www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2016; 5(12): 01-05

www.thepharmajournal.com
Received: 01-10-2016
Accepted: 02-11-2016

**AKA Francis Beranger Angelo**
Department of Nutrition and Pharmacology, University Felix Houphouet Boigny,
22 Bp 582 Abidjan 22, Ivory Coast

**Amonkan Kouao Augustin**
Department of Nutrition and Pharmacology, University Felix Houphouet Boigny,
22 Bp 582 Abidjan 22, Ivory Coast

**Kahou Bi Gohi Parfait**
Department of Animal Physiology, University Felix Houphouet Boigny,
22 Bp 582 Abidjan 22, Ivory Coast

**NENE BI Semi Anthelme**
Department of Animal Physiology, University Felix Houphouet Boigny,
22 Bp 582 Abidjan 22, Ivory Coast

**Correspondence**
**Aka Francis Beranger Angelo**
Department of Nutrition and Pharmacology, University Felix Houphouet Boigny,
22 Bp 582 Abidjan 22, Ivory Coast

# Acute toxicity of the aqueous extract of roasted and ground beans of *Coffea canephora robusta* in the wistar rat

**AKA Francis Beranger Angelo, Amonkan Kouao Augustin, Kahou Bi Gohi Parfait and NENE BI Semi Anthelme**

**Abstract**
The present study was conducted to assess the acute toxicity of the aqueous extract of roasted and ground beans of *Coffea canephora robusta* (aergb*Ccr*) in the rats. The HPLC study of caffeine in the aqueous extract showed a proportion of 7.5%. The evaluation of acute toxicity was done according to the guidelines of the Organization for Economic Cooperation and Development (OECD) 423 for testing chemicals. This study showed that the lethal dose 50 ($LD_{50}$) of this extract would be between 2000 and 5000 mg/kg of body weight (b.w). The relative weights of organs (kidneys, liver and heart) taken from rats at the end of the experiment did not change significantly. The biochemical and hematological parameters showed no significant variation. The aqueous extract of roasted and ground beans of *Coffea canephora robusta* has a relatively low acute toxicity.

**Keywords:** Extract, caffeine, *Coffea canephora robusta*, wistar rat, bio-tolerance, acute toxicity

## Introduction
The coffee beans represent the second most important commodity in the world after oil. The drink resulting from these coffee beans (coffee) is ranked in 4th position after tea, milk and beer [1]. It contains more than a dozen of bioactive compounds, mostly formed during the roasting process of the beans. Three of them are therein in high concentrations, and are important from a physiological point of view. It is the caffeine, diterpene alcohols and phenolic compounds known for their antioxidant effects [2]. The caffeine content of *Coffea canephora robusta* beans varies with the type of beans, method of roasting and brewing method [3]. Caffeine is known principally for its stimulant effects [4]. In the healthy adult, a small amount can increase alertness and concentration. In some individuals, consumption can disrupt the sleep [5]. The maximum amount of caffeine considered as no danger for the health, according to the recent report of the EFSA [6] (European Food Safety Authority) is 200 mg. In some people, quantities of 400 to 800 mg as a single dose may cause adverse biological effects such as insomnia, headache, nervousness, anxiety, tachycardia and tremor [7]. The adverse effects of caffeine and compounds present in coffee oil (cafestol and kahweol) would be reduced by polyphenols coffee [2]. It is therefore essential to take into account the total composition of coffee in different works. The aim of this study was to evaluate the acute oral toxicity of the aqueous extract of roasted and ground beans of *Coffea canephora robusta* in Wistar rats according to the OECD 423 classification system.

## Materials and methods
### Animal material
The Wistar albino female rats were used for this study. These rats, come from the Pasteur Institute Adiopodoumé (Abidjan, Ivory Coast), and are aged 8 to 10 weeks. They weigh between 100 and 120 g. These animals are raised in wire cages airy at 24 ± 3 °C, with a photoperiod of 12 hours and a humidity of 50% for 7 days prior to experimentation. The rats have ad libitum access to water and food.

