

*Full Length Research Paper*

## **Evaluation of *Curcuma zerumbet* (Zingiberaceae) rhizome extracts sub-acute toxicity on Wistar rats**

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Plant extracts have been lately used by the population to treat various types of diseases, and this has been notably encouraged by the World Health Organization (WHO). *Curcuma zerumbet* (Zingiberaceae) belonging to the family of the Zingiberaceae, is herbaceous, perennial and, utilized by the population to treat gastric disorders. However, data on the subacute toxicity of this species are scarce in the literature. Therefore, the present work aimed to ascertain the subacute toxicity of different doses of the aqueous and hydroalcoholic extracts from *C. zerumbet*. These extracts were orally administered through gavage in Wistar rats for 28 consecutive days. This study followed the instructions put forth by Guideline 407 (subacute toxicity) of Organization for Economic Cooperation and Development (OECD). In specific pharmacological tests for acute and subacute toxicity in rats, it can be stated that *C. zerumbet* extracts doses greater than 5 g/kg neither caused mortality nor presented oral toxicity. Therefore, the extracts toxicity parameters analyzed in different doses on the groups of animals have shown no significant difference from those found in the control group. This allows one to conclude *C. zerumbet* (Zingiberaceae) rhizome aqueous and alcoholic extracts in high doses to harbor very low toxicity within a short time.

**Key words:** Curcuma, subacute toxicity, extracts.

### **INTRODUCTION**

The knowledge regarding the medicinal properties of plants has been passed and enhanced from generation to generation in rural and urban communities. The diversity of medicinal plant species has fostered the use of the ones harboring phytotherapy properties due to the belief that all that is "natural" causes no ill effects

(Oliveira et al., 2011). However, the use of plant extracts is not totally free of risks, since in addition to harbouring bioactive compounds, the same plant may contain toxic substances in different amounts and concentrations.

The species *Curcuma zerumbet* (Zingiberaceae) is a perennial herb belonging to the family Zingiberaceae,

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spontaneously occurring in Southeast Asia and Northeast India (DAS et al. 2013). In the state of Amazonas, this plant is found in some municipalities, being utilized in ornamentation or in the form of teas for treating gastric disorders (Castro et al., 2017).

In Brazil, most genera are used as ornamentation, food and pharmaceuticals. They stand out in the treatment of cervical cancer (Epstein et al., 2010), flatulencies (Alsarhan et al., 2012), gastritis (Neamsuvan, 2012), hepatic disorders (Singh et al., 2012), cough (Abe and Ohtani, 2013), rashes skin infections (Rahmatullah et al., 2011) hepatitis and Inflammation (Schaffer et al., 2011). *C. zerumbet* subacute toxicity pharmacological studies have shown very few scientific evidences. Thus, the toxicological study, regardless of pharmacological results, is essential on account of the existing ethnopharmacological and toxicological data. This is why the present work was undertaken with the objective to assess the subacute toxicity of *C. zerumbet* extracts, being orally administered to rats through gavage.

## MATERIALS AND METHODS

### Collection and herbization of *C. zerumbet* Roscoe (Zingiberaceae)

The *C. zerumbet* species samples were collected in the community of Tarumã-mirim, Ramal do Pau Rosa, Latitude 2°43'17"S, Longitude 60°08'19"W, located in the state of Amazonas/Brazil. The exsiccate was sent to the Botany unit of the National Research Institute of Amazonia (INPA) to be identified and deposited in its Herbarium under No. 265800. The rhizomes were hygienized, washed in running water and dried in an air circulating oven at 45°C for 24 h.

### How extracts were obtained

The aqueous extract was obtained by the infusion method using 50 g of *Curcuma* diluted in 1000 mL of distilled water, while the hydroalcoholic extract was obtained by the maceration method using 200 g of *Curcuma* in 2000 ml ethanol: water (1: 1) for one period of 72 h. After extractions, the extracts were dehydrated through lyophilization using the LS 3000 (TERRONI®) lyophilized apparatus for 48 h (Castro et al., 2017).

### Extracts sub-acute toxicity analysis

The determination of the pharmacological activity was done by a test model established by the World Health Organization (WHO) and in accordance with the technical Standards established and approved by the Commission for Ethics in Research on the Use of Animals (CEUA / INPA) under protocol number 003/2013; the animals used in the experiments were albino rats weighing 200 - 400 g, from the Central Bioterium of INPA.

