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*Full Length Research Paper*

# Acute, subacute toxicity and cytotoxicity of *Chrysophyllum perpulchrum*

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**The total methanolic extracts of *Chrysophyllum perpulchrum* were used for toxicological (acute and subacute toxicity) and cytotoxicity studies. The experiment focuses on Swiss mice acclimatized in the animal cages at the University of Cocody-Abidjan. Each cage contains five mices of the same age (6 to 8 weeks), in much the same weight (20 ± 0.5 g) and same sex (male or female). The extract of *C. perpulchrum* is injected to the mice with an insulin syringe intraperitoneally. The doses ranged from 0 to 4000 mg/kg of body weight (bw). According to the term of the study, the blood of the mice used in this second study (20 g of weight) is taken with the view to study the effect of *C. perpulchrum* on the blood formulate numeration (BFN) and also on the serum markers of noble organs that are heart, liver and kidneys. At last, the toxicity of *C. perpulchrum* has been evaluated with some human bronchial epithelial cells. The acute toxicity allowed to determine the maximal tolerated dose (MTD) which is 500 mg/kg of bw, 50% lethal dose ($LD_{50}$) of 1259 mg/kg of bw whatever the method used. The $LD_{50}$ is greater than 500 mg/kg bw. Then, *C. perpulchrum* not inhibiting the cellular proliferation is not over 1 mg/ml. In conclusion, *C. perpulchrum* is moderately toxicic and would entail a light damage of kidney. On the other hand, this substance is not toxic on the human bronchial epithelial cells. She would favor rather the proliferation of these last one. *C. perpulchrum* is thus not cytotoxic.**

**Key words:** *Chrysophyllum perpulchrum*, acute, subacute, toxicity, cytotoxicity.

## INTRODUCTION

The Ivorian flora of which five thousands species have been made an inventory by Adjanohoun and Aké (1979) appears as an emergency exit for elaboration of new remedies which will not only be efficient in the treatment of endemic illnesses peculiar to tropical countries, but also, mainly easily accessible for their big availability. At the present time, teams of researchers obtain some natural substances used for new remedies from plants. It will be the case of *Mansonia altissima* (Sterculaceae), whose mansonine stays the principal purified compound that can be used in the treatment of cardiovascular diseases (Guédé-Guina et al., 1992, 1998). However, the quantity of plants as sources for the production of new remedies is widely unexploited in view of the number of species of superior plants (angiosperms and gymnosperms) on the planet assessed to two hundreds and fifty thousands (250000) (Kong et al., 2003). Only six percent (6%) have been tested for their biological activity and fifteen percent (15%) have been evaluated on the phytochemical level (Verpoorte, 2000). These proportions always stay weak in the eyes of therapeutic potential of plants (Hamburger and Hostettmann, 1991). Besides, it is necessary however to note that in Africa, we are more and more confronted to the reappearance of new illnesses. Among all, we can quote: illnesses linked to

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oxidizing stress. In facts, the oxidative stress is involved in some numerous diseases as triggering factor or associated to some complications. Most of the diseases induced by the oxidizing stress appear with the age because the ageing reduces the antioxidative defenses and increase the mitochondrial reproduction of free radicals (Girodon et al., 1997; Sohal et al., 2002). The oxidizing stress is the principal initial cause of many diseases (Mates and Sanchez-Jimenez, 2000). It is the factor potentiating the appearance of some plurifactorial diseases such as diabetes, Alzheimer's disease, rheumatism and cardiovascular diseases (Sergeant et al., 1998).

In view of the diversity and the gravity of illnesses that induce the oxidizing stress, many teams of researchers have invited themselves in the research of new oxidative in sight of fighting against the oxidizing stress and these associated pathologies. For Bidié (2010), *C. perpulchrum* contains three responsible compounds of a very good oxidative activity similar to this of quercetin. However, *C. perpulchrum* administered orally or intravenously will therefore have access to all body functions. Thus, it seemed interesting to conduct studies of toxicity and biotolerance for a more rational use. The determination of toxicological parameters of the methanolic extract of *C. perpulchrum* therefore seems necessary since it must be administered to living organisms. Since no toxicological study of this extract had been conducted to date, so, as the aim of the present study, we found it is necessary to evaluate acute and subacute toxicity of this extract on laboratory animals. In this study, some hematological and biochemical parameters of this phytomedecine on laboratory mice will be determined too. Indeed, it concerns the blood cells counts, the serum markers of integrity of the heart, the kidney and the liver. Finally, the toxicity of *C. perpulchrum* on the human bronchial epithelial cells will be determined.

