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Hepatoprotective effects of koutoukou *Garcinia kola* seeds extract on the liver of the wistar rats in Abidjan, Cote d'Ivoire

Calixte Bahi[1], Bernadin Dro[2,4*], Allais Venance Ouéméla Ban[1], Bernard Nazaire Djyh[1], Joseph Allico Djaman[1,3]

[1]*Pharmacodynamic Biochemical Laboratory, Félix Houphouët-Boigny University, 22 BP 582 Abidjan 22, Côte d'Ivoire*

[2]*Jean Lorougnon GUEDE University, BP 150 Daloa, Côte d'Ivoire*

[3]*Institut Pasteur de Côte d'Ivoire, 01 BP 490 Abidjan 01, Côte d'Ivoire*

[4]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, 01 BP 1303 Abidjan 01, Côte d'Ivoire*

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Abstract

Garcinia kola is a plant commonly found in West and Central Africa. It has biochemical and physiological properties such as antibacterial, anti-hepatotoxic, hypoglycemic and antioxidant. It is increasingly used in association with other plants and/or certain alcoholic beverages such as koutoukou. Koutoukou is a spirit drink, classified in Groups 4 and 5 of mouthwash because of its high toxicity reported. This study was carried out to assess the safety of koutoukou made from *Garcinia kola* in the liver of Wistar rats. The alcoholic strength showed that the average alcoholic degree of koutoukou varied from 42.33°±4.04 to 62°±3.00. Phytochemical screening revealed the presence of flavonoids, saponosides, polyphenols, polyterpenes, sterols, Catechin tannins in koutoukou samples. Twenty-five Wistar rats divided in 5 groups of 5 rats each was used for 28 days experiment. Bodyweight gain and blood collection for bioassays were performed. Animals were sacrificed after 28 days, and livers were removed for histopathology tests. The weight of animals which received only koutoukou decreased significantly from D21 (160 ± 16) to D28 (120 ±17g). The values of ASAT, ALAT transaminases and Gamma-GT increased significantly during the same period in untreated rats indicating cytolysis. In rats treated with koutoukou and koutoukou from *Garcinia koutoukou* extract, these values decreased significantly indicating a cytoprotective effect of this extract. In the other groups, transaminases and Gamma-GT did not vary significantly during the experiment suggesting an absence of cytolysis. This study revealed that Koutoukou from *Garcinia kola* has a hepatoprotective effect on the liver.

* **Corresponding Author:** Bernadin Dro ✉ droberna@gmail.com

Introduction

Garcinia kola is a plant commonly found in West and Central Africa (Adedeji *et al.*, 2006). Its seed is elliptical or oval, hard and has a slightly aromatic odor (Mazi *et al.*, 2013). The plant is commonly called «bitter cola» or «male cola», respectively, because of its bitter taste or its claimed aphrodisiac effect. Its stem bark is used as a purgative in indigenous people in eastern Nigeria and the latex is applied to fresh wounds to prevent sepsis, helping to heal the wound (Uko *et al.*, 2001).

The biochemical and physiological properties such as antibacterial, anti-hepatotoxic, hypoglycemic and antioxidant of this plant are also reported (Okunji *et al.*, 2007). These activities are supported by its chemical composition in several secondary metabolites such as polyphenols, quinonic substances, tannins, alkaloids (Uko *et al.*, 2001). Moreover, it is increasingly used in association with other plants including *Moringa* and/or certain alcoholic beverages such as koutoukou. Koutoukou (KTK) is a spirit drink, classified in Groups 4 and 5 of mouthwash (Yao, 2009). This traditional alcohol is obtained in several ways, including the distillation of fermented plant products such as palm tree sap and sugarcane juice (Koffi *et al.* 2019). However, the traditional manufacturing procedure of this drink sometimes makes it toxic and harmful for humans (Yao *et al.,* 2011). In addition, when this traditional alcohol is not well prepared, its drink can affect vital organs such as liver. This study was carried out to assess the safety of *Garcinia kola* koutoukou extract in the liver of Wistar rats.