The aqueous and hydroalcoholic extracts subacute toxicity was analyzed using male albino Wistar rats, from the Inpa's Central Bioterium. During the experimental period (28 days), the animals were kept in polypropylene boxes, under controlled photoperiod (12 h light / 12 h dark) at 23 ± 2°C. These animals were fed ration and water with extracts (aqueous or hydroalcoholic) *ad libitum* orally, through gavage (OECD, 2008b). During this period, we analyzed

the ration and water consumption variation as well as the body mass of the animals (OECD, 2008a). The animals were randomly divided into seven groups, with each group containing six rats, according to the following protocol: (i) Group 1: control; (ii) Group 2: fed with 100 mg/Kg of aqueous extract; (iii) Group 3: fed with 1000 mg/Kg of aqueous extract; (iv) Group 4: fed with 5000 mg/Kg of aqueous extract; (v) Group 5: fed with 100 mg/Kg of hydroalcoholic extract; (vi) Group 6: fed with 1000 mg/Kg of hydroalcoholic extract; (vii) Group 7: fed with 5000 mg/Kg of hydroalcoholic extract; After 28 days, the animals were submitted to 12 h of fasting, and blood collection by intracardiac puncture was performed after anesthesia with intraperitoneal administration of ketamine / xylazine (10/10 mg/kg). The blood was stored in collection tubes with 10% sodium EDTA and the following parameters were analyzed:

(i) Hematological: total leukocyte, neutrophils, lymphocytes, eosinophilia, monocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells distribution slope according to the erythrocyte volume (RDW-SD), mean red blood cell volume coefficient of variance (RDW-CV) and mean platelet volume (MPV) platelet count.

(ii) Biochemical: aspartate aminotransferase (AST or TGO), alanine aminotransferase (ALT or TGP), albumin, glucose, urea, creatinine, total cholesterol, triglycerides, uric acid.

At the end of the experimental period, all animals were sacrificed by decapitation and the following organs were removed, weighed, measured and observed macroscopically: liver, small intestine, kidneys, spleen, heart, and lung.

### Statistical analysis

Data were represented as means ± standard deviation of the means of five animals per group of experiment *in vivo*. The differences between the means were determined by one way analysis of variance (ANOVA), followed by the Tukey test. Analyses were performed using the Windows program, Graph Pad Prism version 6.0 (Graph Pad Software). The value of less than 0.05 was considered significant.

## RESULTS AND DISCUSSION

### Extracts sub-acute toxicity analysis

Subacute toxicity assessment is a widely used methodology to ascertain and classify substances as to their ability to cause acute damage to living organisms in high doses, especially pathological and pathological injuries, and may contribute to establish safety parameters - together with other toxicity data – pertaining to human health (Zatta et al., 2009). As regards plants, this method is useful for identifying the toxicity it may present and thereby prevent people from believing natural products to be devoid of toxic or adverse effects (Silveira et al., 2008; Cunha et al., 2009; Farsi et al., 2013). The present work, through experimental trials with rats being orally administered with aqueous and alcoholic extracts at doses of 100 to 5 g/kg, has demonstrated the lack of pharmacological parameters or toxicological effects prone to lead them to mortality. This indicates that extracts from *C. zerumbet* harbor very low toxicity. In Table 1, the results of the biochemical parameters

**Table 1.** Biochemical parameters of Wistar rats after oral application of Curcuma zerumbet rhizome aqueous and hydroalcoholic extract during 28 days of treatment.

Parameter	Groups						
	1	2	3	4	5	6	7
Glucose (mg/dL)	118±2.5	117±1.5	120±1.5	121±1.4	115±2.8	119±2.4	122±1.6
Cholesterol (mg/dL)	88 ±2.7	86±1.8	88±2.4	90±2.5	83±1.9	92±3.6	93±2.5
Triglycerides (mg/dL)	79±2.3	76±1.4	79±1.3	82.9±2.7	75±1.5	78±1.2	86±1.3*
Uremia (mg/dL)	37±4.4	45±2.4	40±2.0	46±1.5*	39±2.3	43±1.7	46±1.8*
Albumina (g/dL)	3.8±1.0	3.1±1.7	3.2±2.8	3.3±1.9	3.3±1.7	3.2±1.8	3.2±1.5
Uric Acid Úrico (mg/dL)	1.2±1.8	1.5±2.7	0.9±1.5	1.3±1.6	2.3±1.3	1.2±1.8	1.1±2.8
Creatinine (mg/dL)	0.78±1.8	0.67±1.5	0.75±1.2	0.69±1.8	0.55±1.0	0.76±1.8	0.79±1.9
TGP/ALT (U/L)	49±4.3	44±3.6	51±2.0	56±1.5	48±2.6	51±2.6	57±2.9
TGO/AST (U/L)	134±5.0	133±2.5	138±1.3	140±3.6	126±1.8	139±2.7	142±1.4