## MATERIALS AND METHODS

### Plant material

*C. perpulchrum* (voucher N° 8375) identified by professor Aké-Assi is a tropical forest plant found in the Republic of Côte d'Ivoire. The active principle is found in every portion of the plant. The methanolic extract from the bark of *C. perpulchrum* was prepared by stirring 50 g of finely ground portions in 1.5 L methanol using a magnetic stirrer (IKAMAG RCT) for 48 h at room temperature (25°C). The methanolic extract was filtered over cotton and whatman N°3 filter paper three times. The filtrate was evaporated at 40°C under reduced pressure with a rotavapor (Buchi 461 watter Batch). The residue obtained, dissolved in a physiological liquid (Mac-Ewen) was used to prepare *C. perpulchrum* solutions.

### Animals

Swiss mices (males and females) aged 6 to 8 weeks and weighing 20 g on average, and rats aged 12 weeks and weighing 150 g (Desfontis, 2004) on average were the pet of the Pasteur Institute of Adiopodoume (Côte d'Ivoire).

### Method of toxicological studies

#### *Acute toxicity*

The mices used for experimentation are the same age (6 to 8 weeks) and are placed in cages containing bedding of wood shavings. This litter is renewed every two days. The experiment consisted of 60 mices divided into 12 batches of 5 mices with 6 batches of male mices and 6 batches of female mices and substantially equal weight (20 ± 0.5 g). Intraperitoneally, different concentrations of *C. perpulchrum* are injected into mice using an insulin syringe of 1 ml. These concentrations ranging from 0 to 4000 mg/kg bw are prepared with a physiological solution (Mac Ewen).

#### *Subacute toxicity*

This experiment was conducted in three (03) months to determine the subacute toxicity of the product on animals by injecting the daily dose and repeated concentrations below the $LD_{50}$ of *C. perpulchrum*. Indeed, we used 4 batches of 3 rats with a control group. For this second part of the study to determine the effect of *C. perpulchrum* on the noble organs (kidney, liver and heart) of animals, varying doses of *C. perpulchrum* were prepared from the MTD (from 0 to 475 mg/kg body weight).

### Method for determining the $LD_{50}$ by calculation

According to Berhens and Karber (1935), the $LD_{50}$ of a product can be determined using the following formula:

$$LD_{50} = 100 - \frac{(a \square b)}{n}$$

With $LD_{100}$ = dose causing death of all animals in experimentation. a = average number of animals dead between two successive doses. b = difference between two successive doses (mg/kg of bw). n = number of animals used per batch or mean animals with n = 5 mice.

### Method for determining the complete blood count (CBC) and serine markers of kidney, liver and heart

#### *Determination of the CBC*

The method used is that described by Yapi et al. (2009). Indeed, whole blood collected in tubes with anticoagulant (EDTA) was used to determine hemoglobin (Hb) concentration and mean blood cells volume (MBCV) using a semi-automatic blood cell counter.

#### *Urea determination*

The enzymatic method used is based on the reaction described by Talke and Schubert (1965). The method has been optimized by Tiffany et al. (1972) who showed that the concentration of urea is proportional to the change in absorbance measured at 340 nm for a given time. The reaction scheme is as follows:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

$$NH_3 + \text{Oxoglutarate} + \text{NADH} + H^+ \xrightarrow{\text{GLDH}} \text{Glutamate} + NAD^+ + H_2O$$

***Uric acid determination***

According to Fossati et al. (1980) and Tietz (1999), uricase acts on uric acid to produce allantoin, carbon dioxide and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with a chromogen (dichlorohydroxybenzene sulfonate and amino-antipyrine) to form a quinonemine which is a red complex. The absorbance measured at 520 nm is proportional to the amount of uric acid.

***Blood glucose determination***

According to the method of Trinder (1969) and Farrance (1987), glucose is oxidized by GOD to form gluconic acid and $H_2O_2$. Then POD reacts with $H_2O_2$; 4-chloro-phenol and the PAP to form a red quinonemine. The absorbance of the colored complex, proportional to the concentration of glucose in the medium was measured at 500 nm.

***Determination of creatinine***

This assay is based on colorimetric reaction (Jaffe reaction without pre-stage processing of the specimen) of creatinine with picric acid in alkaline medium. The kinetics of the reaction are measured at 490 nm. This method has been optimized by Fabiny et al. (1971) and Labbe (1996).

