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Toxicological Study of the Hydroalcoholic Extract of a Recipe of Three Plants Used in Traditional Togolese Medicine

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Abstract

This work consisted of assessing the toxicity of a recipe of three plants used in traditional Togolese medicine. Acute and sub-acute toxicity was assessed according to OECD chemical test guidelines n˚423 dated December 17, 2001 and n˚407 dated October 3, 2008 respectively. Cytotoxicity was assessed using the 3-(4,5-dimethylthiazolyl-2-yl-2,5-diphenyltetrazolium bromide (MTT) test. No significant toxicity was observed after 14 and 28 days, although a dose-dependent decrease in creatinemia was observed in male rats (the recipe to be used to moderate creatinine levels). Cytotoxicity was without effect on NCM 365 cells. The results obtained justify the use of the recipe in traditional medicine.

Keywords

Carica papaya, *Cocos nucifera*, *Persea americana*, Toxicity, Togo

1. Introduction

Today, medicinal plants are renowned for their therapeutic potential. They are increasingly used in our communities as a source of primary health care. The use

of natural substances derived from plants is therefore becoming an obvious solution. The toxicity of medicinal plants needs to be studied.

The toxicity of a substance is its capacity to induce adverse morphological or functional effects in a living organism following administration of a single high dose, or a small dose repeated over a long period. The study of the toxicity of a medicinal plant is the set of pharmacological tests that determine the degree or harmfulness of the plant in order to regulate its use. Toxicity is assessed according to mode of administration, dose, weight variation, mortality rate and variation in biochemical parameters [1]-[3].

A plant-based recipe is used in Togolese traditional medicine to treat typhoid fever [4]. The use of this recipe for therapeutic purposes in no way guarantees its safety. Thus, in addition to the pharmacological effects obtained, a toxicological study must be carried out to determine its safety.

The aim of this study is to evaluate the acute and sub-acute toxicity and cytotoxicity of a recipe based on three plants used in the treatment of typhoid fever in Togo (*Carica papaya* L., *Cocos nucifera* L. and *Persea americana* M.)

2. Material and Method

2.1. Plant Material

The plant material consists of *Carica papaya* roots, *Cocos nucifera* roots and *Persea americana* leaves.

2.2. Animal Material

Ras wistars were used. They were acclimatized for 14 days before any activity. Aeration and lighting conditions were good.

2.3. Extraction

The various plant organs were dried at laboratory temperature. The roots were cut before drying. Once dry, the organs were ground in a mill. The various powders were delipidated in petroleum ether for 24 hours.

After delipidation, extraction was carried out by maceration in a 70% hydroalcoholic solution. Thus, to 200 g of each powder are added 3 liters of hydroalcoholic solution. According to the tradithérapeute's indications, the recipe is composed of 35% Carica papaya, 35% Persea americana and 30% Cocos nucifera.

After 48 hours of contact in a dark place, the mixture is decanted and filtered on filter paper (wattman n˚1). The filtrates obtained are then evaporated under vacuum at 50˚C using a rotavapor (Heidolph type), then freeze-dried to obtain a dry powder.

2.4. Acute Toxicity

The acute oral toxicity of the recipe extract was assessed in rats in accordance with OECD guideline no. 423 for the testing of chemical substances, adopted on

December 17, 2001.

Two groups of 5 rats each were formed, including a control group. Each rat received a dose of 5000 mg/kg body weight; rats in the control group received physiological water only.

The rats were observed for 14 days. Particular attention was paid to observing the various manifestations of tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma.

Weight gains were calculated, recorded and compared on days 1, 7 and 14 post-treatment according to the formula:

$$GP = \frac{P_n - P_{n-1}}{P_{n-1}} * 100 \quad 1 \le n \le \infty$$

GP = weight gain (%); P_n = n^{th} body weight measurement; P_{n-1} = $(n-1)^{th}$ body weight measurement.

2.5. Subacute Toxicity

The subacute oral toxicity of the recipe extract was assessed in rats in accordance with OECD guideline no. 407 for the testing of chemicals adopted on October 3, 2008.

Two (2) doses (500 and 1000 mg/kg) were used. Six (6) groups of 5 rats each were formed, including two 2 control groups.