### Vegetal material
The plant material used is roasted and ground beans of *Coffea canephora robusta*. These beans come from Ivory Coast.
The *robusta* coffee is grown in plains in wetter conditions such as parts of Central and West Africa, Southeast Asia and parts of Brazil [8]. The cherries of this coffee tree are round, small and thicker than those of Arabicas [9]. The grains of this variety are sold cheaper on the market and often enter into the composition of soluble coffees [10].

Robusta coffee accounts for just under 23.6% of world production [11].

**Preparation aqueous extract**

The aqueous extract is obtained by infusion of roasted and ground beans of *Coffea canephora robusta* (*Ccr*). A filter coffee machine of Philips brand Daily collection insulated stainless timer HD7479/20, was used to prepare coffee. The infusion was made with 30 g of roast and ground beans of *Ccr* in 175 ml of distilled water. The filtrate obtained is evaporated in an oven at a temperature of 60 °C. The crystals obtained are pulverized. The captured fine powder is kept refrigerated in sterile glass jars sealed. This technique yielded 3g of dry extract, corresponding to a yield of 10%.

**Extraction and Analysis of caffeine**

To extract caffeine, one gram of freeze-dried coffee ground powder is transferred to a volumetric flask of 50 ml. An amount of 35 ml of methanol is added and the resulting suspension was immersed in an ultrasonic bath for 10 minutes. The suspension is subsequently cooled to room temperature and filtered on Whatman paper N°4. The HPLC system (Shimadzu Corporation, Japan) consisting of a pump (Shimadzu LC-20A Liquid Chromatograph), a UV detector (Shimadzu SPD-20A UV spectrophotometry detector), and controlled by a computer (software) is used for assaying the proportion of caffeine in the extract. Qualitative analysis of caffeine is obtained by comparison of retentions times of the compounds eluted to the retention times of the reference solutions. The concentrations are determined from the average of the peak areas of the reference solutions. Caffeine content being the average of 3 tests, the concentration of caffeine in the extract is given by the following formula:

$$SC = \frac{S\ area\ \ x\ \ CW}{W\ area}$$

*SC*: Sample Concentration
*CW*: Concentration Witness
*W* area: peak area Witness
*S* area: peak area Sample

**Evaluation of the acute toxicity**

**Administration of aqueous extract**

A limit test is conducted at a dose of 2000 mg/kg of b. w over a period of 2 weeks. Another dose of 2000 mg/kg of b. w is administered to a second set of 3 rats for confirmation during the same time. Finally a dose of 5000 mg/kg of b, w is administered to a third rat batch. The control group (4th batch) receives distilled water. The different solutions are administered orally using a stomach tube.

**Effect of the extract on the general appearance of rats**

The observations were carried on various manifestations, such as diarrhea, lethargy, aggressiveness, irritability, drowsiness and the mortality.

**Effect on weight**

The weight of each animal is determined before administration of aqueous extract of roasted and ground beans of *Ccr* and then every third day. The weight changes are calculated and recorded. At the end of the study the animals were sacrificed and organs such as the kidney, liver and heart were removed and weighed.

**Effect on biological parameters**

**Biochemistry**

The determination of these biochemical parameters was carried out by different enzymatic methods according the data sheet (Bio Systems). Blood samples collected in anticoagulant-free tubes are centrifuged at 3000 revs/ min for 10 min. The serum are then stored at -20 °C. They are used to respectively assaying alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by the methods of coupled reactions of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) and measured by spectrophotometer at 340 nm [12, 13, 14]. The creatinine is measured by the Jaffe [15, 16] compensated method. Glucose is assayed by the method of Trinder [17] and measured in a spectrophotometer at 500 ± 20 nm. Finally, the determination of urea is done by the urease kinetic method and measured in a spectrophotometer at 340 nm [18, 19].

**Hematologic and leukocyte formula**

Blood samples are collected in tubes containing anticoagulant (EDTA) and used to determine hematological parameters and differential count using a PLC (Beckmann Coulter Act Diff 2) of blood count with a volumetric system associated to a photometric system for counting different blood cells in 60 seconds.

