15</td>
<td>0.91 ± 0.15</td>
<td>0.92 ± 0.15</td>
<td>0.91 ± 0.15</td>
<td>0.92 ± 0.15</td>
<td>0.92 ± 0.15</td>
</tr>
<tr>
<td>triglyceride (mg/dl)</td>
<td>1.11 ± 0.33</td>
<td>0.93 ± 0.24</td>
<td>0.61 ± 0.28</td>
<td>0.55 ± 0.11</td>
<td>0.53 ± 0.08</td>
<td>0.52 ± 0.06</td>
<td>0.48 ± 0.38</td>
<td>0.47 ± 0.18</td>
<td>0.47 ± 0.08</td>
</tr>
<tr>
<td>triglyceride (mg/dl)</td>
<td>0.25 ± 0.05</td>
<td>0.28 ± 0.15</td>
<td>0.23 ± 0.08</td>
<td>0.25 ± 0.09</td>
<td>0.24 ± 0.08</td>
<td>0.29 ± 0.04</td>
<td>0.29 ± 0.04</td>
<td>0.29 ± 0.04</td>
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</tr>
<tr>
<td>triglyceride (mg/dl)</td>
<td>0.25 ± 0.12</td>
<td>0.10 ± 0.13</td>
<td>0.04 ± 0.09</td>
<td>0.22 ± 0.09</td>
<td>0.21 ± 0.15</td>
<td>0.08 ± 0.20</td>
<td>0.16 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>triglyceride (mg/dl)</td>
<td>0.19 ± 0.40</td>
<td>19.3 ± 3.85</td>
<td>19.5 ± 4.54</td>
<td>19.1 ± 4.39</td>
<td>19.2 ± 4.26</td>
<td>18.7 ± 4.68</td>
<td>19.3 ± 17.25</td>
<td>19.2 ± 4.26</td>
<td>19.1 ± 4.26</td>
</tr>
<tr>
<td>cholesterol (mg/dl)</td>
<td>18.9 ± 0.56</td>
<td>13.6 ± 0.49</td>
<td>14.2 ± 1.70</td>
<td>13.8 ± 0.49</td>
<td>14.1 ± 1.55</td>
<td>13.3 ± 2.14</td>
<td>13.2 ± 1.55</td>
<td>13.5 ± 0.75</td>
<td>13.5 ± 0.75</td>
</tr>
<tr>
<td>cholesterol (mg/dl)</td>
<td>119.3 ± 2.20</td>
<td>122.4 ± 3.11</td>
<td>119.3 ± 2.20</td>
<td>114.2 ± 2.56</td>
<td>113.2 ± 2.69</td>
<td>116.9 ± 3.67</td>
<td>113.9 ± 3.53</td>
<td>117.6 ± 4.67</td>
<td>114.7 ± 4.67</td>
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<tr>
<td>triglyceride (mg/dl)</td>
<td>119.4 ± 3.53</td>
<td>70.9 ± 8.32</td>
<td>68.8 ± 5.90</td>
<td>58.4 ± 8.06</td>
<td>56.3 ± 6.54</td>
<td>64.4 ± 5.98</td>
<td>61.2 ± 7.70</td>
<td>56.2 ± 12.32</td>
<td>48.5 ± 3.36</td>
</tr>
<tr>
<td>triglyceride (mg/dl)</td>
<td>7.55 ± 0.93</td>
<td>7.88 ± 1.25</td>
<td>7.41 ± 1.25</td>
<td>7.72 ± 0.97</td>
<td>7.89 ± 0.97</td>
<td>7.62 ± 0.45</td>
<td>7.39 ± 0.97</td>
<td>6.53 ± 0.39</td>
<td>7.38 ± 0.08</td>
</tr>
</tbody>
</table>

The values are expressed as means followed by the standard error on the mean (S.E.M.) in n = 5 rats per batch; the means assigned to the same letter in a column are not significantly different (p > 0.05); **= highly significant (p < 0.01) and ***= very highly significant (p < 0.001) when compared to the same batch of traditional Koutoukou; TK= (received 125 ml equivalent of traditional Koutoukou) and InK= (received 125 ml equivalent of industrial Koutoukou); IC= (received 250 ml equivalent of traditional Koutoukou) and InK= (received 250 ml equivalent of industrial Koutoukou); ALP= alkaline phosphatase; APAP= serotonin demethylase; ALAT= alanine transaminase; cholinesterase= HBD (High density lipoprotein).
**DISCUSSION**

Hematological parameters analysis of animals showed significant differences in both female and male rats at different doses administered for all *Koutoukou* types ingested. The leukocyte lineage composed of leukocytes, lymphocytes, monocytes and granulocytes showed significant variations according to doses, *Koutoukou* type consumed and animals sex. In female rats, the increase in leukocyte lineage is observed from 50 g PA, for improved and industrial *Koutoukou*. However, with traditional *Koutoukou*, there was an increase from 12 g PA before observing a significant decrease in this line from 50 g PA dose. For male rats, the increase in leukocyte lineage only appears at 80 g PA dose and the decrease from 12 g PA for three (3) *KTK* types. Thus, samples administered at 12 g PA of traditional *Koutoukou* and 50 g PA of improved *Koutoukou* disrupt the animal welfare system. The difference in results at leukocyte lineage level shows that the body is experiencing deterioration of immune system and that white blood cells are fighting this aggression. These results are agreement with those of Téhoua et al (2011) work. These authors found high values of leukocytes in animals consuming drinking water containing 12 % vol traditional *Koutoukou*. After *Koutoukou* weaning, the deterioration of immune system seems irreversible for animals treated with traditional *Koutoukou*; which shows an increase in leukocyte lineage level compared to animals treated with improved and industrial *Koutoukou* (reference). The stimulation of immune system leading to these increases could be explained by lesions or damage caused by heavy metals present in this drink (Alex et Lawrence, 2003).