Material and methods

Animal material

The animal material consisted of albino rats of the Wistar strain with an average of 163 ± 15 g weight. They were kept in nonoxidative iron cages and acclimatized for 3 days under experimental pressure and temperature conditions at Polytechnic University of Abomey-Calavi in Benin. They had free access to water and were fed with pellets obtained from Benin Vet Services. The pet store was regularly cleaned to keep the animals healthy.

Plant material

The fresh seeds of *Garcinia kola* were collected in Anyama and Azaguié, localities around Abidjan, Cote d'Ivoire and identified by two botanists from National Floristic Center of Félix Houphouët-Boigny University, Abidjan, Cote d'Ivoire.

Technical equipment

The technical equipment consisted of screening equipment, extraction items (knives, spatulas, porcelain capsules, glass funnel, hydrophilic cotton, wattman paper), histological cutting equipment (electronic grinder, magnetic stirrer, sand bath, rotavapor, 50° C oven, alcoholmeter, UV spectrophotometer) and glassware (test tubes, Erlenmeyer flasks and test tubes,).

Solvents and chemical reagents

The extract was made of koutoukou, a drink traditionally made in Dabou, Abidjan agglomerate (Cote d'Ivoire). Phytochemical screening required various reagents depending on the chemical group. Stiasny's reagent was used to characterize catechic tannins. Stiansy's reagent, sodium acetate and ferric chloride were used for Gallic tannin characterization. Hydrochloric alcohol, magnesium shavings and isoamylic alcohol were used to detect flavonoids. Acetic anhydride and concentrated sulphuric acid were required for sterols and polyterpenes and a solution of 2% ferric chloride alcoholic for polyphenols. The Bornstraëgen reagent, chloroform, 2-fold diluted ammonia and hydrochloric acid were used for quinonic substances. The dragendorff, bouchardat and valser-mayer reagents and 60° alcohol were used to identify alkaloids.

Determination of alcoholic strength

The alcoholic strength of koutoukou samples was determined using an alcoholometer. A volume of 500 mL of koutoukou was poured into a test tube. Then, the alcoholometer was inserted into the test tube and the alcoholic strength of the Koutoukou extract was read at 20°C using a thermometer.

Extraction

The seeds of *Garcinia kola* were kept from sun and dust for 3 days, cut into small pieces using a knife and dried at room temperature. They were reduced in fine powder using an electronic grinder due to 2500 tr/min. Then, 100 g of the powder was added to 1000 mL of koutoukou in an Erlenmeyer flask. After adding the magnetic stirrer, the mixture was allowed to maceration for 24 hours and filtered using water-repellent cotton. The supernatant was evaporated using a rotavapor for evaporation and completely dried into an oven at 50°C (Zirihi *et al.*, 2003). The dried extract was used for the experiment.

Phytochemical screening

The identification of different chemical groups was carried out using techniques described by Ronchetti and Russo (1971), Hegnauer (1973) and, Wagner (1983), Békro *et al.* (2007).

Sterols and polyterpenes were investigated by evaporated 5 mL of each extract without charring on a sand bath. The residues were hot dissolved in 1 mL of anhydride acetic, and 0.5 mL of concentrated sulphuric acid was added to the triturate. The appearance of a purple or purple ring at the interphase turning from blue to green showed the presence of Sterols and polyterpenes.

Polyphenols were investigated by adding a drop of 2% ferric chloride alcoholic solution to 2 mL of Koutoukou extract. The presence of these component was shown by the appearance of a darker or darker blue-blackish or green color.

Flavonoids were highlighted using cyanidine reaction. Two (2) mL of each extract was evaporated. The residue was twice returned to 5 mL of diluted hydrochloric alcohol and 2-3 magnesium shavings were added to release heat induce an orange-pink or purplish coloration. The addition of 3 drops of isoamylic alcohol intensifies this coloring and confirms the presence of flavonoids.