**Statistical Analysis**

The statistical analysis was performed using the Graph Pad Prism 5 software (San Diego, USA). The analysis of variance ANOVA (One-way ANOVA) followed by the Tukey-Kramer test was used for comparison of results. The difference is considered statistically significant when $P < 0.05$.

**Results and discussion**

A reference solution is used to calculate the caffeine content in aergb*Ccr*. The surface corresponding to the caffeine content in the reference solution is shown in Figure 1. The caffeine content in the aergb*Ccr* is 7.5% (Figure 2). This content is greater than those found in the roasted coffee beans of robusta by Y. Chu-F[20] (2.4-2.5%), by Debry [21] (2%) and [Clarke [22]; Viani [23]; Debry [24]; Ky *et al*. [25]; Vasconcelos *et al*. [26]] which is (1.2-2.6%). This high proportion of caffeine may be due to the origin of the grain, the time, and roasting temperature of grains on the one hand and to the method of preparing coffee[3]the other hand.

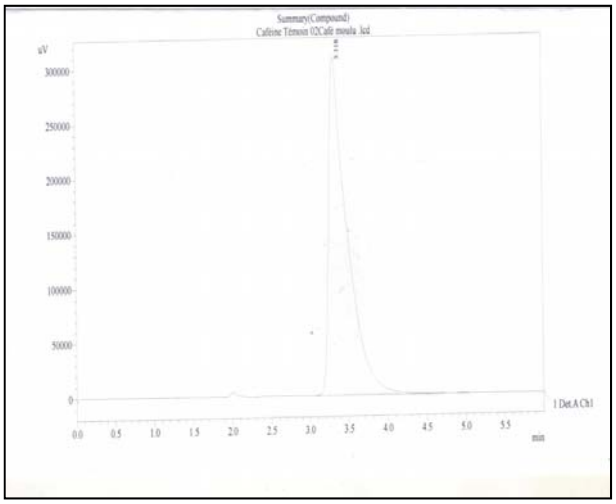


**Fig 1:** Witness the caffeine dosage by HPLC

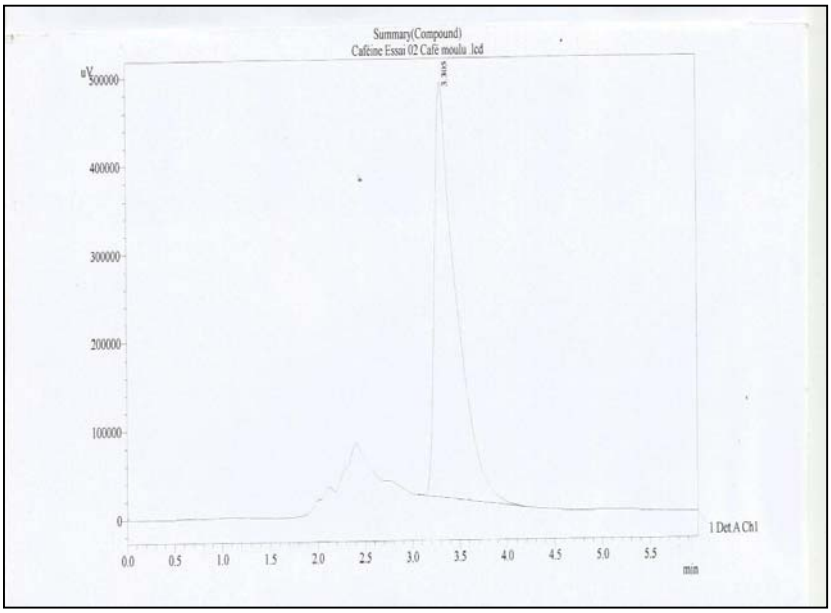


**Fig 2:** The caffeine by HPLC assay in the aergbCcr

During the study of acute toxicity, the various clinical signs observed in rats after administration of aergb*Ccr* at the doses of 2000 and 5000 mg/kg of b.w are presented in Table I.