1, control; 2, dose of 100 mg/kg of the aqueous extract of the rhizome of *C. zerumbet*; 3, dose of 1000 mg / kg of the aqueous extract of the rhizome of *C. zerumbet*; 4, 5000 mg/ kg dose of *C. zerumbet* rhizome aqueous extract; 5, dose of 100 mg/kg of the hydroalcoholic extract of the rhizome of *C. zerumbet*; 6, dose of 1000 mg/Kg of the hydroalcoholic extract of the rhizome of *C. zerumbet*; 7, 5000 mg/kg dose of the hydroalcoholic extract of the rhizome of *C. zerumbet*; values expressed as mean ± S.D.1.

after the application of the extracts for a period of 28 days of experiment. Table 1 shows that the study groups total cholesterol did not differ significantly but triacylglycerol differ significantly when hydroalcoholic extract was administered orally at the dose of mg/kg whereas no change was observed when the rats were fed with the aqueous extract.

This finding may be both related to hydroalcoholic extract concentration and the animals feeding, since the latter was not controlled.

Urea and/or creatinine are eliminated by the kidneys and; in chronic kidney disease there is reduced functioning of the kidneys, with both substances being accumulated in the blood (Dusse and Freitas, 2015). Many factors can significantly change the plasma urea values without being related to the renal function especially protein-rich diet, such as the one found in rodents ration. Elevated urea values in conjunction with creatinine indicate processes that lead to decreased renal blood flow or to gastrointestinal bleeding with obstructive post-renal processes such as tumors and stenosis of the urinary tract (Sodré et al., 2007). Since there were no significant increases on urea levels, uremia is possibly related to total protein content and not to renal disease. Proteins are degraded to urea and as still observed in Table 1. The latter had their levels significantly increased for groups (4) and (7) as compared to control (1).

Renal (pre-renal, renal and post-renal) and extra-renal (protein intake) factors may interfere with increased serum urea concentration. Therefore, this increase in urea levels in the animals that consumed higher doses of the extracts, both aqueous and hydroalcoholic, may be suggested by extra renal factors such as the ingestion of dietary protein by animals, since urea is the main end product of protein catabolism or toxicity index for the

high dose groups of aqueous and hydroalcoholic extracts.

Plasma albumin is a good indicator of nutritional status and, in this case, the values obtained in this research are within the normality standards for the species under study. According to the literature, healthy rats bear albumin content in the range of 3.4 to 4.3 g dL<sup>-1</sup> (Gautier et al., 2014). There was no effect on AST and ALT levels, which are considered sensitive indicators of hepatocellular damage and when within limits may provide a quantitative assessment of the degree of liver damage (Al-habori et al., 2002). Therefore, it is possible to deduce, that the aqueous and hydroalcoholic extract caused no damage to the kidneys or liver, at doses below 5000 mg / kg. The hematological parameters assessment can be used to determine the extent of deleterious effects brought about by strange compounds, including plant extracts, on an animal's blood constituents (Ashafa et al., 2012).

