

Effect of aqueous extracts of *Curcuma longa* and *Syzygium aromaticum* on some biological parameters of non-diabetic rats

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Abstract

Introduction: Medicinal plants are used to cure various kinds of illnesses. This work aimed to evaluate the metabolic property of *Curcuma longa* and *Syzygium aromaticum*, two medicinal spices used by traditional therapists in Benin.

Study methods: The aqueous extracts of *Curcuma longa* rhizome and the *Syzygium aromaticum* seed were screened. Single dose 2000 mg/kg body weight acute oral toxicity tests were performed in female Wistar rats. Animal weight, serum creatinine, ALT transaminase and hemoglobin level were determined on day 0 and then on day 14. Histological sections were taken from the liver, kidneys and spleen. For the metabolic activity test, the extracts were administered to rats at a concentration of 300 mg/Kg of body weight for 21 consecutive days. The blood tests performed were blood sugar, triglycerides and cholesterol.

Results: The phytochemical screening revealed the presence of tannins, mucilages and flavonoids in both species. In terms of toxicity, there were no deaths. These extracts did not significantly change rat body weight, creatinine, ALT, hemoglobin, and did not alter the histology of the liver, kidneys, and spleen. Regarding metabolic activity, *Curcuma longa* extract significantly increased transiently total cholesterol and triglyceride.

Conclusion: *Curcuma longa* and *Syzygium aromaticum* were not acutely toxic and *Curcuma longa* transiently increased cholesterolemia and triglyceridemia. Any use of these plants should take into account their metabolic properties.

Keywords: *Curcuma longa*, *Syzygium aromaticum*, cholesterol, triglycerides, toxicity.

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1. Introduction

Medicinal plants are used around the world to treat various kinds of illnesses [1]. Almost 80% of the African population uses medicinal plants for health needs because of their effectiveness [2,3]. Various secondary metabolites contained in these plants are thought to be responsible for their properties [4].

The present study was initiated with the aim of evaluating some metabolic properties of two spices, *Curcuma longa* and *Syzygium aromaticum*, in female rats of wistar strains.

2. Material and methods

2.1 Plant material

The rhizomes of *Curcuma longa* and the seeds of *Syzygium aromaticum* were collected respectively in Savalou and Cotonou. The organs were dried in the laboratory at room temperature (20 °C - 25 °C) out of direct sunlight and moisture for 14 days. They were then reduced to powder and stored in suitable bottles. Maceration was used to obtain the various aqueous extracts. The extracts once filtered were evaporated at 60 °C with an evaporator [5].

2.2 Ethics statement

The study was approved by the National Research Ethic review Boards of Benin. The Wistar rats used in this study were handled according to the institutional animal safety guidelines (Animal facility, National School of Applied Biosciences and Biotechnologies, National University of Sciences, Technologies, Engineering and Mathematics, Benin).

2.3 Animal material

The animal material consisted of female albino rats of Wistar strains weighing between 113 and 163g. There were sixteen (16) rats from the ISBA animal facility. These rats were acclimatized to ambient conditions in the animal house of the Experimental and Clinical Biology laboratory at the National School of Applied Biosciences and Biotechnologies in Benin. They had access to sufficient food and water. They were lit for 12 hours a day and were put in spacious cages. The cage was cleaned regularly and the water was renewed very often. The behavior of the animals was observed during the two weeks of acclimatization.

2.4 Identification of secondary metabolites

The metabolites were identified by coloring and precipitation reactions specific to each metabolite family [6].

2.5 Acute oral toxicity

An acute toxicity test (AOT) was performed as recommended by the Organization for Economic Co-operation and Development guideline 423 for the testing of chemicals [7]. Three groups of rats were formed for, namely the control group and the two tests groups. Each group consists of three female Wistar rats. Each animal in the control group received by force gavage and in a single dose of distilled water and the animals in the tests groups received respectively by force gavage and in a single dose 2000 mg / kg body weight of the aqueous extract of

Curcuma longa and *Syzygium aromaticum*. Animals were observed carefully for four hours and then daily for 14 days. They were weighed and the blood was collected by orbital puncture at the start of the experiment and then after 14 days [5] the following blood tests were performed. Serum creatinine for the exploration of kidney function. ALT was assayed for hepatic function. The hemoglobin level was determined [5].