Catechic tannins were determined using the Stiasny reagent. Five (5) ml of each extract was dried up in capsules and 5 ml of Stiasny reagent was added to the residue. The mixture was kept at 80°C for 30 min in a water bath and left cool. The observation of a precipitate in large flakes showed the presence of catechic tannins. For gallic tannins, the previous solution was filtered.

The filtrate was collected and saturated with sodium acetate. Then, the addition of 2% FeCl3 drops induced an intense blue-black coloration attesting the presence of gallic tannins.

Quinonic substances were investigated from the Bornstraëgen reagent. Two (2) mL of each of the 2 extracts were dry evaporated and the residue was crushed in 5 mL of 1/5 hydrochloric acid. The crusher was poured into a test tube and washed for 30 min. After cooling, it was extracted with 20 ml of chloroform and 0.5 mL of diluted Ammonia was added twice. A red or violet coloration showed the presence of quinones.

The alkaloids were characterized by the Bouchardat, Dragendorff and valser-mayer reagents. Six (6) mL of each solution were dried up in a test tube. The residue is taken up by 6 mL of alcohol at 60° and distributed in three (3) different tubes. In the first tube, 2 drops of the Dragendorff reagent were added to the alcoholic solution causing a precipitate or orange coloration. The addition of 2 drops of Bouchardat's reagent on the alcoholic solution in the second tube, caused a precipitate of reddish-brown coloration and indicates a positive reaction.

In the third tube, an addition of 2 drops of valser-mayer reagent leads to the appearance of a precipitate or a white-cream coloration reflecting a positive reaction.

For Saponosides, 15 mL of aqueous extract was poured into a test tube 15 cm high and 15 mm in diameter. Then the tube was shaken until foam appears, then left to rest for 10 mn. The persistence of the foam at a height of more than 3 cm confirmed the presence of Saponosides.

Animal testing

The experiment was carried out for 28 days.

The animals were fed with to the same diet and divided into 5 groups of 5 rats each. Group 1 was control and it has not been processed. The remaining 4 groups received koutoukou, silymarin (a hepatitis reference substance) and koutoukou extract from *Garcinia kola* at different doses. Table 1 presents the different group and the related treatment.

Body weight gain and blood collection for bioassays were performed recorded at day 0(D0), 2(D2), 7(D7), 14(D14), 21(D21), and 28(D28). After 28 days, the animals were sacrificed, their livers were collected and stored in 10% formalin for histopathology examination.

Blood sample collection and tests

Blood samples were collected using a hematocrit capillary tube through the Retro-orbital sinus in the orbital vein of rats anesthetized with chloroform. Biochemical analyses were performed using the kinetic method in accordance with the methodology of Sodipo *et al.*, (2012) using the RAYTO brand semi-automaton. Blood tests consisted of the determination of transaminases (Alanine aminotransferase (ALAT); Aspartate aminotransferase (ASAT) and Gamma-GT).

Histological slices

The histological slides involved a macroscopic observation of the whole livers and a microscopic examination of the livers of experimental animals. At the death of each animal and at the end of the observation time, the removal of the whole liver was immediately carried out. Macroscopic observation was qualitative and limited to the liver external characteristics such as color, consistency, and texture. Slices of liver were collected in each animal for microscopic study. They were fixed for 4 hours in sterile single tubes with DuboscqBrazil liquid (Alcoholic slug). Two types of staining were carried out. They were the classic topographic staining with haematoin-eosin and that of polysaccharides by the reaction to the periodic acid of Schiff (method of Hotchkiss-Mc Manus-Lillie) accompanied by background coloring of basophilic substances by Harris haematoxylin (Martoja and Martoja, 1967).