- Group 1: male rats, 1000 mg/kg body weight
- Group 2: male rats, 500 mg/kg body weight
- Group 3: female rats, 1000 mg/kg body weight
- Group 4: female rats, 500 mg/kg body weight
- Group 5: male rats, physiological water
- Group 6: female rats, physiological water

Animals received a daily dose of the recipe by gavage through an esophageal tube for 28 days. Gavage sessions were conducted at the same time of day. Animals were observed twice a day for 28 days.

Weight gains were calculated, recorded and compared on days 1, 7, 14, 21 and 28 post-treatment.

At the end of treatment, the animals were fasted for 12 hours, but given water ad libitum. They were then anesthetized with ether. Blood samples were collected by retro-orbital puncture using capillary tubes (Waynforth, 1980), in EDTA (Ethylene-Diamine-Tetra-Acetic) and dry tubes for hematological and biochemical studies, respectively

2.6. Cytotoxicity

Celltoxicity was assessed using the 3-(4,5-dimethylthiazolyl-2-yl-2,5-diphenyltetrazolium) bromide (MTT) assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA) [5].

For this purpose, a total of 3×10^4 NCM 365 cells were seeded per well in a 96-well plate and allowed to adhere for 24 h. Then, they were treated with the

extracts at different concentrations 2, 5, 10, 50 and 100 µg/ml and with a control (not cultured with the extract) for 48 h at 37˚C. Finally, the culture medium was replaced by 90 µL/well of fresh medium + 10 µL/well of MTS solution and incubated for 3 - 4 h at 37˚C under 5% CO_2, with absorbance measured at 490 nm on a plate reader. The assay was repeated three times ($n = 3$).

2.7. Statistical Analysis

Excel spreadsheets were used to process the data and plot the curves. Statistical analysis of the results was carried out using Graph Pad Prism 8.00 software using ANOVA One Way followed by Dunnett's test. Differences were considered significant at $P < 0.05$.

3. Results

After 14 days of observation (Figure 1), we recorded no deaths or dangerous signs following oral administration of the recipe at a dose of 5000 mg/kg body weight. According to the Globally Harmonized System of Classification and Labelling of Chemicals [6], the hydroethanol extract of the recipe is non-toxic by the oral route in Wistar rats. This suggests that the LD50 is greater than 5000 mg/kg. From this result, it appears that the recipe can be considered not to cause acute toxicity.

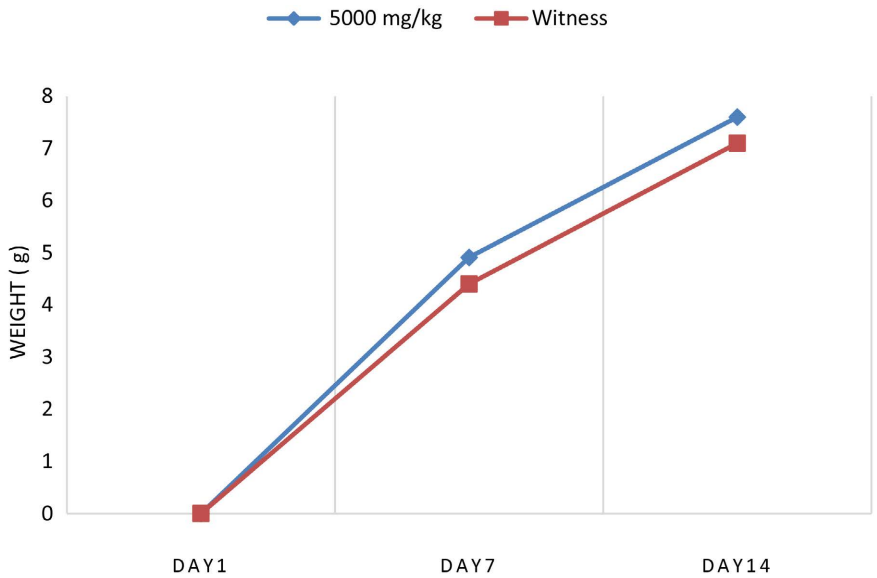

Figure 1. Effect of the recipe on rat weight for 14 days.