2.6 Histology

At the end of the experiment, the animals were dissected. The liver, the kidney and the spleen were removed, fixed in 10% buffered formalin, and embedded in paraffin. The specimens sections (5 µm) were stained with hematoxylin and eosin (H&E), following a standard protocol [8]. The pictures were taken at 400X magnification.

2.7 Action of extracts on certain biological parameters

Two batches of rats were formed, namely batch 1 for *Curcuma longa* and batch 2 for *Syzygium aromaticum*. Each batch consisted of three female Wistar rats. The animals in the batches received by forced gavage the aqueous extract corresponding to their batch at 300 mg / kg of body weight, daily for 21 consecutive days. They were weighed and blood was taken by orbital puncture at the start of the experiment (D0), on day fourteenth (D14) and then on day twenty-one (D21). The blood tests performed are blood sugar, triglycerides and cholesterol.

2.8 Statistical analysis

The results of the biological parameters were expressed as the mean \pm 2 times the standard error on the mean (Mean \pm 2 SEM). For each parameter the results of days 14 or days 21 are compared with those of Day 0 using the Dunn's multi-comparison test or Mann Whitney test. The significance level was set at 5%. The graphs were drawn using Graphpad software.

3. Results

3.1 Phytochemical composition of spices

Table 1 showed the phytochemical screening of the two spices. The screening revealed the presence of catechetal tannins, flavonoids, anthocyanins, alkaloids, mucilages, quinone derivatives, free antracenes, C-heterosides and O-heterosides in the rhizomes of *Curcuma longa*. As for the seeds of *Syzygium aromaticum*, the secondary metabolites found were gallic tannins, flavonoids, leuco-anthocyanins, mucilages, steroids and quinone derivatives.

Table 1: Phytochemical screening of the two spices

CHEMICAL GROUPS	<i>Curcuma longa</i>	<i>Syzygium aromaticum</i>
Catechetical tannins	+	-
Gallic tannins	-	+
Flavonoids	+	+
Leuco-Anthocyanins	-	+
Leuco-Anthocyanins	+	-
Alkaloids	+	-
Reducing compounds	-	-
Mucilage	+	+
Saponoside	-	-
Cyanogenicderivatives	-	-
Triterpenes	-	-
Steroids	-	-
Coumarins	-	-
Quinone derivatives	++	+
Free antracenic	++	-
C-Heterosides	++	-
O-Heterosides	++	-
Cardiotonic derivatives	-	-
Quinone derivatives	++	+
Free antracenic	++	-
C-Heterosides	++	-
O-Heterosides	++	-
Cardiotonic derivatives	-	-

3.2 The aqueous extract of *Curcuma longa* and *Syzygium aromaticum* were not acutely toxic

Table 2 and 3 showed the physical, biochemical and hematological assessments of the acute oral toxicity test of *Curcuma longa* and *Syzygium aromaticum* extracts respectively.

The average weight of the rats was 143 ± 19 g or 135 ± 21 g on D0 and did not significantly change on D14, after treatment with the extracts at 2000 mg/Kg of body weight, indicating no physical disturbances. The mean creatinine level was 9.4 ± 2.2 mg/mL or 9.1 ± 0.95 mg/mL

rats on D0 and did not significantly increase on D14, after treatment with the extracts at 2000 mg/Kg of body weight, suggesting no disturbance in kidney function. The mean level of ALT transaminase was 25 ± 6 U/L or 32 ± 7 U/L in rats on D0 and did not significantly increase on D14, after treatment with the extracts at 2000 mg/Kg of body weight, suggesting no hepatic cytolysis. The average hemoglobin level was 13.2 ± 0.8 g/dL or 14.3 ± 0.8 g/dL on D0 and did not decrease on D14, after treatment with the extracts at 2000 mg/Kg of body weight, indicating that the extracts did not create anemia.