***Determination of transaminases***

The serum glutamic oxaloacetic transaminase (SGOT) method was developed by Karmen et al. (1955) and optimized by Henry et al. (1960) [consistent with the recommendations of the IFCC (1986)]. The reaction scheme is as follows:

$$\text{L-Aspartate} + \text{2-Oxaloacétate} + \text{L-Glutamate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{L-Malate} + NAD^+$$

The decrease in absorbance due to conversion of NADH to $NAD^+$ and proportional to the AST activity in the specimen is measured at 340 nm. The serum glutamic transaminase-pyruvic (SGTP) method was developed by Wrobleski and La Due, and optimized by Henry (1960) and Bergmeyer et al. (1978) [consistent with the recommendations of the IFCC (1986)]. The reaction scheme is as follows:

$$\text{L-Alanine} + \text{2-Oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+$$

The decrease in absorbance measured at 340 nm due to conversion of NADH to $NAD^+$ is proportional to ALT activity in the specimen.

***Determination of alkaline phosphatase (ALP)***

The activity of ALP was determined according to the method of Withold (1996) to the following reaction:

$$\text{Phenylphosphate} \xrightarrow{\text{ALP}} \text{Phenol} + \text{Phosphate}$$

The phenol liberated by hydrolysis of the substrate reacts in the presence of 4-amino-antipyrine and ferricyanide potassiumn to form a red complex whose absorbance measured at 510 nm is directly proportional to ALP activity in the specimen. Sodium arsenate was used to stop the enzymatic reaction and prevents instability of the color observed with previous methods.

***Determination of lactate dehydrogenase (LDH)***

The method of Henry et al. (1974) (consistent with the recommendations of SFBC) was used:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+$$

The decrease in absorbance (measured at 340 nm) due to conversion of NADH to $NAD^+$ was directly proportional to LDH activity in the specimen.

***Determination of creatinine phosphokinase (CPK)***

The activity of CPK was determined according the method of Fabiny and Ertingsthausen (1971) and optimized by Labbé et al. (1996). Yapi et al. (2011) have also determined the activity of CPK. It is a colorimetric reaction of creatinine with picric acid under alkaline conditions, the kinetics of development of which is measured at 490 nm.

**Cytotoxicity of *C. perpulchrum***

***Cellular culture***

The cellular line H292 is a line of human bronchial epithelial cells. The cells have been maintained in a saturated atmosphere in wate rat five percent (5%) of $CO_2$ at 37°C. The mid of complete culture is from RPMI 1640 complemented with 5% fetal calf serum 2 mM of L-glutamine, cocktail diantibiotic (100 U/ml of penicillin and 50 µg/ml de streptomycin). Products were supplied by Invitrogen (California, USA). *C. perpulchrum* has been dissolved to 10 mM in water and kept to 4°C. The different concentrations used for the cultures have been realized in complete RPMI 1640.

**Test of cellular lyse - determination de la cytotoxicity**

The cytotoxicity of *C. perpulchrum* has been evaluated on the

human bronchial epithelial cells by measuring the liberation of cytoplasmic enzyme lactate deshydrogenase (LDH) in the field of culture. The liberation of LDH correlates with the number of lysed cells and is useful for the studies of cytotoxicity (Tipton et al., 2003). The mixture has been realised; thanks to kit CytoTox 96® Assay Kit following the indications of the provider (Promega, Charbonnieres, France). Briefly, the Human bronchial epithelial cells have been cultured in sheets 96 well to the concentration of $1 \times 10^4$ and $2.5 \times 10^4$ cells/well to study, respectively, the cytotoxicity of *C. perpulchrum* on some cells in division and some confluence cells, not divising any more. After a night at 37°C, the mid has been replaced by complet mid containing different concentrations of *C. perpulchrum*. The activity of LDH has been measured 48 h later on 50 µL of floating cells. The absorption (DO experimental) has been measured at 492 nm on a reader of sheet ELISA (Tecan, Trappes, France). The maximum liberation's rate of LDH (DO of total lyse) has been obtained from cellular carpet with 1% of Triton X-100. The results are expressed in percentage of total lyse, corresponding to [(D.O. experimental - D.O. of the mid without cell)/(D.O. of the total lyse - D.O. value of the mid without cell) × 100].

#### Test of cellular proliferation - MTS test

The effect of *C. perpulchrum* on the proliferation of human bronchial epithelial cells has been studied by the colorimetric test thanks to kit CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay Kit (Promega). The human bronchial epithelial cells have been cultured to the concentration of $3 \times 10^3$ cells/well in 100 µl of complete mid in sheets 96 wells. After 24 h, some growing concentrations of *C. perpulchrum* which are 1 and 10 mg/ml have been added to the cells. After 48 h of incubation, 20 µl from a freshly prepared solution of MTS/PMS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophe-nyl)-2H tetrazolium/ phenazine methosulfate) have been added on cells. The new step of incubation has been realised for 2 to 4 h. The quantity of formazan soluble produced by cellular reduction of MTS has been determined by measure of absorption at 492 nm a reader of sheets ELISA.

