



## Acute and sub-acute toxicity of *Pithecellobium dulce* (Roxb.) Benth. stem bark hydroalcoholic extract on Wistar rats

[Toxicidad aguda y subaguda del extracto hidroalcohólico de la corteza del tallo de *Pithecellobium dulce* en ratas Wistar]

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### Abstract

**Context:** *Pithecellobium dulce* (PD) is an annual herbaceous plant commonly used in African traditional medicine as a purgative, antipyretic, anti-ulcer and wound dressing agent.

**Aims:** To evaluate the acute and sub-acute toxicity of *P. dulce* stem bark hydroethanolic extract in Wistar rats.

**Methods:** In the acute test, a single dose of 5 g/kg body weight was administered to Wistar rats afterwards they were observed individually 4 hours post-dosing, and at least once daily for 14 consecutive days. The sub-acute toxicity was evaluated by daily oral administration of 0.5 and 1 g/kg extract, for 28 days. Biochemical and hematological parameters assessment as well as body and organ weights of the rats were carried out.

**Results:** The limit dose of 5 g/kg did not cause any mortality or signs of acute toxicity on the rats during the experimentation period. In the sub-acute test, uterus-ovary-trompe (UOT) weight decreased dose-dependently: Control group (0.82 ± 0.03 g); Extract 0.5 g/kg (0.57 ± 0.06 g); Extract 1g/kg (0.48 ± 0.01 g) (p < 0.01). Extract lowered urea values in female group treated with 1 g/kg (p < 0.01). Lymphocytes percentage was dose dependently increased in treated male groups: Control group (53.00 ± 0.58%); extract 0.5 g/kg (58.67 ± 0.67%) and extract 1 g/kg (60.67 ± 2.41%).

**Conclusions:** These findings suggest that PD is relatively safe when administered orally in rats but is slightly atrophic for female reproductive organs.

**Keywords:** animal model; medicinal plants; pharmacology; toxicology.

### Resumen

**Contexto:** *Pithecellobium dulce* (PD) es una planta herbácea anual comúnmente utilizada en la medicina tradicional africana como purgante, antipirético, anti-ulceroso y cicatrizante.

**Objetivos:** Evaluar la toxicidad aguda y subaguda del extracto hidroetanólico de la corteza del tallo de *P. dulce* en ratas Wistar.

**Métodos:** En el ensayo agudo se administró una dosis única de 5 g/kg de peso corporal a ratas Wistar, que se observaron individualmente 4 horas después de la dosificación y, al menos, una vez al día durante 14 días consecutivos. La toxicidad subaguda se evaluó mediante administración oral diaria de 0,5 y 1 g/kg de extracto, durante 28 días. Se realizó la evaluación de los parámetros bioquímicos y hematológicos, así como los pesos de órganos y órganos de las ratas.

**Resultados:** La dosis límite de 5 g/kg no causó muerte o signos de toxicidad aguda en las ratas durante el período de experimentación. En la prueba subaguda, el peso del útero-ovario-trompa (UOT) disminuyó dependiendo de la dosis: Grupo control (0,82 ± 0,03 g); Extracto 0,5 g/kg (0,57 ± 0,06 g); Extracto 1 g/kg (0,48 ± 0,01 g) (p < 0,01). El extracto disminuyó los valores de urea en el grupo de las hembras tratado con 1 g/kg (p < 0,01). El porcentaje de linfocitos aumentó de forma dependiente de la dosis en los grupos masculinos: Grupo control (53,00 ± 0,58%); Extracto 0,5 g/kg (58,67 ± 0,67%) y Extracto 1 g/kg (60,67 ± 2,41%).

**Conclusiones:** Estos hallazgos sugieren que *P. dulce* es relativamente segura cuando se administra oralmente en ratas, pero es ligeramente atrófica para los órganos reproductores femeninos.