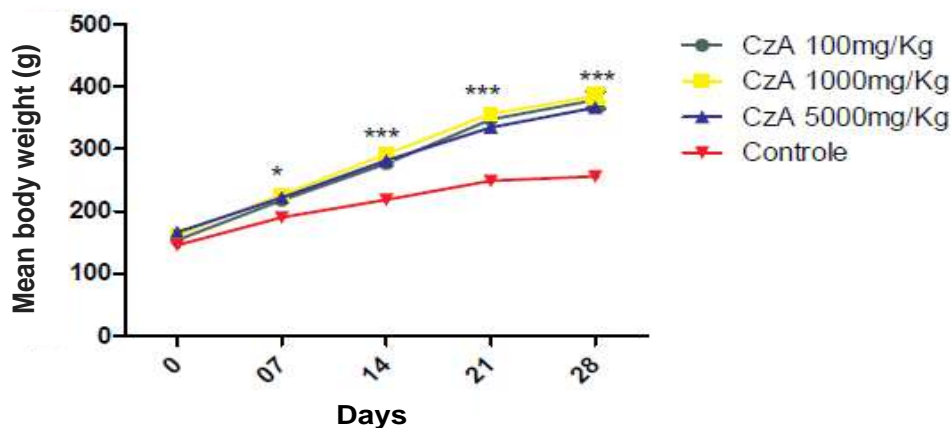
Low hemoglobin, erythrocytes and hematocrit concentrations may indicate anemia, recent bleeding or fluid retention, causing hemodilution. Yet, a decreased platelet count (thrombocytopenia) can result from a series of pathological situations, such as the increased destruction of these cells, due to the use of certain drugs, immune disorders, disseminated vascular coagulation and even mechanical lesions (Dougan et al., 2008). The leukocyte differential is used to assess the distribution and morphology of white blood cells, providing more specific information about the immune system than the leukocyte count alone does (Prinyakupt and Pluempitiwiriyaewej, 2015).

Hematological parameters were measured in this study after 28 days of administration of the extracts. The treated animals showed no significant decreases in red blood cell, hemoglobin, hematocrit and platelets levels (Table 2) and the differential count of white cells and lymphocytes, as compared to control's.

**Table 2.** Wistar rats hematological parameters after oral application of the aqueous and hydroalcoholic extract during 28 days of experiment.

Parameter	Groups						
	1	2	3	4	5	6	7
Leu. ( $10^3/\mu\text{L}$ )	8.8±2.5	8.2±1.9	7.0±2.7	5.3±2.9	9.4±1.4	9.4±1.5	9.5±1.5
Hem. ( $10^6/\mu\text{L}$ )	6.3±1.6	5.8±2.6	5.9±1.3	5.6±1.5	6.4±1.4	6.1±1.6	5.9±1.3
Hemo. (g/dL)	13.9±1.5	13.3±2.2	13.9±2.0	12.7±1.3	14.6±2.0	14.3±2.0	13.6±1.6
Hema. (%)	37.7±1.5	37.1±1.9	38±2.0	35.9±2.1	40.6±1.7	39.5±2.4	38.4±1.4
M.C.V. (f)	59±2.5	63.8±1.7	63±1.3	63.2±1.6	63.4±2.3	62.5±1.9	63.7±1.6
M.H.C. (pg)	21.7±1.9	22.8±2.3	23.3±2.4	22.3±1.7	22.8±1.4	23.3±1.6	22.9±2.0
M.C.H.C. (g/dL)	36.8±1.6	35.8±1.9	36.5±1.4	35.3±1.6	35.9±1.7	36.2±1.8	35.4±1.4
R.D.W. (%)	12.1±2.0	13±1.3	12.9±1.6	13.6±2.0	12.7±1.4	12.6±1.5	13.9±1.6
Plat. ( $10^3/\mu\text{L}$ )	899±1.5	711±1.5	722±1.5	773±1.4	723±1.5	669±1.5	906±1.5
Neu. (%)	11.4±2.3	12±2.6	12.1±1.7	14±1.4	13±0.3	12±0.7	11.9±1.4
Lymph. (%)	81.2±1.7	77.0±2.2	80.7±1.9	77±1.4	77±0.8	76±1.5	82.2±2.7
Mon.(%)	6.5±1.3	3±1.1	7.0±1.4	5±2.3	5±1.6	5±1.7	3.3±1.5
Eus. (%)	0.6±0.6	1±0.5	0.0±0.2	0.1±1.5	0.0±1.0	0.1±2.0	0.4±2.4
Bas.	0.3±0.4	0.0±1.0	0.2±0.1	0.0±1.2	0.0±0.2	0.0±1.7	0.2±1.7