Table 2: Physical, biochemical and hematological results in the acute oral toxicity test of *Curcuma longa* extracts

Parameters	Average at D0	Average at D14	P-value
Body weight (g)	143 ± 19	165 ± 16	0.4
Creatinine (mg / L)	9.4 ± 2.2	11.1 ± 2.02	0.2
Transaminase ALT (U / L)	25 ± 6	26 ± 8	0.8
Hemoglobin level (g / dL)	13.2 ± 0.8	14.7 ± 0.4	0.1

Table 3: Physical, biochemical and hematological results in the acute oral toxicity test of *Syzygium aromaticum* extract

Parameters	Average at D0	Average at D14	P-value
Body weight (g)	135 ± 21	151 ± 15	0.3
Creatinine (mg / L)	9.1 ± 0.95	6.6 ± 2,02	0.1
Transaminase ALT (U / L)	32 ± 7	40 ± 6	0.2
Hemoglobin level (g / dL)	14.3 ± 0.8	14.5 ± 1.2	0.9

3.3 The aqueous extracts of *Curcuma longa* and *Syzygium aromaticum* did not induce liver, kidney and splenic damage.

The liver of rats did not show atypia in the acute oral toxicity test with extract of *Curcuma longa* (Figure 1B) and *Syzygium aromaticum* (Figure 1C). The hepatocytes

(arrows) were neatly arranged in cords separated by the venous sinusoids (S) as in the controls (Figure 1A). Centrilobular (CV) veins were characteristic.

The renal parenchyma of rats in the acute oral toxicity test of *Curcuma longa* extract (Figure 1E) and *Syzygium aromaticum* (Figure 1F) kept the normal

architecture of the controls (Figure 1D). The glomeruli (G), proximal and distal tubules (T) did not show atypia.

The splenic architecture of the rats was not altered in the acute oral toxicity test of *Curcuma longa* extract (Figure 1H) and *Syzygium aromaticum* (Figure 1I). In the

white pulp, the periarteriolar (AS) sleeves around the central arterioles (CA) and the germinal centers (GC) were characteristic as in the control rats (FIG. 1G). The same in the red pulp where venous sinusoids and Bilroth's cords were typical.

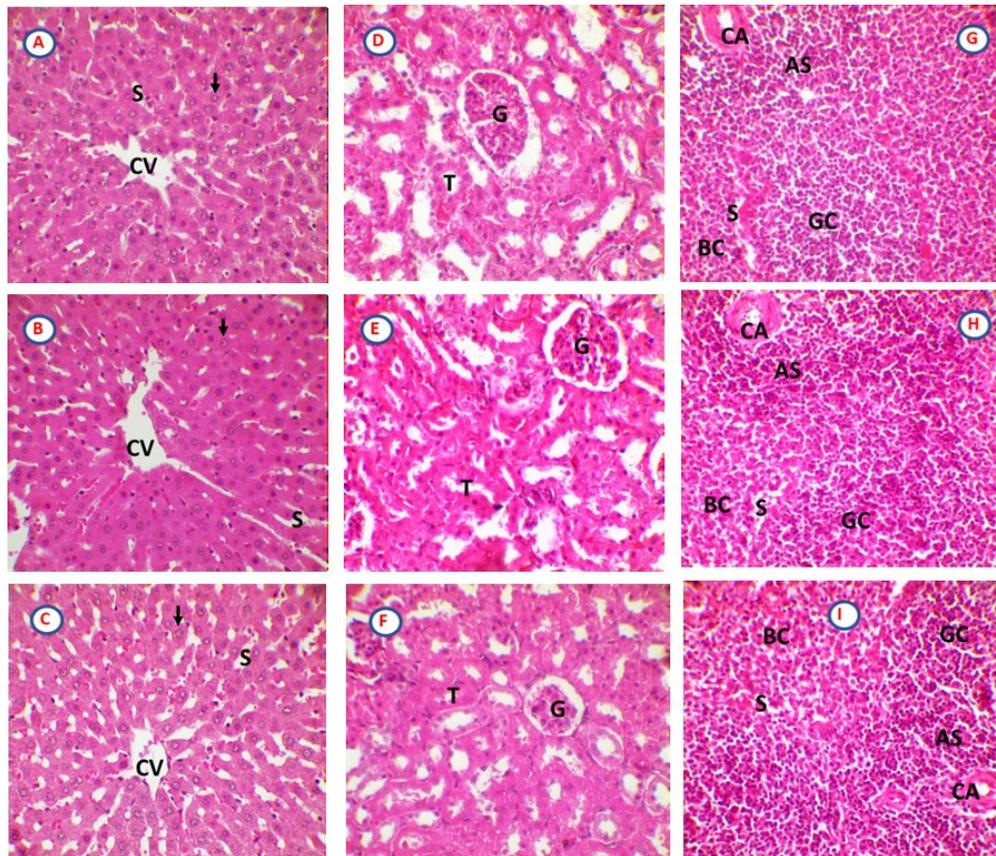


Figure 1: Liver, renal and spleen histology in acute oral toxicity tests of the aqueous extracts of *Curcuma longa* and *Syzygium aromaticum*