#### Statistical analysis

Experiments were run in duplicate and statistical analyses were performed using the Graph Pad Prism 5.0 software (Microsoft). Analyses of variance (ANOVAs) were conducted and multiple comparisons between means were performed by Tukey and Dunnett procedure. Significance level was defined as $P < 0.05$. Results were given as means ± standard deviation.

## RESULTS

### Acute toxicity

#### *Animal behavior*

Injection doses of total methanolic extract of *C. perpulchrum* between 250 and 4000 mg/kg bw to the animals allowed us to observe the following events: -Regrouping trends followed by immobilization of the mouse can be extended over a period of 3 min to 10 h depending on the doses administered, accelerated respiratory rate (tachypnea); Tremor; -Disruption of feeding behavior (loss of appetite); -Immobilization and respiratory disorder are signs preceding the death of mice. These animals die lying on their stomach or side. The deaths are recorded in batches 4, 5 and 6 of male and female mice at doses respectively 1000, 2000, 4000 mg/kg of bw (Table 1). The first dead mice were recorded in a batch of female mice. In control batches of mice, no apparent abnormal signs were observed. The mice kept the same aspect, either on behavioral or nutritional. After 14 days 24 h observation, all mice died in batch 5 (males and females) over a 3days period. We observed the gradual recovery of mice from other lots, which is illustrated by a return to normalization of respiratory rate and feeding behavior similar to control groups of mice.

### Subacute toxicity

#### *Behavior of rat after 21 days of observation*

This second part of this study was conducted on rats divided into 4 groups of 5 rats with a control group. The doses used were based on the MTD. Thus the doses ranged from 0 to 475 mg/kg body weight. Observation of rats for 21 days after repeated administration of the product showed no mortality in control groups of rats. However, in the experimental batches (batches 2 and 3) of rats, there were deaths. The dose 250 mg/kg bw resulted in death of 1 rat during the first day after 5 days repeated injection of the product. The dose 500 mg/kg bw caused the death of 2 rats on the second day after 5 days of repeated injection of the product. On the 5th day of treatment, rats from batches 2 and 3 were all weakened. They were motionless with rapid breathing. In addition, there was a change in their nutritional status, which was characterized by a loss of appetite. This change was more pronounced at batch 3. During the following 14 days, mice that survived had resumed their usual feeding habits as in controls.

#### *Behavior of mice after 3 months of observation*

During the 40 days following the first 21 days of observation of animals in control groups and experimental batches (2 and 3), no conduct disorder or change in their general appearance and nutritional status was observed. No mortality was recorded. 30 days (1 month) of observation after these 2 months, no death was recorded, only some signs of weakness. The animals have metabolized the product.

### Effect on blood cells counts

Our study reveals a variation of white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb) and platelets after treatment of animals with *C. perpulchrum* (Table 3). Indeed, the WBC went from 8.48 ± 1.03 to 12.27 ± 0.48,

**Table 1.** Mortality of male mice (M) and females (F) as a function of levels of total methanolic extract of *C. perpulchrum* and time.

| Lots | Group 1 0 (mg/kg bw) | | Group 2 250 (mg/kg bw) | | Group 3 500 (mg/kg bw) | | Group 4 1000 (mg/kg bw) | | Group 5 2000 (mg/kg bw) | | Group 6 4000 (mg/kg bw) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time after injection | M | F | M | F | M | F | M | F | M | F | M | F |
| | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 15 min | / | / | / | / | / | / | / | / | / | / | 3 | 3 |
| 30 min | / | / | / | / | / | / | / | / | / | / | 3 | 3 |
| 1 h | / | / | / | / | / | / | / | / | / | 1 | 3 | 3 |
| 5 h | / | / | / | / | / | / | / | / | 1 | 2 | 4 | 4 |
| 10 h | / | / | / | / | / | / | 1 | 1 | 2 | 3 | 5 | 5 |
| 15 h | / | / | / | / | / | / | 1 | 1 | 2 | 4 | 5 | 5 |
| 20 h | / | / | / | / | 1 | 1 | 2 | 3 | 4 | 4 | 5 | 5 |
| 24 h | / | / | / | / | 1 | 1 | 3 | 3 | 4 | 4 | 5 | 5 |
| Mortality (%) | / | | / | | 20 | | 60 | | 80 | | 100 | |

RBC from 7.26 ± 0.41 to 8.19 ± 0.18 before coming down in 7.23 ± 0.29. As for Hb, it was observed that a non significant increasing which goes from 12.27 ± 0.31 to 14.23 ± 0.14 and platelets from 1113 ± 9.70 to 1199 ± 104.4. For other elements of the BFN except the HCT and Neutrocytes that increase, we see a general reduction of all other elements (MCV, MCH, MCHC, lymphocytes, monocytes Eosinocytes and Basocytes) (Table 3).