Statistical analysis

The collected data were analyzed and plotted to the Graphpad software. The parameter values were expressed as a mean plus or minus twice the standard error on the mean (Mean ± 2 SEM). In each group and for each parameter, the values of the different days (D2, D7, D14, D21 and D28) were compared to that of day zero (D0) by ANOVA, the Dunnett Multiple Comparisons Test. The threshold of significance has been set at 5%.

Results

Alcoholic degree

The alcoholic strength of the koutoukou samples used for the study were 40 and 47 for Koutoukou and the koutoukou extract from *Garcinia Kola,* respectively (Table 2). The average for the sample studied was = 42.33 ± 4.04.

Table 1. Groups of animals with received treatment.

Group	Number of rats	Treatment received	Doses
I	5	Distilled water (Control Group)	-
II	5	Koutoukou	50 µL/Kg BW
III	5	Silymarin (a hepatitis reference substance)	6 mg/kg BW
IV	5	Koutoukou from *Garcinia kola*	200 mg/kg BW
IV	5	Koutoukou from *Garcinia kola*	300 mg/kg BW

BW: Bodyweight.

Phytochemical screening of koutoukou extract

The phytochemical characterization showed that the major chemical component of Koutoukou extracted from *Garcinia Kola* seeds are polyphenols. In addition, Sterols and polyterpenes, flavonoids, catechic tannins, and saponosides are significantly

present. However, gallic tannins, and quinonic Substances were not found in the koutoukou extract scereened (Table 3).

Body weight

The bodyweight of animals varies from 160±24 to 185±18 g in the different groups at D0. Its value increases not significantly from D21 to 215±27 g at D28 in group IV and V which received the Koutoukou extract in *Garcinia kola* (Fig. 1). In group III which received the reference substance (Legalon, Silymarin) and the Group I (control) all animals were recovered.

The weight increased slightly from D8 to 163±13 and was almost stable until D28. However, the animals of the group II (koutoukou), have a significant decrease in their weight from D21 (160 ± 16 g) to D28 (120 ±17g).

Table 2. Alcohol Content of Koutoukou.

Samples	Ec_1	Ec_2	Ec_3
Ethanol (°)	40	47	40

Average = 42.33 ± 4.04; N = 3 (number of Koutoukou samples), Ec_1, Ec_2, Ec_3, Consumable samples taken from different sites in the suburbs of Abidjan.

ASAT transaminase

The ASAT transaminase ranges from 107±14 to 124±14 U/L in different groups to D0. Its value increased significantly from D21 to 175 ± 16 U/L at D28 in the control group (Fig. 2). ASAT transaminase decreased in groups II, IV and V, which received koutoukou (from *Garcinia kola* or not). However, this parameter did not vary significantly from D0 during the experimental period in other groups (I, III).

Evolution of ALAT transaminase during the experiment

ALAT transaminase decrased from 47±10 (in different groups) to 57±14 U/L (D0). It increases significantly at D28 in the treated and untreated koutoukou groups (88±18 U/L). In contrast, in the group II which received koutoukou and group V treated with koutoukou from *Garcinia kola* extract at 300 mg/Kg weight/day, ALAT transaminase decreased significantly at D28 (37 ± 8 U/L). In the other groups, ALAT transaminase did not vary significantly from D0 during the experimental period (Fig. 3).

Evolution of the Gamma GT

The average Gamma GT level (Fig. 4) varied from 124 to 134 U/L to D0 in the different groups. It increases significantly from D21 in groups II and IV. On Day 28, the Gamma GT averages varied from 57±6 to 23±4 U/L respectively in these groups. In the other groups, the gamma GT did not vary significantly from during the experimental period.

Table 3. Compounds of koutoukou extract of *Garcinia kola* seeds.

Extract	Metabolites									
	Sterols Polyterpenes	Polyphenols	Flavonoides	Tannins		Quinonic Substances	Alkaloids			Saponosides
				Gal	Cat		D	B	VM	
(EKGK)	++	++ +	++	-	++	-	++	++	-	++

+: Positive Test, ++: Significantly present, +++: Widely present, -: Negative test, E2 (EKGK) : Koutoukou extract from *Garcinia kola*, Gal: Gallic, Cat: Catechics, D: Dragendorff, B: Bouchardat, V-M: Valser-Mayer.