3.4 *Curcuma longa* transiently increases cholesterolemia and triglyceridemia

Figures 2, 3 and 4 showed the average blood glucose, triglyceridemia and total cholesterol levels, respectively, during treatment with *Curcuma longa*.

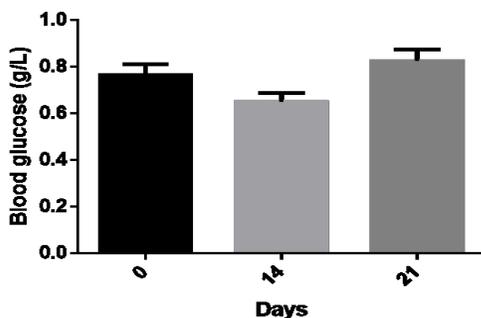


Figure 2: The average blood sugar level over time in rats treated with *Curcuma longa*

The average blood sugar level was 0.77 ± 0.04 g/L on day zero (D0); 0.65 ± 0.03 g/L on day fourteen (D14) and 0.83 ± 0.04 g/L on day twenty one (D21) in rats treated at 300 mg/Kg/Days with *Curcuma longa*. Compared to day

0, the mean blood sugar level did not change significantly in the rats on D14 and D21.

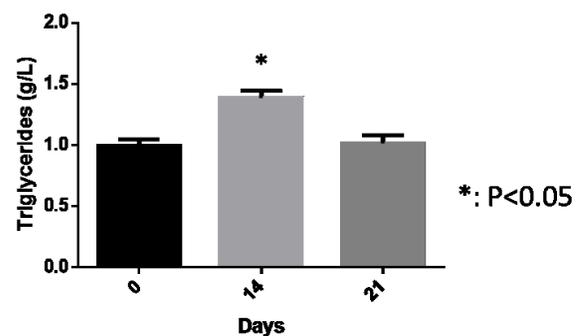


Figure 3: The average triglyceride level over time in rats treated with *Curcuma longa*

The average triglyceride level was 0.99 ± 0.04 g/L on day zero; 1.38 ± 0.05 g/L on day fourteen and 1.01 ± 0.06 g/L on day twenty one in rats treated at 300 mg / Kg/D with *Curcuma longa*. Compared to day 0, the mean triglyceride level increased significantly on day 14 in rats treated with *Curcuma longa* ($P < 0.05$).

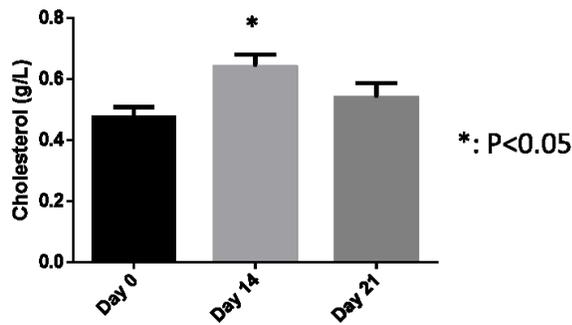


Figure 4: The mean total cholesterol level over time in rats treated with *Curcuma longa*

The average total cholesterol level was 0.47 ± 0.03 g/L on day zero; 0.64 ± 0.03 g/L on day fourteen and 0.54 ± 0.04 g / L on day twenty one in rats treated at 300 mg/Kg/Day with *Curcuma longa*. Compared to day 0, the mean total cholesterol level increased significantly on day 14 in rats treated with *Curcuma longa* (P <0.05).

3.5 *Syzygium aromaticum* did not affect blood sugar, triglyceridemia and cholesterolemia

Figures 5, 6 and 7 showed respectively the average level of blood glucose, triglycerides and total cholesterol over time in rats treated with *Syzygium aromaticum*.