### Effect on the kidneys

Biochemical parameters that allow us to get an idea about the state of the kidneys or renal activity are: urea, uric acid and creatinine. We find an increase of the three parameters compared to the control group. Indeed, for urea, uric acid and creatinine, control values are (for 0 mg/kg bw of *C. perpulchrum*): 0.28 ± 0.02; 38.33 ± 2.18 and 6.00 ± 0.57 respectively. These values become 0.27 ± 0.05; 49.33 ± 0.66 and 06.66 ± 0.66 respectively; for the dose of 475 mg/kg bw of *C. perpulchrum* (Table 4).

### Effect on the heart

We determine activities of transaminases (GOT, GPT), creatinine phosphokinase (CPK) and lactate deshydrogenase (LDH) as markers of integrity of heart. Indeed, our results reveal a change from control values (GOT = 169.3 ± 51.50; GTP = 76.33 ± 0.4; CPK = 165.0 ± 20.0 and LDH = 262.3 ± 10.90). The experimental values indicate: (GOT = 141.0 ± 15.28; GTP = 63.67 ± 7.05; CPK = 101.7 ± 3.71 and LDH = 199.3 ± 5.54) after treatment with 475 mg/kg bw of *C. perpulchrum* (Table 4).

### Effect on the liver

In addition to the activity of serum transaminases, CPK and LDH, blood glucose and serum activity of ALP have been identified. Thus, after treatment with *C. perpulchrum* (475 mg/kg of bw), we see a change in blood glucose from 1.04 ± 0.17 (control) to 0.69 ± 0.07 and the activity of ALP from 294.7 ± 45.61 to 199.3 ± 5.54 (Table 4).

## STUDY OF CYTOTOXICITY OF *C. PERPULCHRUM*

### Cytotoxic effects of *C. perpulchrum* on some human bronchial epithelial cells

The human bronchial epithelial cells have been cultivated during 2 days with different concentrations of *C. perpulchrum* and the cytotoxic effect of plant's extract has been evaluated by measure of liberation of LDH. The cellular lyse is inversely proportional to the cellular proliferation (Figure 1). Thus, *C. perpulchrum* does not induce the cellular lyses on some cells devising itself, for some concentrations going from 0 to 1 mg/ml. To the strongest concentrations (10 mg/ml), the cytotoxic effect observed has

**Table 2.** Toxicological parameters of *C. perpulchrum*.

| Parameters | MTD | $LD_{50}$ | $LD_{100}$ |
|---|---|---|---|
| Dose (mg/kg bw) of total methanolic extract of *C. perpulchrum* | 500 | 1259 | 4000 |

MTD: maximal tolerated dose; $LD_{50}$: 50% lethal dose; $LD_{100}$: 100% lethal dose.

**Table 3.** Effect of *C. perpulchrum* on blood formulate numeration.

| Samples / Parameters | Control | Group 1 | Group 2 | Group 3 |
|---|---|---|---|---|
| GB | 8.48±1.03 | 11.40 ±0.76 | 13.67 ± 0.14$^{ns}$ | 12.27±0.48$^{ns}$ |
| GR | 7.26±0.41 | 8.19±0.18$^{ns}$ | 7.62±0.63$^{ns}$ | 7.23±0.29$^{ns}$ |
| HbG | 12.27±0.31 | 12.97±0.23$^{ns}$ | 14.00±1.40$^{ns}$ | 14.23±0.14 |
| HCT | 37.03±1.09 | 44.44±0.47$^{ns}$ | 41.37±4.08$^{ns}$ | 39.30±1.68$^{ns}$ |
| VGM | 54.40±0.77 | 53.37±0.50 | 52.40±0.95 | 52.47±1.40 |
| TCMH | 18.03±0.08 | 16.97±0.23$^{ns}$ | 17.27±0.23$^{ns}$ | 17.00±0.26$^{ns}$ |
| CCMH | 33.17±0.60 | 31.80±0.20$^{ns}$ | 32.90±3.6n$^{s}$ | 32.53±0.52$^{ns}$ |
| Plaquettes | 1113±9.70 | 1116±52.74$^{ns}$ | 1173±60.38$^{ns}$ | 1199±104.4$^{ns}$ |
| Lymphocytes | 62.93±2.42 | 59.10±3.49$^{ns}$ | 56.86±5.44$^{ns}$ | 53.67±8.48$^{ns}$ |
| Monocytes | 5.66±0.88 | 4.66±1.76$^{ns}$ | 4.00±2.0$^{ns}$ | 3.33±0.33$^{ns}$ |
| Neutrophiles | 33.00±2.08 | 37.33±3.71$^{ns}$ | 38.33±0.88$^{ns}$ | 40.33±1.20 |
| Eosinophiles | 2.33±0.33 | 2.00±0.57$^{ns}$ | 1.66±0.33$^{ns}$ | 1.33±0.33$^{ns}$ |
| Basophiles | 00 | 00 | 00 | 00 |