Histological slices

Fig. 5 shows the hepatic histology inaccurate acute and sub-chronic oral toxicity tests of koutoukou extract from *Garcinia kola* seeds. In the oral toxicity tests Aigüe (C) and Sub-chronic (D), the liver of rats fed with *Garcinia kola* koutoukou extract showed no visible atypia. Normal-looking hepatocytes (arrows) were well arranged in radial cords around the

centrilobular vein (VC). Venous sinusoids (S) are well visible, as seen in control rats (B). In untreated koutoukou gavees (A), there is an accumulation of numerous vacuoles in some hepatocytes (B).

Discussion

This study was carried out to evaluate the effect of koutoukou extract from *Garcinia kola* seeds on the liver of Wistar rats. The alcoholic strength of the koutoukou samples used for the study averaged was 42.33°±4.04. This result showed that this traditional drink produced by strict traditional practices has alcohol almost similar to corresponding to whisky which has alcoholic strength between 40 and 60° (Pequignot and Trémolières, 2020). Indeed, the koutoukou from the distillation of fermented sweet juices including sugar cane, sugar-water-yeast mixture, and palm oil sap, as in our case, has various alcoholic strength due to several factors like the origin of the raw material and the time of fermentation (Camara, 2002). These findings are consistent with Yao *et al.* (2012), who showed the incidence of alcoholism induced by 'Koutoukou' ranging from 42 to 72° on the vital organs of adult albino rats.

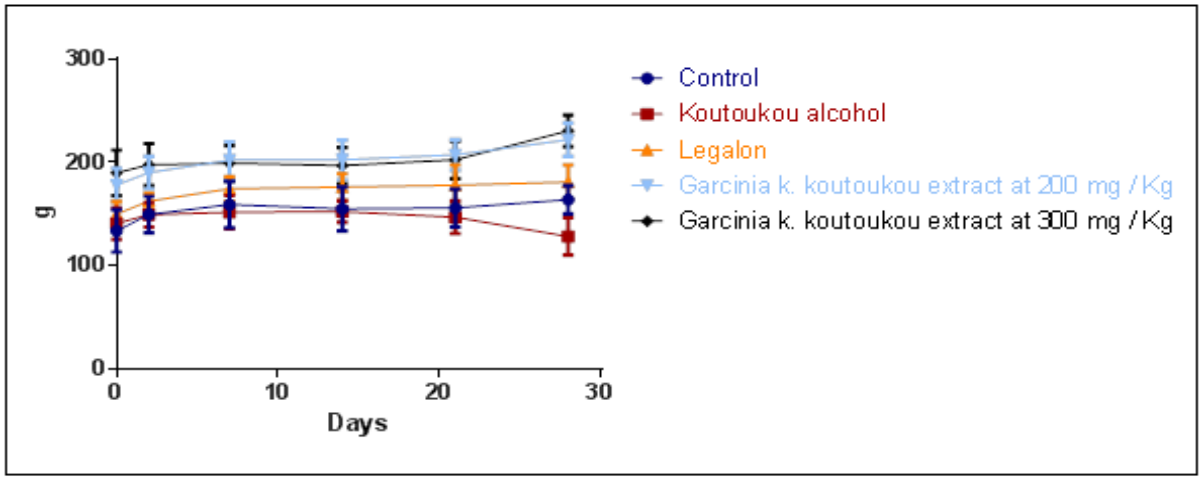

Fig. 1. Evolution of bodyweight in animals during the experiment.