Treated male and female rats received doses of 500 and 1000 mg/kg body weight for 28 days (Figure 2 and Figure 3), while control rats received physiological water only. During this period, no deaths, dangerous signs, changes in locomotor activity, or food and water consumption were recorded. All rats gained weight. Weight gain was slightly higher in treated rats than in control rats after 28 days.

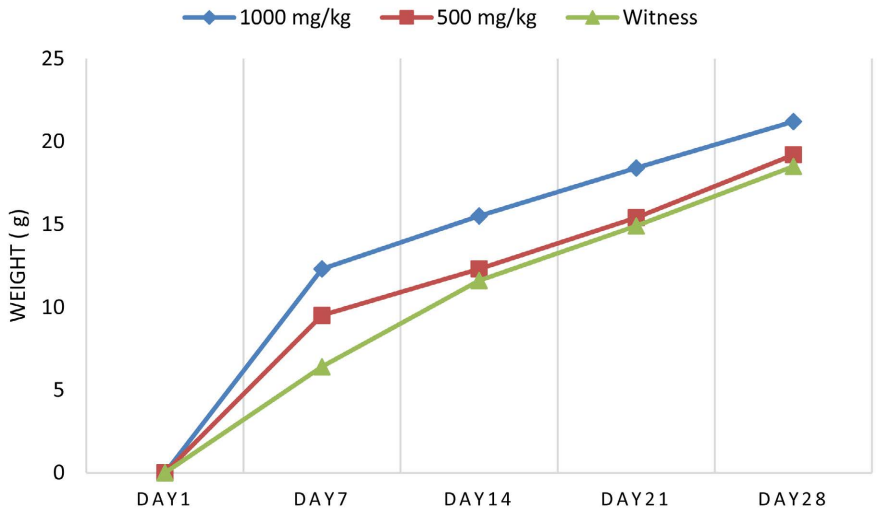

Figure 2. Effect of the recipe on weight of male rats for 28 days.

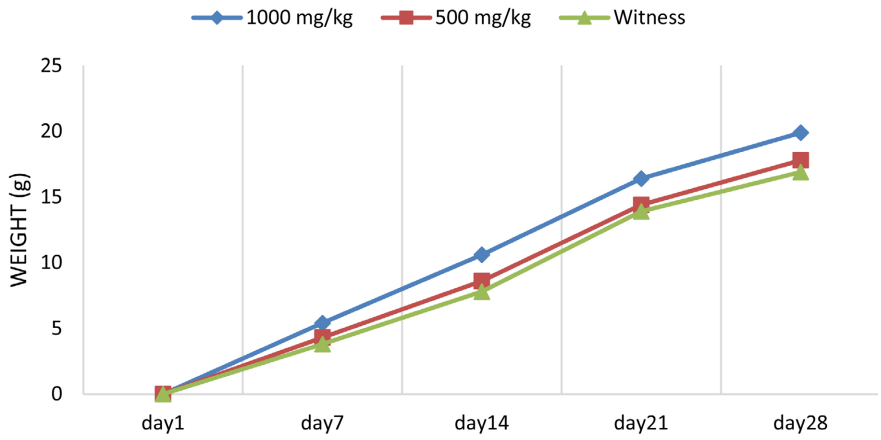

Figure 3. Effect of the recipe on weight of female rats for 28 days.

The various organs—liver, kidney, testes, lungs, heart, ovaries and spleen—were sampled, weighed and compared with those of control rats. The results showed that there was no significant difference between the various organs of treated rats compared with controls, except in the liver and lungs of female rats at a dose of 1000 mg/kg body weight. (Table 1)

Table 1. Organ weight (*g*) variation.