**Palabras Clave:** farmacología; modelo animal; plantas medicinales; toxicología.

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## INTRODUCTION

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*Pithecellobium dulce* (Roxb.) Benth is one of numerous plants used by ethnomedicine practitioners in Togo to overcome various diseases. It has been commonly used for fencing and tanning, as fodder for feed and pods for food (Manna et al., 2011; Radji et al., 2011). It is an evergreen medium sized, branched, spiny tree that reaches 22 meters heights (Orwa et al., 2009) and noted for its tolerance of heat, drought, salinity and impoverished soils (Raju and Jagadeeshwar, 2014). The plant is well known for its edible fruits and they have been consumed for various ailments in a traditional manner (Kaviyaran et al., 2014). All plant parts elaborate a vast array of biologically active compounds and have been demonstrated to exhibit antidiabetic, locomotor, anti-venom, free radical scavenging, protease inhibitor, anti-inflammatory, anti-bacterial, anti-dysentery, anti-mycobacterial, abortifacient, spermicidal, anti-convulsant, anti-ulcer, anti-diarrheal, anti-fungal, anti-tubercular, anti-tumor and anti-oxidative properties (Manna et al., 2011; Sukantha et al., 2011; Megala and Geetha, 2012b; Shweta and Mehta, 2013; Mule et al., 2016) but no significant information about its stem bark is available (Katekhaye and Kale, 2012). Information about safe use of the stem bark is poor even if data about edible plant parts toxicity are available (Megala and Geetha, 2012a). This study was planned to determine the acute and sub-acute toxic effect of stem bark hydro-ethanolic extract of *Pithecellobium dulce* Benth.

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## MATERIAL AND METHODS

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### Plant material

The stem bark of *Pithecellobium dulce* was collected at the Klikame district (Lomé) (geographic coordinates: 6°33'0" N 1°39'0" E ~67 m.a.s.l.) in September 2014. The plant materials were botanically authenticated by Professor Komlan Batawila at the department of Plant Science and Ecology of the University of Lomé (Togo) where specimens were deposited (herbarium specimen number: TOGO 04216). Plant samples were dried in laboratory at room temperature and reduced into powder to pass a sieve of 1 mm. The powder was then submitted to

percolation with ethanol-water (70:30, V/V) for 72 h. The mix was filtered through Whatman 1 paper and evaporated to dryness under vacuum with an evaporator type Heidorph (Hei-VAP, Germany).

### Animals

Female Wistar rats (90-120 g) and male (150-180 g) provided by the Department of "Microbiology Laboratory and Quality Control of Foodstuffs" of University of Lomé (Togo) were used. They were housed in a standard environmental condition and fed with rodent standard diets and water *ad libitum*. They were kept under alternative cycle of 12 h of light and darkness, at  $26 \pm 2^\circ\text{C}$ . The study was approved by the Committee for Animal Experimentation Ethics of ESTBA-UL (N° 0002/2016-04/ESTBA-UL) A minimum number of animals were used to obtain reliable results in respect to (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996).

### Acute toxicity

The animals were fasted overnight with water at free access. For the acute toxicity, they were randomly divided in two groups of males ( $n = 5/\text{group}$ ) each sequentially dosed at intervals of 48 h. The control group received orally distilled water. The test group was orally treated with stem bark hydroethanolic extract of *Pithecellobium dulce* with unique dose of 5 g/kg. Animals were observed for general behavioral and body weight changes, hazardous symptoms and mortality within a period of 4 hours for immediate signs of toxicity and at least once daily for 14 days for delayed signs of toxicity (Diallo et al., 2014). After anesthesia by inhalation with ether, the spinal-cord of rats was dislocated by stretching.

### Sub-acute toxicity

Repeat-dose oral toxicity study was carried out according to OECD guideline 407 (OECD, 2008) with slight modification. The animals were divided into three groups of 6 animals each (3 males and 3 females). Group 1 received 10 mL/kg body weight of distilled water and served as control. Groups 2 and 3 received extract doses of 0.5 and 1 g/kg body

weight, respectively. The extract was administered daily for 28 days the same time and observed at least twice daily for morbidity and mortality. Body weights of the animals were evaluated daily. On the 29<sup>th</sup> day, after an overnight fast, the rats were anaesthetized with ether and blood samples were obtained by retro-orbital puncture, using capillary tubes for hematological and biochemical studies, with and without EDTA, respectively. Blood tubes without EDTA were centrifuged at  $1500 \times g$  for 10 min to obtain serum stored at  $-20^{\circ}\text{C}$  until use (Diallo et al., 2014).

### Morphological study

Necropsy of all animals was carried out to analyze the macroscopic external features of heart, lungs, liver, kidney, spleen and reproductive organs (uterus-ovary or testicles) were recorded. These organs were carefully removed and weighed individually with analytical balance (AE Adam, Model AAA 300L, Adam equipment).