**Table 4.** Effects of *C. perpulchrum* on serum markers of the integrity of the liver, the heart and the renal function.

| Samples / Parameters | Control | Group 1 | Group 2 | Group 3 |
|---|---|---|---|---|
| Urea | 0.28±0.02 | 0.27±0.05$^{ns}$ | 0.29±0.02$^{ns}$ | 0.27±0.05$^{ns}$ |
| Uric acid | 38.33±2.18 | 37.67±0.88$^{ns}$ | 40.00±2.51$^{ns}$ | 49.33±0.66$^{ns}$ |
| Creatinine | 6.00±0.57 | 7.33±0.33$^{ns}$ | 7.66±0.33$^{ns}$ | 8.66±0.66$^{ns}$ |
| GOT | 169.3±51.50 | 152.0±13.28$^{ns}$ | 145.3±14.15$^{ns}$ | 141.0±15.28 |
| GPT | 76.33±4.84 | 71.67±8.83$^{ns}$ | 66.33±4.37$^{ns}$ | 63.67±7.05$^{ns}$ |
| Blood glucose | 1.04±0.17 | 0.99±0.10$^{ns}$ | 0.95±0.04$^{ns}$ | 0.69±0.07$^{ns}$ |
| ALP | 294.7±45.61 | 239.3±22.84 | 208.3±28.36 | 197.0±5.29 |
| CPK | 165±20.0 | 148.7±14.53$^{ns}$ | 133±8.66$^{ns}$ | 101.7±3.71$^{ns}$ |
| LDH | 262.3±10.91 | 234±15.95$^{ns}$ | 211.7±4.80$^{ns}$ | 199.3±5.54 |

increased proportionally with the increase of *C. perpulchrum* concentration added to the culture's medium on confluence cells inversely to Figure 2. *C. perpulchrum* does not induce any liberation of LDH excepted for the strongest concentration of the extract used (10 mg/ml).

### Effect of *C. perpulchrum* cellular growth of human bronchial epithelial cells

Using the concentrations of *C. perpulchrum* without cytotoxicity, we have analyzed some human bronchial epithelial cells. This has been realized by determining the number of actives cells by a colorimetric method (MTS test). Results presented in Table 2, show that *C. perpulchrum* reduces the number of cells in dose-dependant way. The toxic effect of *C. perpulchrum* is visible ($p < 0.05$) to 10 mg/ml. In fact, some concentrations from 1 to 10 mg/ml of *C. perpulchrum,* an enriched middle and a positive control constituted by triton (the toxin) have been used. This test reveals that: to the concentration of 1 mg/ml of methanolic total extract of *C. perpulchrum,* the cellular proliferation is identical to that of enriched domain. However, for a high

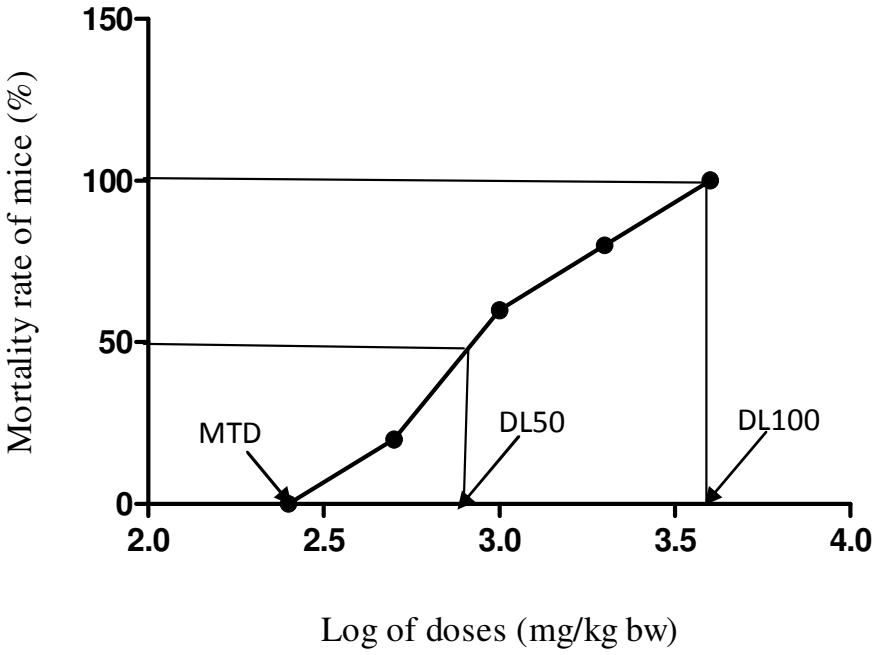


**Figure 1.** Mortality rate of mice as a function of levels of total methanolic extract of *Chrysophyllum perpulchrum.* MTD: maximum tolerated dose, $LD_{50}$: lethal dose 50 and $LD_{100}$: lethal dose 100.