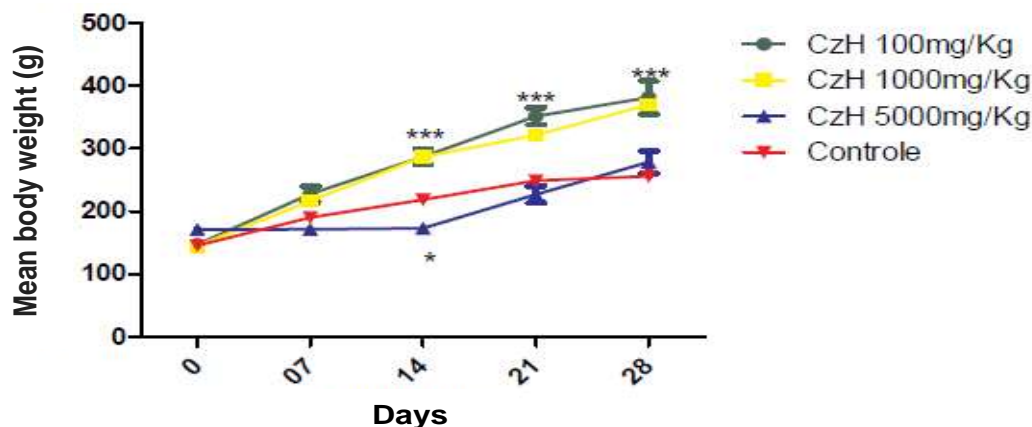
1, control; 2, Dose of 100 mg/kg of the aqueous extract of the rhizome of *C. zerumbet*; 3, dose of 1000 mg / kg of the aqueous extract of the rhizome of *C. zerumbet*; 4, 5000 mg/ kg dose of *C. zerumbet* rhizome aqueous extract; 5, dose of 100 mg/kg of the hydroalcoholic extract of the rhizome of *C. zerumbet*; 6, dose of 1000 mg/Kg of the hydroalcoholic extract of the rhizome of *C. zerumbet*; 7, 5000 mg/kg dose of the hydroalcoholic extract of the rhizome of *C. zerumbet*; values expressed as mean  $\pm$  S.D.1.

**Figure 1.** Animals body mass assessment for 28 days of oral treatment with aqueous extract from *Curcuma zerumbet*.

In the study by Hossen et al. (2017), the MCV and HCM concentration levels increased when animals were fed for a period of 28 days with species *Curcuma Longa*. While the other hematological data remained unchanged. In this study, this was not observed. In the study by Salama et al. (2013) this species biochemical and hematological presented no significant difference between the control groups. Thus, corroborating the findings put forth by the present work.

For daily habits (feed consumption and fecal production), there was no significant variation between the groups treated with the aqueous and hydroalcoholic

extract and the control group. However, there was change in the body mass parameter, when compared to that shown by the control. The mean body mass presented by the animals treated with the aqueous extract at doses of 100, 1000 and 5000 mg/Kg increased from  $155 \pm 5.8$  to  $363 \pm 7.8$ ;  $166 \pm 8.4$  to  $370 \pm 7.5$  and  $166 \pm 6.9$  to  $350 \pm 8.7$  respectively; while that presented by the control increased from  $145 \pm 5.7$  to  $241 \pm 6.5$  showing a statistical difference from the seventh day of application onwards (Figure 1) while the mean body mass of the animals observed in the hydroalcoholic extract experiment increased from  $147 \pm 8.9$  to  $366 \pm 7.8$



**Figure 2.** Animals body mass assessment for 28 days of oral treatment with the hydroalcoholic extract from *C. zerumbet*.

at the dose of 100 mg / kg;  $144 \pm 8.8$  to  $345 \pm 9.8$  at the dose of 1000 mg / kg and  $171 \pm 7.0$  to  $252 \pm 7.8$  at the dose of 5000 mg / kg. These findings may be related to the extracts-borne protein and carbohydrate levels. Figure 2, shows the animals to present a significant difference in the body mass from the 14<sup>th</sup> day of the experiment onwards, as compared to that of the control at doses of 100 and 1000 mg/kg. The animals evaluated at the dose of 5000 mg/kg exhibited a constant body mass in the first weeks of the experiment. They exhibited the same gain of body mass as that of control animals, from the 14th day onwards. It is known that systemic toxicity can be identified by the decrease in the body mass of the animals and by changes in water and feed intake, which have shown to be paramount when undertaking a substance or extract's toxicity assessment, since it provides data pertaining to the animals' health status as a whole (Valadares, 2006). Nevertheless, these animals showed no changes on their food and water intake. The macroscopically analyzed and weighed organs exhibited no statistical differences between the control groups and the experimental doses. Thus, the tested *C. zerumbet* extracts showed no evidence of harboring any toxicity.

## Conclusion

It can be concluded that the aqueous and hydroalcoholic extract from the rhizome of *C. zerumbet* (Zingiberaceae) harbors low, short term toxicity in high doses. In cases of prolonged use, further studies are required to ascertain the outcome of such histopathologic changes.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENT

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