### Hematological and biochemical analysis

Hematological analysis was performed using an automated hematological analyzer (Sysmex KX21, Tokyo, Japan). This included: red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets count (PT). The differential leukocyte count was performed with an optical microscopy (Olympus CH 30 Made in Japan) after staining blood smear (fixed 3 min by MayGrünwald and colored during 20 min with Giemsa hematological stain from Cypress Diagnostic Langdorp, Belgium) and, in each case, 100 cells were counted (Silva et al., 2011).

Biochemical analysis has been done on stored sera, the following parameters were determined: glucose, blood urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, triglycerides, alkaline phosphatase (ALP), total proteins, chloride, potassium and sodium. Dosages were possessed using MINDRAY BS 200 automated biochemistry analyzer with Mindray clinical chemistry reagent kits (Shenzhen Mindray Biomedical Electronics SN WN-

34104444 Co. Ltd GmbH Europe) from the same laboratory (Silva et al., 2011).

### Statistical analysis

Values were expressed as mean value  $\pm$  standard deviation). The data were analyzed by one-way analysis of variance (ANOVA) followed by *post hoc* Fisher's Least-Significant-Difference test using least squares means matrix of pairwise comparison probabilities with SYSTAT11 software. Statistical significance was set at  $p < 0.05$ . Graphs were plotted with GraphPad Prism 6.1 software®.

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## RESULTS

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### Acute toxicity

After the rats were orally given a single dose of hydroethanolic stem bark extract of *Pithecellobium dulce* at 5 g/kg, no deaths or hazardous signs were recorded for a period of 4 h and during 14 days of observation when compared to the control group.

### Subacute toxicity

Animals in all groups survived up to 28 days without any apparent adverse symptoms such as piloerection, alteration in the locomotor activity or food and water consumption. Fig. 1A showed the weight variation expressed in grams during the periods of treatment recorded for males Wistar rats. In the first week of gavage there was no variation in all groups. All animals started gaining weight from the second week. The same weight evolution between the two groups treated with different dosages of hydroethanolic extract of PD was observed. As showed on Fig. 1B, after seven days, the two-treated group lost weight, especially the one treated with PD extract at dose 1 g/kg, but no significant statistical difference was observed when compared with control group ( $p > 0.05$ ).

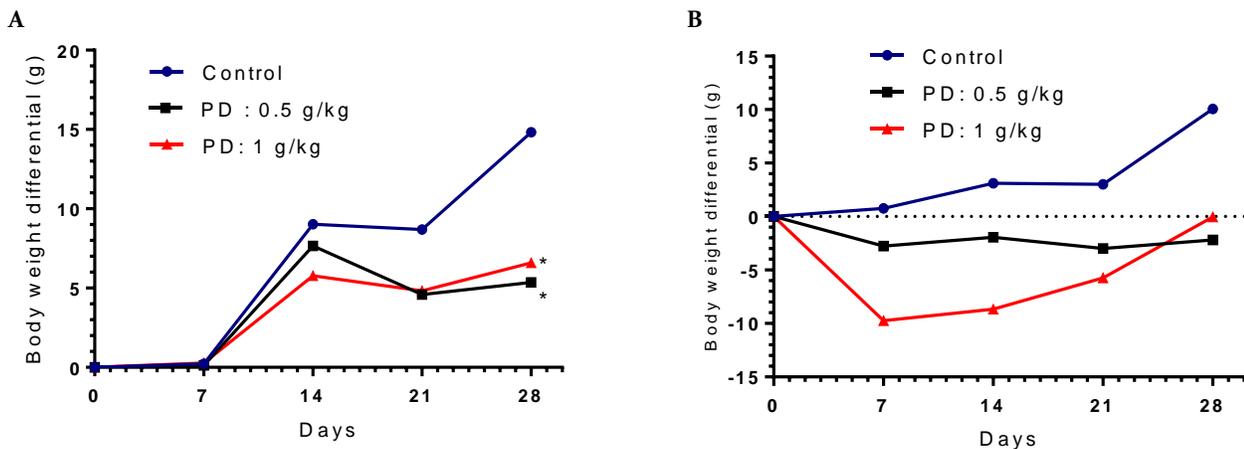
Organs weights are presented on Table 1 for male Wistar rats. Kidneys weight increased in group treated with PD extract at 0.5 g/kg ( $p < 0.05$ ). Testicles weight decreased in group that received PD 0.5 g/kg but the difference with the control group was not statistically significant ( $p > 0.05$ ).