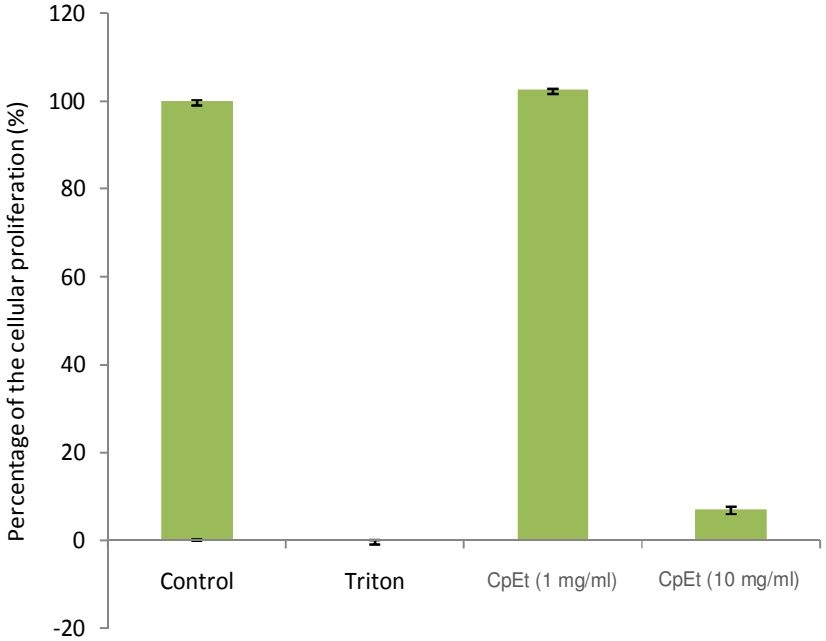


**Figure 2.** Effect of *C. perpulchrum* on the proliferation of the human bronchial epithelial cells. CpEt: total extract of *C. perpulchrum*.

concentration (10 mg/ml) of the same extract, the cellular proliferation is very weak, even null as those registered with the triton (Figure 2).

## DISCUSSION

The results of acute toxicity show that the increasing of

the doses of total extract of *C. perpulchrum* leads to the increase of mice's mortality submitted to the test.

Moreover, the proportion of affected animals raise with the dose. So, there is a relationship dose-response between the dose of the administrated extract and the mortality registered (Fatima et al., 2010). This study determined the toxicological parameters of total methanolic extract after intra peritoneal injection in mice. These quantities are: the MTD (500 mg/kg of bw), the $LD_{50}$ (1259 mg/kg of bw) and $LD_{100}$ (4000 mg/kg bw). These parameters allow us to say that the administration of doses below the MTD induced no mortality as also shown by Lapointe (2004) and Fatima et al. (2010). However, doses above the MTD, induced deaths for which the rate varies depending on the dose (Kouassi, 2006). At doses between MTD and the $LD_{50}$, the mortality rate after 24 h of observation of the female mice is the same that of the males. At doses above the $LD_{50}$, the mortality rate is always the same for all the mices (Laroche et al., 1986; Sitwell, 2007). Generally, the $LD_{50}$ reflected the lethal nature of the dose of a substance (Langley et al., 2003). Moreover, the time of action of the product is faster in female mice than in male mice. This suggests that female mice are more sensitive to the total methanolic extract of *C. perpulchrum* than male mice, and the metabolism of the product would be slower in male mice, where from the mortality delayed at these last ones. The sensitivity of the animal towards a given drug may vary according to sex (Laroche et al., 1986; Sitwell, 2007).

In addition, the value of $LD_{50}$ estimated at 1259 mg/kg bw (3 times that of the MTD) as well in male mice than females helps to qualify the total methanolic extract of *C. perpulchrum* as moderately toxic substance according to the scale of $LD_{50}$ classification by Cotonat (1996). The clinical signs of toxicity presented by the mice are:

i) Respiratory rate acceleration.
ii) Tremor.
iii) Immobilization of the animal.