Organs/Doses	1000 mg/kg	500 mg/kg	Witness
		Males	
Liver	6.02 ± 0.60	5.90 ± 0.84	5.52 ± 0.44
Kidneys	0.80 ± 0.21	0.76 ± 0.14	0.78 ± 0.16
Heart	0.65 ± 0.07	0.75 ± 0.13	0.67 ± 0.16
Spleen	0.96 ± 0.17	1.14 ± 0.37	1.17 ± 0.13
Lungs	1.61 ± 0.25	1.62 ± 0.25	1.57 ± 0.14

Continued

		Females	
Liver	5.77 ± 0.47***	4.87 ± 0.50	4.09 ± 0.40
Kidneys	0.71 ± 0.09	0.67 ± 0.04	0.68 ± 0.10
Heart	0.70 ± 0.04	0.67 ± 0.11	0.74 ± 0.13
Spleen	1.14 ± 0.31	0.95 ± 0.16	1.15 ± 0.13
Lungs	2.06 ± 0.58***	1.57 ± 0.24	1.31 ± 0.17
Ovaries	2.55 ± 0.42	2.45 ± 0.31	2.29 ± 0.24

***P < 0.001.

As with the organs, biochemical parameters (Table 2) were measured in the different batches of rats studied. A dose-dependent decrease in creatinemia compared with the control was observed in male rats.

Table 2. Variation of biochemical parameters.

	1000 mg/kg	500 mg/kg	Witness
		Males	
Gly (mg/dl)	89.00 ± 17.61	91.52 ± 16.49	94.4 ± 6.09
Urea (mg/dl)	36.00 ± 5.10	34.42 ± 11.83	40.0 ± 9.19
Creat (mg/dl)	0.75 ± 0.12**	0.82 ± 0.21**	1.08 ± 0.21
ASAT (UI/L)	45.6 ± 24.23	41.6 ± 18.42	48.6 ± 14.43
ALAT (UI/L)	73.4 ± 30.78	64.0 ± 31.08	60.6 ± 9.05
GGT (UI/L)	1.5 ± 1.02	2.0 ± 1.41	1.8 ± 1.16
PAL (UI/L)	313.6 ± 45.11	350.4 ± 139.18	287.4 ± 52.09
Bil T (mg/dl)	1.76 ± 2.29	1.77 ± 0.78	1.55 ± 0.64
Bil D (mg/dl)	0.51 ± 0.10	0.48 ± 0.21	0.52 ± 0.15
CHOT (mg/dl)	89.6 ± 15.27	7.4 ± 9.75	98.8 ± 7.22
Trig (mg/dl)	78.5 ± 16.59	93.6 ± 25.85	72.8 ± 10.68
		Females	
Gly (mg/dl)	94.6 ± 5.92	96.2 ± 8.61	93.8 ± 9.45
Urea (mg/dl)	44.6 ± 4.41	42.6 ± 8.93	48.8 ± 5.42
Creat (mg/dl)	0.80 ± 0.21	0.88 ± 0.22	0.89 ± 0.28
ASAT (UI/L)	68.6 ± 13.32	59.2 ± 11.09	49.8 ± 10.76
ALAT (UI/L)	69.6 ± 10.19	67 ± 24.35	68.4 ± 11.70
GGT (UI/L)	2.01 ± 0.75	1.7 ± 1.05	1.6 ± 1.36
ALP (UI/L)	223.8 ± 38.84	244.6 ± 45.33	209.4 ± 18.59
Bil T (mg/dl)	1.29 ± 0.08	1.40 ± 0.54	1.39 ± 0.54

Continued

Bil D (mg/dl)	0.48 ± 0.10	0.42 ± 0.49	0.51 ± 0.23
CHOT (mg/dl)	89 ± 7.24	80 ± 10.18	88 ± 11.16
Trig (mg/dl)	77.3 ± 8.48	71.4 ± 11.79	76.6 ± 6.68

**P < 0.01. Gly: Blood glucose, Creat: creatinemia, ASAT: Aspartate amino-transferase, ALAT: Alanine aminotransferase, GGT: Gammaglutamyl transferase, ALP: Alkaline phosphatase, Bil T: Total bilirubin, Bil D: bilirubin direct, CHOT: total cholesterol, Trig: triglycerides.

Blood cells are called upon when a foreign agent enters the body. Toxicity may induce a change in the number, shape or size of these cells. To this end, we performed blood counts in rats from the three (3) batches studied. No significant differences were observed between treated and control rats, regardless of sex. (Table 3)

Table 3. Variations of haematological parameters.