Organs weight for three groups of female Wistar rats were recorded on Table 2. When compared to

the control group, lungs weight decreased from  $0.93 \pm 0.01$  g to  $0.73 \pm 0.01$  g ( $p < 0.01$ ). The decreasing values were also observed with the same statistical significant difference in UOT for both treated groups dose-dependently: Control group ( $0.82 \pm 0.03$  g); PD 0.5 g/kg ( $0.57 \pm 0.06$  g); PD 1 g/kg ( $0.48 \pm 0.01$  g).

Table 3 contains data about biochemical parameters of male Wistar rat under effect of PD extract. Results showed significant increase in total cholesterol levels at the dose of 0.5 g/kg b.w. ( $p < 0.01$ ).

On Table 4, biochemical parameters were recorded on Wistar female rat. The hydroethanolic stem bark extract of *Pithecellobium dulce* lowered urea values in group treated with dose 1 g/kg ( $p < 0.01$ ). Table 5 contains the hematological parameters recorded on male Wistar rats. Lymphocytes percentage count dependently increased in both treated groups: Control group ( $53.00 \pm 0.58\%$ ); PD 0.5 g/kg ( $58.67 \pm 0.67\%$ ) and PD 1 g/kg ( $60.67 \pm 2.41\%$ ). However, the same hematological parameters were noted modified in female groups (Table 6).



**Figure 1.** Body weight loss of male (A) and female (B) rats administered with two doses of hydroethanolic stem bark extract of *Pithecellobium dulce* (PD, 0.5 and 1 g/kg) by oral route for 28 consecutive days.

The values were expressed as mean  $\pm$  SEM ( $n = 3$  animals/group). \* $p < 0.01$  in (A) when compared to the controlled group. In (B) there was no significant statistical difference ( $p > 0.05$ ).

**Table 1.** Effects of hydroethanolic stem bark extract of *Pithecellobium dulce* on male Wistar rat organs.

Organs	Control	Extract dose (g/kg)	
		0.5	1
Liver	$5.43 \pm 0.67$	$5.39 \pm 0.17$	$5.39 \pm 0.30$
Lungs	$0.99 \pm 0.02$	$1.03 \pm 0.01$	$1.68 \pm 0.55$
Heart	$0.60 \pm 0.06$	$0.62 \pm 0.02$	$0.63 \pm 0.05$
Testicles	$2.20 \pm 0.12$	$1.26 \pm 0.02$	$1.83 \pm 0.47$
Kidneys	$1.15 \pm 0.05$	$1.91 \pm 0.27^*$	$1.15 \pm 0.07$
Spleen	$0.41 \pm 0.08$	$0.41 \pm 0.01$	$0.34 \pm 0.01$

\* $P < 0.05$  vs. control (one-way analysis of variance (ANOVA) followed by Fisher's Least-Significant-Difference Test). Values represent the mean  $\pm$  SEM ( $n = 3$ /group).

**Table 2.** Effects of hydroethanolic stem bark extract of *Pithecellobium dulce* on female Wistar rat organs.

Organs	Control	Extract dose (g/kg)	
		0.5	1
Liver	3.86 ± 0.10	3.64 ± 0.29	3.71 ± 0.16
Lungs	0.93 ± 0.01	0.73 ± 0.01*	0.97 ± 0.05
Heart	0.47 ± 0.03	0.85 ± 0.30	0.45 ± 0.02
UOT	0.82 ± 0.03	0.57 ± 0.06*	0.48 ± 0.01*
Kidneys	0.97 ± 0.05	0.92 ± 0.01	0.90 ± 0.03
Spleen	0.30 ± 0.03	0.35 ± 0.02	0.26 ± 0.01

UOT: uterus-ovary-trompe; \* $p < 0.01$  vs. control (one-way analysis of variance (ANOVA) followed by Fisher's Least-Significant-Difference Test). Values represent the mean ± SEM (n = 3/group).

**Table 3.** Effects of hydroethanolic stem bark extract of *Pithecellobium dulce* on male Wistar rat biochemical parameters.