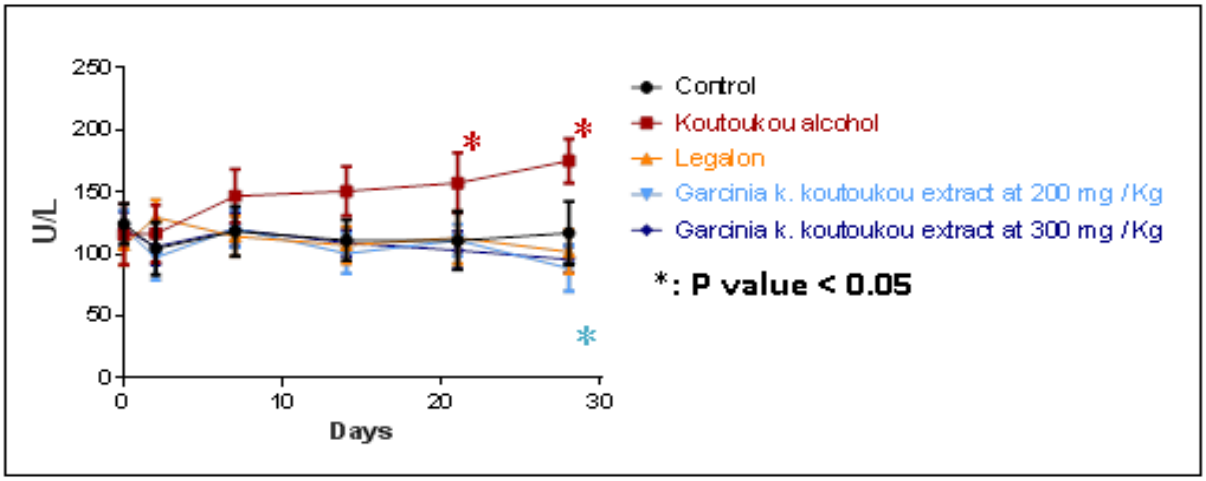

Fig. 2. Evolution of ASAT activity in different groups.

The phytochemical screening of the koutoukou extracted from *Garcinia kola* recorded several components as flavonoids, saponosides, polyphenols, polyterpenes, sterols, catechic tannins and alkaloids. These results are in line with Denen *et al.* (2015). These authors found the same secondary metabolites (saponosides, polyphenols, polyterpenes, sterols, tannins) in *Garcinia kola* ethanolic extract. These compounds confer several properties such as antihypertensive, anti-thrombic, antibacterial,

antiviral, antiallergic, antioxidant (Lagnika *et al.*, 2012), anti-tumoral and anti-hepatotoxic (Milane, 2004). At D0 the weight of the animals ranged from 160±24 to 185±18 g in the different groups. In the batches which received the extract from Koutoukou extracts from *Garcinia kola* at 200 and 300 g/pc, the weight value increased significantly from D21 to D28. This variation can be explained by the fact that the extract protects animals from the deleterious effects of koutoukou.