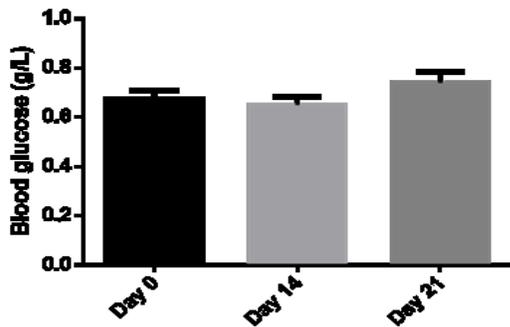


Figure 5: The average blood sugar level over time in rats treated with *Syzygium aromaticum*

The average blood sugar level was 0.67 ± 0.03 g/L on day zero; 0.65 ± 0.03 g/L on day fourteen and 0.74 ± 0.04 g/L on day twenty one in rats treated at 300 mg/Kg/Day with *Syzygium aromaticum*. Compared to day 0, the mean blood sugar level did not change significantly in the rats treated on D14 and D21 (P<0.05).

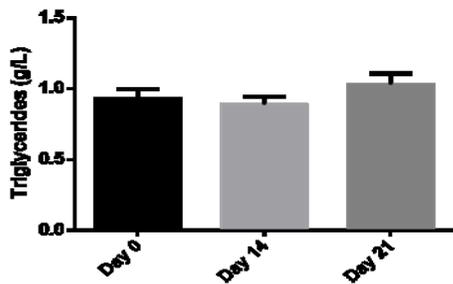


Figure 6: The average triglyceride level over time in rats treated with *Syzygium aromaticum*

The average triglyceride level was 0.93 ± 0.06 g/L on day zero (D0); 0.89 ± 0.06 g/L on day fourteen and 1.03 ± 0.08 g/L on day twenty one in rats treated at 300 mg/Kg/Day with *Syzygium aromaticum*. Compared to day 0, the mean triglyceride level did not change significantly in the rats treated on D14 and D21.

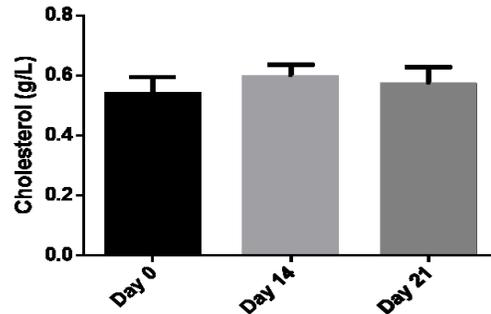


Figure 7: The mean total cholesterol level over time in rats treated with *Syzygium aromaticum*

The average total cholesterol level was 0.54 ± 0.05 g/L on day zero; 0.59 ± 0.04 on day fourteen and 0.57 ± 0.06 g/L on day twenty one in rats treated at 300 mg/Kg/Day with *Syzygium aromaticum*. Compared to day 0, the mean total cholesterol level did not change significantly in the rats treated on D14 and D21.

4. Discussion

The phytochemical analysis of *Curcuma longa* revealed polyphenols known for various therapeutic properties [9]. The phytochemical analysis of *Syzygium aromaticum* revealed tannin, flavonoid, leuco anthocyanins, quinone derivatives, also reported by other authors [10,11]. The hepatic, renal and haematological tests of extracts of the two spices showed their safety in the acute oral toxicity test, also confirmed by histology. This result is similar to those of Etame-Loe[12] and Agbaje [13] on *Curcuma longa*, *Syzygium aromaticum*. The two extracts did not have hypoglycemic activities, contrary to the findings of Kuroda [14] and Chien [15]. *Curcuma longa* extracts displayed a transient hypertriglyceridemia and hypercholesterolemia, which contrasts with the results of Olatunde [16], Budiman [17], Ika [18] and Myung-A [19] who instead showed hypotriglyceridemic and hypocholesterolemic activities for *Curcuma longa*, *Syzygium aromaticum*. These differences could be explained by the facts that these authors worked on models of diabetic rats whereas it was about non-diabetic rats in this work.

5. Conclusion

The aqueous extracts of *Curcuma longa* rhizome *Syzygium aromaticum* seeds were not toxic to the liver, kidneys, red blood cells and spleen. They increase cholesterolemia and triglyceridemia transiently. This suggests control in the use of these spices by the population.

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