	1000 mg/kg	500 mg/kg	Witness
		Males	
GB (10^3/µl)	9.1 ± 1.52	8.4 ± 3.04	8.96 ± 2.31
Lymp (%)	40.7 ± 9.56	44.44 ± 10.60	42.4 ± 9.21
Gran (%)	51.1 ± 9.32	49.44 ± 12.35	50.76 ± 8.16
HGB (g/dl)	14.2 ± 1.33	13.28 ± 0.44	13.28 ± 1.25
GR (10^6/mm^3)	8.11 ± 0.80	7.99 ± 0.22	7.81 ± 0.64
HCT (%)	40.34 ± 3.02	37.38 ± 0.92	38.98 ± 3.29
VGM (Fl)	47.92 ± 1.73	46.86 ± 1.97	50.28 ± 5.94
TMH (pg)	17.1 ± 1.26	16.62 ± 0.85	17.12 ± 1.88
CCMH (g/dl)	35.88 ± 1.64	35.48 ± 0.61	34.06 ± 1.05
PLT (10^3/µl)	955.80 ± 4 5.53	911.4 ± 85.23	979.8 ± 69.10
		Females	
GB (10^3/µl)	10.84 ± 1.09	9.48 ± 0.86	9.36 ± 1.81
Lymp (%)	42.02 ± 10.06	40.32 ± 7.88	45.8 ± 8.87
Gran (%)	52.72 ± 10.07	54.3 ± 8.18	48.28 ± 9.34
HGB g/dl	13.98 ± 1.16	13.72 ± 1.15	13.66 ± 0.84
GR (10^6/mm^3)	7.842 ± 0.50	7.67 ± 0.61	7.81 ± 0.6
HCT (%)	39.62 ± 3.39	38.52 ± 3.04	39.84 ± 2.26
VGM (Fl)	50.48 ± 1.65	50.24 ± 0.68	51.38 ± 5.03
TMH (pg)	17.76 ± 0.51	17.82 ± 0.19	17.36 ± 1.70
CCMH (g/dl)	35.24 ± 0.17	35.56 ± 0.87	34.22 ± 0.95
PLT (10^3/µl)	914.40 ± 56.82	933.00 ± 25.57	859.80 ± 42.98

GB: white blood cells, Lymp: Lymphocyte, Gran: Granulocytes, HGB: Hemoglobin, HCT: hematocrit, GVM: mean corpuscular volume, TMH: mean corpuscular hemoglobin content, CCMH: mean corpuscular hemoglobin concentration, PLT: Blood Platelets.

Cytotoxicity was assessed in vitro on normal NCM 365 colon cells (Figure 4) using the 3-(4,5-dimethylthiazolyl-2-yl-2,5-diphenyltetrazolium bromide (MTT) assay. The results show that the recipe is non-toxic to normal colon cells at a concentration of 100 µg/ml, with a crosis percentage of 9.41% according to NF EN ISO 10993-5 classification. The cytotoxic concentration (CC50) is 59,224 µg/ml or 59.22 mg/ml.

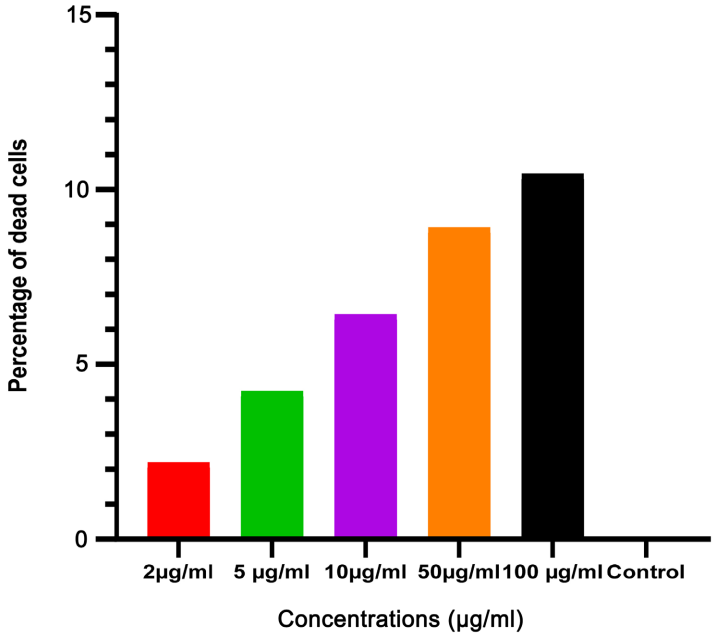

Figure 4. Toxicity of hydroethanol extract of the recipe on NCM 365 cells.