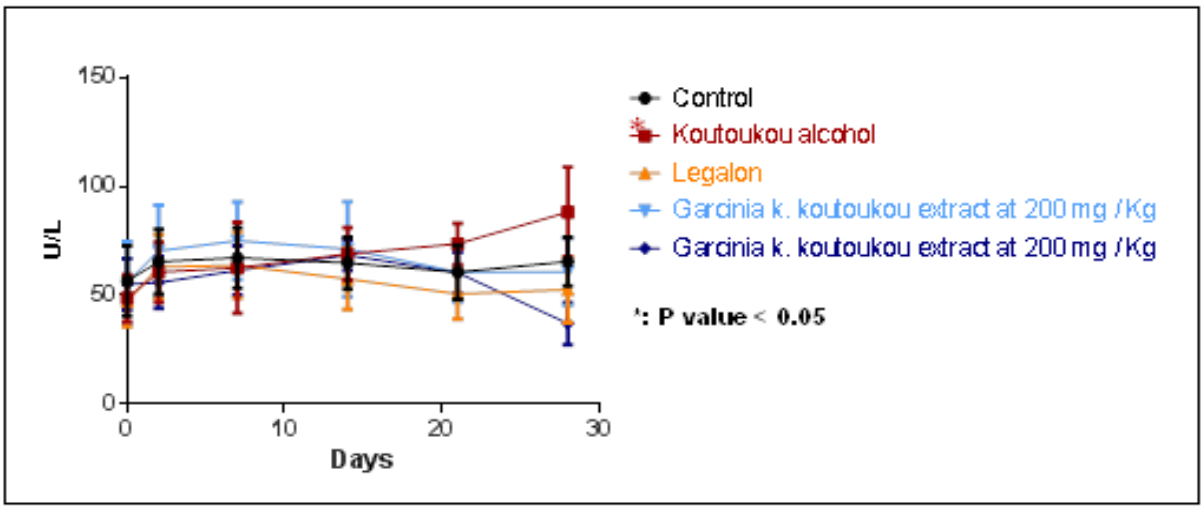

Fig. 3. Evolution of ALAT activity during the experiment.

These results are in line with Tchogou *et al.*, 2017, who showed the invariance of the weight of animals treated with *Cocos nucifera* extract. However, the animals which received the koutoukou only had their weights significantly reduced from D21 (160±16 g) to D28 (120±17 g). This decrease might reflect a homeostatic imbalance. These results might be due to diclofenac ingestion which is able to induce a significant decrease in weight reflecting abnormalities (Alabi *et al.* 2017). Toxicities tests were conducted according to OECD recommendations (423).

The parameters analyzed were transaminases (biochemical liver markers) and liver histology.

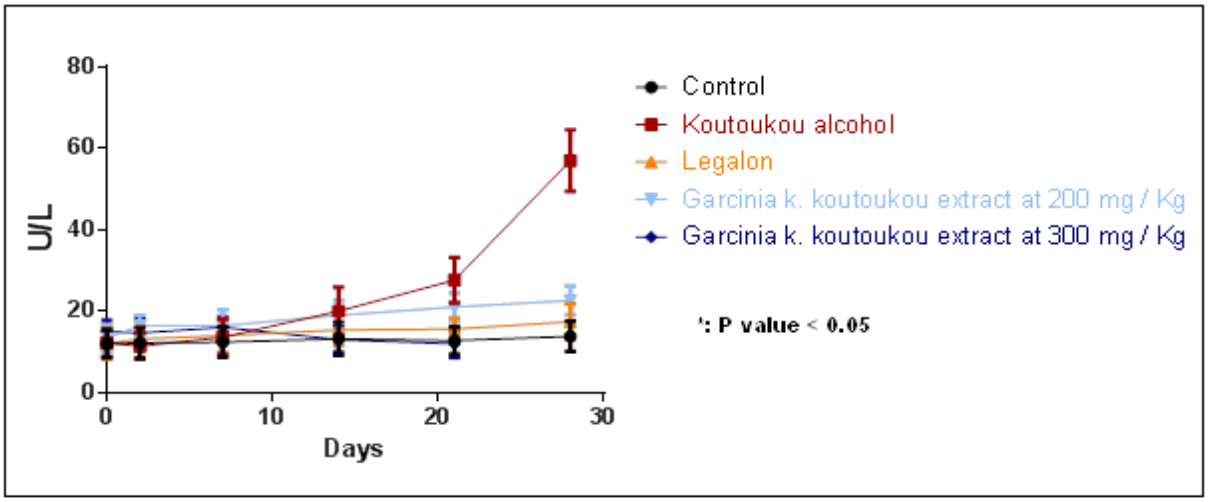

Fig. 4. Evolution of Gamma-GT activity.

The evolution of the rate of Transaminases (G- GT, ASAT, ALAT) made it possible to highlight the state of the liver. Transaminases increased significantly by D21 in groups IV (57 ± 6), V (175 ±16) and the control (88±18) at D28. These results showed that Koutoukou induces inflammation or liver cytolysis.