**Table 1:** Different clinical signs observed after a single gavage of aergb*Ccr* at the doses of 2000 and 5000 mg/kg b.w.

| | **30 Minutes** | | **1 Hour** | | **4 Hours** | | **8 Hours** | | **24 Hours** | | **48 Hours** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose in mg/kg of b.w | 5000 | 2000 | 5000 | 2000 | 5000 | 2000 | 5000 | 2000 | 5000 | 2000 | 5000 | 2000 |
| diarrhea | no | no | no | no | no | no | no | no | no | no | no | no |
| Lethargy | no | no | no | no | no | no | no | no | no | no | no | no |
| Aggressiveness | no | no | no | no | no | no | no | no | no | no | no | no |
| Excitability | yes | yes | yes | yes | no | no | no | no | no | no | no | no |
| Drowsiness | no | no | no | no | yes | yes | no | no | ------ | no | ----- | no |
| Death | no | no | no | no | no | no | no | no | yes | no | yes | no |

During the first hour after administration of the extract, the rats of experimental batches were stirred in the cages. This excitation is due to caffeine which increases, neuromuscular transmission and increases the neural excitability reducing the discharge threshold of motor neurons and/or by reducing the inhibitory effect of adenosine on the discharge timing [21]. Gradually, as time elapses, the rats were less agitated. After four hours, a general state of drowsiness sets in. This drowsiness resulting in difficulty moving. They could be attributed to the sedative and anesthetic properties, and especially psychoactive of alkaloids [27] contained in the aqueous extract. Two deaths occurred at the dose of 5000 mg/kg of b. w after 24 hours and 48 hours. There are no deaths related to the administration of the extract at the dose of 2000 mg/kg of b. w is recorded during the test. This indicates that the aergb*Ccr*, has a lethal dose 50 ($LD_{50}$) of between 2000 and 5000 mg/kg of b. w. Bringing back these doses at the content of caffeine in the aergb*Ccr* (7.5% caffeine), the lethal dose 50 ($LD_{50}$) would be between 150 and 375 mg/kg of b.w of caffeine. These results are in conformity with those of Josef M. Petres [28]. who found a lethal dose 50 ($LD_{50}$) of 192 mg/kg of b.w of caffeine in the rat. Referring to the globally harmonized OECD [29] classification system, the aergb*Ccr* is classified as danger substances 5. Compared to the OECD, aergb*Ccr* is biotolerant according to Alain [30] toxicity scale.

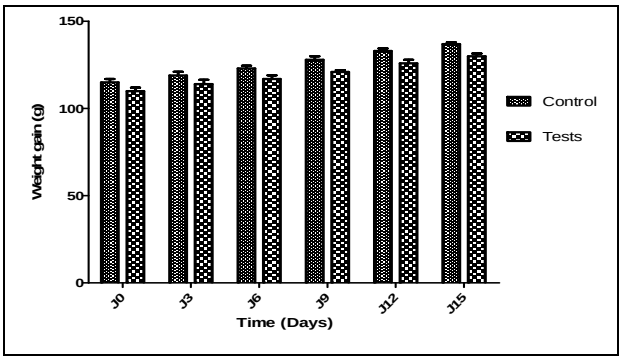


**Fig 3:** Body weight gain for the variation in the animals having received the aergbCcR to 2000 mg/kg b.w (experimental rat) and distilled water (control rats).

**Table 2:** Effect of aergb*Ccr* to 2000 mg/kg of b.w administered orally on the organs in the rats.

| **Organs (g)** | **Control rats** | **Experimental rats** |
|---|---|---|
| Liver | 4.08±0.41 | 4.17±0.28 |
| Heart | 0.43±0.26 | 0.47±0.14 |
| Kidney | 0.64±0.025 | 0.67±0.02 |

All animals in the experiment had a weight gain during the study period (*p>0, 05*) both in the control group and in the treated batch.

**Table 3:** Effect of aergb*Ccr* to 2000 mg/kg b.w administered orally on biochemical parameters in the rats.

| parameters | Control rats | Experimental rats |
|---|---|---|
| Urea (g/l) | 0.19 ± 0.01 | 0.18 ± 0.02 |
| Blood sugar (g/l) | 0.95 ± 0.16 | 1.05 ± 0.20 |
| Creatinine (mg/l) | 8.67 ± 0.95 | 7.67 ± 1.04 |
| Transaminases ASAT (U/L) | 35.33 ± 3.06 | 32 ± 4.57 |
| Transaminases ALAT (U/L) | 39.33 ± 1.67 | 37.67 ± 2.62 |

Biochemical parameters of rats treated with aergb*Ccr* have not significantly change during the two weeks of experiment.