4. Discussion

The aim of our work is to evaluate the acute and subacute toxicity and cytotoxicity of a recipe of 3 plants (Carica papaya L., Cocos nucifera L., Persea americana M.) used in traditional medicine to treat typhoid fever.

The pharmacological properties of medicinal plants have led us to evaluate the toxicity of the recipe.

As the extract's action on pathogens can cause damage to consumers' organs, it is appropriate to carry out a toxicological study of our recipe. With this in mind, we assessed the acute and sub-acute toxicity and cytotoxicity of our recipe. The acute toxicity results indicate that the LD50 is greater than 5000 mg/kg body weight. According to [7], any substance with an LD50 greater than 5 g/kg can be considered non-toxic, so the recipe made from Carica papaya roots, Cocos nucifera and Persea americana leaves is not acutely toxic. According to [8], the limit test is carried out only when there is sufficient evidence that the test substance is not likely to be toxic or is of low toxicity. Consequently, [9]-[11], obtained LD50 values at 2000 mg/kg and 5000 mg/kg using Carica papaya roots, seeds and leaves respectively. On the other hand, [12] [13] showed that Cocos nucifera roots are not toxic at a concentration of 2000 mg/kg and [14] showed

that Persea americana leaves are not toxic at a dose of 2000 mg/kg.

The hydroalcoholic extract showed no sign of significant toxicity for 28 days in the rats studied. The change in body weight is a sensitive indication of the state of health of the animals [15] [16]. Thus, the increase in liver and lung weights in female rats could be explained by weight gain in female rats treated at 1000 mg/kg.

Analysis of blood parameters is important for toxicity risk assessment, as any changes in biochemical and haematological systems have a higher predictive value for toxicity [17] [18]. The absence of significant changes in transaminases and renal function tests suggests that the recipe did not cause liver or kidney damage. Transaminases are enzymes that catalyze the transfer of alpha-amino radicals from alanine and aspartic acid to alpha-ketoglutaric acid [16]. They are synthesized in the cytoplasm and released into the general circulation when cells are damaged [1] [19] [20]. In addition, the dose-dependent reduction in creatinine levels in treated male rats may allow the recipe to be used to moderate creatinine levels in males. The absence of significant variations in haematological parameters suggests that the recipe did not induce a toxic effect on bone marrow, considered to be one of the most sensitive targets of toxic compounds. Moreover, any changes in the haematological systems of treated rats are more predictive of toxicity in humans than in rats when extrapolated from animal study data [17]. *In vitro* toxicity on normal NCM 365 colon cells showed no cell necrosis.

The results obtained would suggest that plants taken in isolation did not show significant toxicity at repeated dose for 28 days, so [21] [22], did not observe mortality or abnormalities in liver and kidney markers using Carica papaya leaves similarly [9] [12] used the immature inflorescence of cocos nucifera for 28 days of exposure at a dose ranging from 1.75 to 14 mg/kg body weight, and lastly [23], obtained no significant signs of toxicity using Persea americana seeds. On the other hand, [24] obtained liver degeneration following administration of 750 mg/kg of Persea americana aqueous extract.

5. Conclusion

The recipe made from *Carica papaya* L., *Cocos nucifera* L. and *Persea americana* M. is used in traditional medicine to treat typhoid fever. Our study assessed the recipe's acute and subacute toxicity and cytotoxicity. At the end of the study, it emerged that the recipe, prepared using the traditional method, did not induce any significant toxicity. The results obtained justify the use of this recipe in traditional medicine for the treatment of typhoid fever. These results could constitute a database that could guide the development of new drugs for the treatment of typhoid fever.

Conflicts of Interest

The authors have declared no conflicts of interest.

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