These alterations may support the increase observed in liver volume and the weight loss of the animal (Boussahel, 2011). Chemical compounds of koutoukou, including methanol, butanol, and heavy metals, are toxic (Koffi *et al.,* 2019), have deleterious effects on vital organs such as the liver (Yao, 2012). The histology of the hepatic parenchyma confirms this observation in rats fed with koutoukou and not treated. Indeed, there is an accumulation of numerous vacuoles in some hepatocytes (arrows), indicating cellular suffering. These results are like those of Yao (2012), who highlighted the toxic effects of koutoukou on the liver. Moreover, like the control group, a non-significant variation of the Transaminases from Do to D28 is observed at the level of the groups that received the koutoukou and treated with the extract with the koutoukou of *Garcinia kola* and legalon (Silymarine).

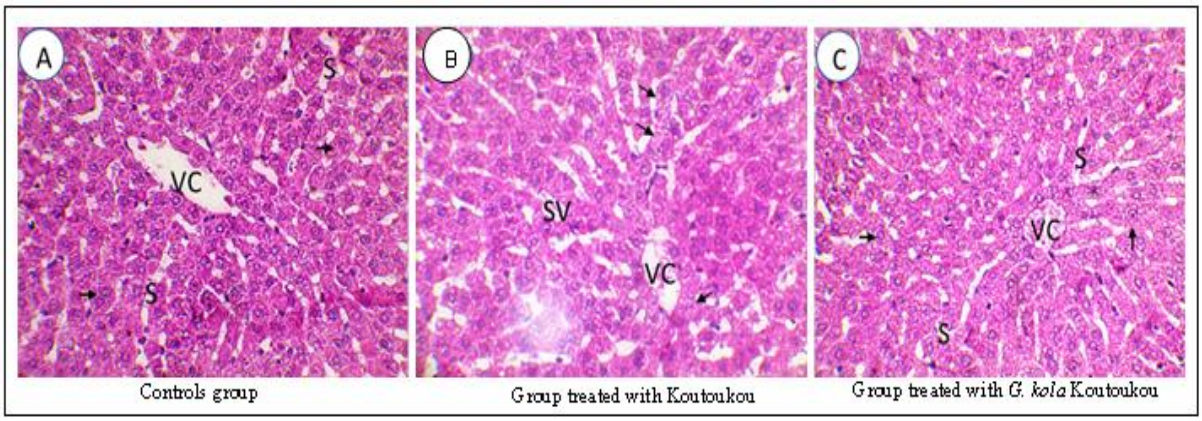

Fig. 5. Hepatic histology in acute and sub-chronic oral toxicity tests of *Garcinia kola* koutoukou extract (400 X).

This invariance of transaminases shows the safety of *Garcinia Kola*'s Koutoukou extract, hence its hepatoprotective effect. Denen *et al.* (2015) agreed, highlighting the effect of *Garcinia kola* ethanolic extract on biochemical markers of the liver. In addition, Alade and Ani (1990) showed the hepatoprotective effect of *Garcinia kola* extract during paracetamol-induced poisoning that resulted in non-variance of biochemical markers of the liver.

The histological section of the liver of the rats in the *Garcinia kola* koutoukou extract group has no cellular atypia. Normal-looking hepatocytes (arrows) that are well arranged in radial cords around the centrolobular vein and good visibility of venous sinusoids as recorded in control rats. Tchogou *et al.* (2017) demonstrated similar hepatoprotective effects of *Cocos nucifera* extract against anemia.

Conclusion

This study showed that the chemical groups present in the koutoukou extract are involved in biological activities such as hepatoprotection. The consumption of only Koutoukou by rats, resulted in deleterious effects on their liver. These effects resulted in increased transaminases and cellular necrosis of the hepatic parenchyma. Furthermore, the ingestion of Koutoukou-*Garcinia kola* by rats did not reveal any cellular atypia. *Garcinia kola* koutoukou extract is not harmful and has a hepatoprotective activity like Silymarin contained in legalon 70 mg.

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