**Table 4:** Effect of aergb*Ccr* at 2000 mg/kg b.w administered orally on formula leukocyte parameters in the rats.

| parameters | Control rats | Test rats |
|---|---|---|
| Polymorphonuclear neutrophils (%) | 14.0 ± 2.65 | 12.67 ± 1.15 |
| Polymorphonuclear Eosinophils (%) | 1.67 ± 0.58 | 1.33 ± 0.58 |
| Polymorphonuclear basophils (%) | 00 ± 0.00 | 00 ± 0.00 |
| Lymphocytes (%) | 79.33 ± 3.08 | 81 ± 2.00 |
| Monocytes (%) | 5 ± 1.00 | 5 ± 1.00 |

The parameters of the leukocyte formula in rats treated with aergb*CcR* did not change significantly during the test.

**Table 5:** Effect of aergb*Ccr* to 2000mg/kg b.w administered orally on hematological parameters in the rats.

| parameters | Control rats | Experimental rats |
|---|---|---|
| Leukocytes ($10^9$/L) | 8.43 ± 2.21 | 10.53 ± 1.77 |
| Erythrocytes ($10^{12}$/L) | 6.93 ± 0.63 | 7.17 ± 0.24 |
| Hemoglobin (g/dl) | 13.07 ± 0.57 | 13.73 ± 0.40 |
| Hematocrit (%) | 37.10 ± 1.81 | 39.47 ± 1.40 |
| MCV (fl) | 51.60 ± 1.15 | 54.37 ± 1.30 |
| MCH (pg) | 17.87 ± 0.75 | 18.57 ± 1.07 |
| MCHC (g/dl) | 34.40 ± 1.02 | 34.67 ± 0.85 |
| Platelets($10^9$/L) | 728 ± 109 | 753 ± 127 |

In rats treated with the aqueous extract of roasted and ground beans of *Ccr*, these parameters were not significantly changed during the test.

The aqueous extract of roasted and ground beans of *Coffea canephora robusta* has no effect on biochemical and hematological parameters. Normal values of urea and creatinine suggest that this extract did not alter the structure and renal functions. Indeed, studies have shown that these values are high when kidney are damaged[31].In addition, the transaminases (ALT) and (AST) are not disturbed during this experiment. This shows that the liver and to a lesser degree the muscles have not been affected. Given therefore to the results obtained, the aqueous extract of roasted and ground beans CcR is non-toxic for most parameters tested, therefore has no influence on the quality and function of blood and the organs vital as the liver and kidneys. The aergb*Ccr* to 2000mg/kg b.w, caused no changes in hematological and leukocyte levels.

## Conclusion

This study aims to determine the acute toxicity of the aqueous extract of roasted and ground beans of *Coffea Canephora robusta* in the Wistar rat. The value of the lethal dose 50 ($LD_{50}$) in this study is between 2000 and5000 mg/kg b.w. The administration of aqueous extract of roasted and ground beans of *Coffea Canephora robusta* has no significant effect on the physiology organization which caused no change in biochemical and hematological parameters of the animals. Studied at the dose of 2000 mg / kg b.w. These results demonstrate the importance and the use of this drink (coffee) in the world.