The erythrocyte line consists of erythrocytes, hemoglobin, hematocrits, mean globular volumes (MGV), mean corpuscular hemoglobin rate (MCHR) and mean globular hemoglobin concentrations (MCHC). *Koutoukou* samples also resulted in significant difference in erythrocyte, hemoglobin, hematocrit and mean globular hemoglobin concentration (MCHC) levels depending on dose, drink type and animals genus for 28 days. In female rats, a significant decrease in erythrocyte lineage is observed at 80 g PA for traditional *Koutoukou* while at same dose an increase in erythrocyte lineage is observed for industrial *Koutoukou* (reference). But no significant changes were recorded at this lineage level after ingesting the improved *Koutoukou* (IK) in female rats. In male rats, only one increase is significant at 12 g PA for traditional *Koutoukou*. According to Bladé et al (2007), these markers are used to determine discrete anemia in alcoholics. Thus, the decrease in erythrocyte lineage to 80 g PA could mean that the content, morphology and osmotic fragility of erythrocytes would have been altered at this dose [16]. Thus, the traditional *Koutoukou* consumed at four (4) "rounds" (80 g PA.) would have caused anemia in female rats for 28 days. While no anemia was revealed in male rats during the entire experiment, regardless of dose consumed. These results corroborate those of Téhoua et al (2011) who found no decrease in erythrocyte counts at 12% vol dose of traditional *Koutoukou* in drinking water. After weaning in *Koutoukou*, no abnormalities were detected during the reversibility phase. This could be due to the reversibility of anemia contracted during the subacute toxicity phase. As for thrombocyte line (blood platelets), the results showed significant decreases from 50 g PA for traditional and industrial *Koutoukou* (reference) and 80 g PA for improved *Koutoukou* in male rats. According to Elwood et al (1993), alcohol consumption reduces platelet aggregation in part by increasing prostacyclin synthesis. Thus, this decrease may be due to thrombocytopenia observed at these doses.

Regarding serum biochemical parameters, differences are observed depending on dose, drink type and animal sex. Indeed, in female rats, serum enzymatic markers (PAL, ASAT, and ALAT) increase significantly from 12 g PA for traditional, improved and industrial *Koutoukou*. The latter has a decrease in alkaline phosphatase (ALP) to 80 g PA in female rats. In male rats, a decrease in ALP levels and increases in transaminases are observed for traditional *Koutoukou*. As for improved and industrial *Koutoukou*, significant increases are observed in ASAT from 12 g PA. The increase in transaminases could be explained by alcoholic hepatitis. This anomaly is believed to be caused by cell membrane damage due to direct toxic action of ethanol on mitochondria (Kintz et al, 2009). According to Nalpas et al (1986), an increase in this serum fraction would lead to liver disease in alcoholics. The decrease in ALP observed at 80 g PA would have led to hepatocellular insufficiency (Kew, 2000). Thus, the *Koutoukou* consumption could be the cause of liver damage. After weaning, the return to normal concentration (transaminase) is gradual. But an increase in ALP may be due to an increase in bone ALP, due to weanlings rats growth (Leveille-Webster, 2000). In terms of *KTK* effect on serum metabolites, HDL-cholesterol concentration increased for traditional and industrial *Koutoukou* from 12 and 50 g PA and decreased significantly to 80 g PA for three *KTK* types in females.

Thus, *Koutoukou* consumed in moderation could help prevent cardiovascular disease by increasing of good cholesterol (HDL) level. This would reduce the risk of blood clots forming in coronary arteries (vessels that supply the heart), which is phenomenon responsible for heart attacks. Our results are agreement with Sacco et al (1999), who found high concentrations of HDL-cholesterol in rats after moderate alcohol consumption (less than 30 g per day). According to Gaziano et al (1993), this increase is due to an increase in HDL-cholesterol types 2 and 3 and moderate alcohol consumption.

These results indicate that consuming small amounts of *Koutoukou* could prevent cardiovascular disease. However, chronic alcoholization with *Koutoukou* (CAK) would lead to decrease in triglyceridemia from 80 g PA. *Koutoukou* absorption would disrupt the metabolism of very light density lipoproteins (VLDL), high-density lipoproteins (HDL) and apoproteins A (Sugimoto et al, 2002).

In male rats, chronic alcoholization with *Koutoukou* (CAK) induces hypoproteinemia from 50 g PA for traditional *Koutoukou* and 80 g PA for improved *Koutoukou*. This could be due to decrease in release of hepatitis proteins due to an anomaly in microtubular system of hepatocytes. This results in accumulation of hepatitis proteins, which would contribute to formation of hepatoesegaly (Nalpas et Berthelot, 1984).
CONCLUSION

At end of this study, it appears that the moderate daily consumption of improved Koutoukou (50 g PA or 125 ml) induces beneficial effects on cardiovascular diseases. On the other hand, the Koutoukou doses greater than 50 g PA, would cause adverse effects with disruption (increase or decrease) of certain hematological and biochemical parameters (leukocytes, platelets, cholesterol, and transaminases) in rats. Improved Koutoukou is less toxic because of low and reversible effects induced when consumed in high doses compared to traditional Koutoukou. The production of improved Koutoukou according to techniques and quality standards developed in laboratory could be extended to trained traditional producers. This action project will make this traditional drink less harmful to consumer health in the future.

References

Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, (1993). Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk


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