According to the relationship table of clinical signs of intoxication and organs or Laroche et al. (1986) systems and Hachette (1989), one might infer that the total methanolic extract of *C. perpulchrum* have an effect on the respiratory system. This finding is in agreement with the work performed by Bidié (2005) (University of Cocody, Côte d'Ivoire, Thesis) on the effect of MYTA on rabbit breathing. According to the author, MYTA induced excessive degradation of lungs. It would also have an action on the neuromuscular system. The results obtained during the 14 days following the 24 h observation have shown that the total methanolic extract of *C. perpulchrum* given as a single dose, has a toxic action that extends over a period of 15 min to 3 days.

Beyond 24 h, mice that survived gradually recovered after 5 days. Given this observation, the product accumulates in the body and its elimination is difficult, resulting in a longer recovery time. This suggests that biotransformation of *C. perpulchrum* is slow, resulting in the accumulation or difficult elimination of metabolites. This could be the basis of damage to organs and tissues. The study of the subacute toxicity at doses 250 and 475 mg/kg bw in mice found the following clinical signs: weakening, immobilization, loss of appetite, respiratory rate increased of mice. These signs of intoxication lasted 2 to 3 days. This is due to the accumulation of *C. perpulchrum* in the body. The return to normal of mice treated after 3 days, compared to controls is evidence that animals have eliminated the product that had been administered. However, the deaths could be explained by the fact that these animals could not eliminate the entire product accumulated in the body in time. This suggests that the metabolism of *C. perpulchrum* is difficult. Furthermore, the results obtained with the enzymes (GOT, GPT, ALP, CPK and LDH) in serum after administration of *C. perpulchrum* in the doses of 250 and 475 mg/kg bw, showed that no significant change of activity occurred. This would allow us to say that the heart and liver would not have suffered damage. So, the not significant variation of the activity of enzymes in the blood serum could be the cause either of an ageing of tissues or cells, or an overactive muscles or a normal serum enzyme activity. Thus, *C. perpulchrum* would have a potential hepatoprotective effect. Nevertheless, we see an increase in urea, uric acid and creatinine in blood serum. While this increase is not very high it could be due to an accumulation of these metabolites in blood serum. This suggests that the kidneys have suffered a decline in kidney filtration, there by causing the non-renal elimination of waste.

Indeed, the decline in glomerular filtration is linked to lower blood pressure by carotid vessel described by Bidié et al. (2010). This would cause the accumulation of metabolites (uric acid, urea and creatinine) in blood serum. We conclude therefore that the kidneys have suffered a malfunction that is due to the moderately toxic effect of *C. perpulchrum*. Also, the results of cytotoxicity's study of *C. perpulchrum* on the human bronchial epithelial cells come to reinforce those obtained previously (acute and subacute toxicity). In fact, the respiratories troubles observed during the previous study would account for by the cytotoxicity induced by *C. perpulchrum* to the strong concentrations (10 mg/ml). To this concentration, the human bronchial epithelial cells undergo an inhibition of their proliferation as observed with the triton (positive witness). However, to the concentration of 1 mg/ml, the proliferation of bronchial epithelial cells is comparatively maximum to the cellular proliferation due to enriched domain. *C. perpulchrum* is not so cytotoxic. This result come to confirm that obtained with the study of rabbit's carotidian arterial. In fact, this study has shown that at 10 mg/kg bw (minimal dose inducing an hypotension), the methanolic total extract of

*C. perpulchrum* induces a light hypotension (09 mmHg) which last only 20 s. Thus there is no accumulation of the product in the organism. The toxicity of the product at this concentration is not so recognized. This affirmation is in harmony with Bouchet et al. (2004) when they studied the cytotoxicity of Fumonisine $B_1$ on porcine intestinal epithelial cells. These authors have shown that the cytotoxicity has been observed to a minimal concentration of 200 µM. This product according to them is so considered as non cytotoxic on the cells studied. In the present study, *C. perpulchrum* at the concentration of 1 mg/ml, corresponding to 1000 µg/ml does not present cytotoxicity for the same tested on human bronchial epithelial cells. Moreover, at this concentration, the proliferation of human bronchial epithelial cells is maximal. This concentration is widely superior to that obtained with the works of Bouchet et al. (2004). *C. perpulchrum* is not cytotoxic.

## Conclusion

The study of acute and subacute toxicity of *C. perpulchrum* has revealed that this plant is moderately toxic. Moreover, the evaluation of these effects on the blood formule shows that there is no notable variation of blood formule. Moreover, the biochemical parameters and the serum enzymes do not vary insignificatively. It is only observed for the kidney damage. The study of cytotoxicity has shown that *C. perpulchrum* is not cytotoxic. In an ulterior study, we propose a histopathological study to clear the damaging of kidneys enounced from the activity of serum enzymes.

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