## References


1. Wolf A. A short history of beverages and how our body treat them. Obesity Reviews 2008; 9:151-64.
2. Bonita JS, Mandarano M. Coffee and cardiovascular disease: *in vitro*, cellular, animal, and human studies. Pharmacol Res 2007; 55(3):187-98.
3. http://www.passeportsante.net/fr/Nutrition/EncyclopedieAliments/Fiche.aspx?doc=cafe_nu]. [Visité le 25 mai, 2016].
4. Philip P, Taillard J. The effects of coffee and napping on nighttime highway driving: a randomized trial. Ann Intern Med. 2006; 144(11):785-91.
5. http://www.Coffeeandhealth.org.
6. European Food Safety Authority (EFSA). EFSA panel on dietetic products, nutrition and allergies (NDA). Scientific Opinion on the safety of caffeine. EFSA Journal 2015; 13:4102.
7. Fredholm BB. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 1999; 51:83-133.
8. http://www.ico.org/prices/m1.htm I.C.O (2011)
9. Lambard Ch. « Le café de la terre à la tasse». Centre de Caféologie. Bordeaux (France). 2003.
10. FAO. (Food and Agriculture Organisation) Manual on the application of the HACCPsystem in mycotoxin prevention and control. Food and Nutrition 2001, 73. ISSN 0254-4725.
11. Costro L, Mourad AL, Oliveira PAPLV, Braddini JPOA, Kletecke RM. Environmental profile of Brazilian green coffee. International Journal J.L.C.A. 2006; 11:16-21.
12. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C, Part 5. Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clin Chem Lab Med. 2002; 40:725-733.
13. IFCC reference procedures for measurement of catalytic concentrations of enzymes: corrigendum, notes and useful advice. Clin Chem Lab Med. 2010; 48:615-621.
14. Gella FJ, Olivella T, Cruz Pastor M, Arenas J, Moreno R, Durban R *et al*. A simple procedure for routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. Clin Chim Acta 1985; 153:241-247.
15. Weber JA, Van Zanten AP. Interferences in current methods for measurements of creatinine. Clin Chem 1991; 37:695-700.
16. Peake M, Whiting M. Measurement of serum creatinine-current status and future goals. Clin Biochem. 2006; 27:173-184.
17. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem. 1969; 6:24-27.
18. Talke H, Schubert GE. Enzymatische harnstoffbestimmung in blut und sermimoptischen test nach Warburb. Klinische Wochenschrift. 1965; 43:174-175.
19. Gutmann I, Bergmeyer HU. Methods of enzymatic Analysis, edBergmeyer HU, Academic Press, NY, 1974; 4:1794-1798.
20. CHU YF. «Coffee: Emerging Health Effects and Disease Prevention ». Ames, Iowa: Wiley-

Blackwell, 2012, 352.
21. Debry G. Le café et la santé. 1993.
22. Clarke RJ. Quality Control in the Food Industry (Coffee Technology), 2è ed. Academic Press, London, 1987, 4.
23. Viani R. The composition of coffee. In: S. Garattini Ed. Caffeine, Coffee and Health. Raven Press New York. 1993, 17-41
24. Debry G. Le café. Sa composition, sa consommation, ses incidences sur la santé » Centre de Nutrition Humaine. 1995.
25. Ky CL, Louarn J. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild Coffeaarabica L. and C. canephora P. accessions. Food Chemistry 2001; 75:223-230.
26. Vasconcelos ALS, Franca AS. A comparative study of chemical attributes and levels of amines in defective green and roasted coffee beans. Food Chemistry 2007; 101:26-32.
27. LUIS BLANC F, CACERES J, SACO-MENDES S. Cure magique par les guerisseurs andins : le recours a des plantes psycho actives. Psychotropes, T. IV, n°3, 1988, 53-58. (somnolence).
28. Josef M. Peters, « Factors Affecting Caffeine Toxicity: A Review of the Literature », The Journal of Clinical Pharmacology and the Journal of New Drugs, nº 3, 1967; 7:131-141.
29. OCDE. Sous-comité d'experts du système général harmonisé de classification et d'étiquetage des produits chimiques : dangers pour la santé et l'environnement – toxicité aiguë. UN/SCEGHS/2/INF. 2001, 11-13.
30. ALAIN P. *Les médicaments*. 3eme ed. CdM 2000, 500.
31. Coulibaly FA, Djyh BN, Guédé-Guina F, Djama AJ. Evaluation des marqueurs sériques des reins chez les lapins traités par Phyllanthus amarus (Euphorbiaceae). Ann. Botaniq. Af. Ouest. 2007; (5):69-78.