Biochemical parameters	Control	Extract dose (g/kg)	
		0.5	1
Glucose (g/L)	1.03 ± 0.03	1.01 ± 0.05	1.05 ± 0.04
Urea (g/L)	0.28 ± 0.03	0.24 ± 0.01	0.23 ± 0.04
Creatinine (mg/dL)	0.30 ± 0.00	0.33 ± 0.02	0.30 ± 0.01
Uric acid(g/L)	0.51 ± 0.03	0.61 ± 0.01	0.64 ± 0.03
AST (U/L)	98.00 ± 2.65	104.67 ± 1.45	103.33 ± 5.24
ALT (U/L)	43.67 ± 3.39	54.67 ± 9.88	44.33 ± 3.29
Total cholesterol(g/L)	0.65 ± 0.01	0.89 ± 0.05*	0.67 ± 0.01
Triglycerides(g/L)	0.59 ± 0.08	0.63 ± 0.06	0.79 ± 0.08
Alkaline phosphatase (U/L)	244.33 ± 31.71	181.33 ± 23.41	211.67 ± 15.74
Total protein (g/dL)	9.13 ± 0.79	8.19 ± 0.22	8.02 ± 0.31
Potassium(mmol/L)	3.83 ± 0.03	4.01 ± 0.14	4.02 ± 0.09
Sodium (mmol/L)	145.07 ± 0.15	145.17 ± 0.56	146.90 ± 0.12
Chlorine (mmol/L)	99.17 ± 0.46	100.27 ± 0.47	103.67 ± 0.92
Magnesium (mg/dL)	2.76 ± 0.09	2.22 ± 0.09	3.00 ± 0.32
Calcium (mg/dL)	9.24 ± 0.62	9.57 ± 0.33	9.72 ± 0.04

Values represent the mean ± SEM (n = 3/group). \* $P < 0.01$  vs. control (one-way analysis of variance (ANOVA) followed by Fisher's Least-Significant-Difference Test).

**Table 4.** Effects of hydroethanolic stem bark extract of *Pithecellobium dulce* on female Wistar rat biochemical parameters.

Biochemical parameters	Control	Extract dose (g/kg)	
		0.5	1
Glucose (g/L)	0.89 ± 0.05	0.91 ± 0.07	0.95 ± 0.03
Urea (g/L)	0.23 ± 0.01	0.22 ± 0.02	0.15 ± 0.01*
Creatinine (mg/dL)	0.40 ± 0.01	0.39 ± 0.04	0.31 ± 0.01
Uric acid (g/L)	0.83 ± 0.01	0.77 ± 0.09	0.79 ± 0.01
AST (U/L)	107.67 ± 13.73	101.67 ± 9.97	107.67 ± 5.55
ALT (U/L)	44.33 ± 5.70	35.00 ± 8.75	61.67 ± 1.86
Total cholesterol (g/L)	0.62 ± 0.01	0.62 ± 0.02	0.70 ± 0.06
Triglycerides (g/L)	0.67 ± 0.07	0.56 ± 0.04	0.50 ± 0.01
Alkaline phosphatase (U/L)	194.67 ± 25.30	196.00 ± 55.26	298.67 ± 7.87
Total protein (g/dL)	8.48 ± 0.25	8.59 ± 0.30	8.28 ± 0.04
Potassium (mmol/L)	3.98 ± 0.05	3.77 ± 0.01	4.02 ± 0.01
Sodium (mmol/L)	144.80 ± 0.25	144.90 ± 0.12	144.17 ± 0.61
Chlorine (mmol/L)	99.63 ± 0.90	101.03 ± 1.30	100.37 ± 0.73
Magnesium (mg/dL)	2.72 ± 0.28	3.00 ± 0.49	2.34 ± 0.03
Calcium (mg/dL)	9.87 ± 0.37	10.02 ± 0.89	9.75 ± 0.05

Values represent the mean ± SEM (n = 3/group); \*P < 0.01 vs. control (one-way analysis of variance (ANOVA) followed by Fisher's Least-Significant-Difference Test)

**Table 5.** Effects of hydroethanolic stem bark extract of *Pithecellobium dulce* on male Wistar rat hematological parameters.

Parameters	Control	Extract dose (g/kg)	
		0.5	1
WBC (10 <sup>3</sup> μL)	8.57 ± 1.15	8.47 ± 0.52	9.30 ± 1.16
RBC (10 <sup>6</sup> μL)	8.43 ± 0.25	7.98 ± 0.11	8.57 ± 0.23
Hemoglobin (g/dl)	14.27 ± 0.19	13.90 ± 0.10	14.53 ± 0.32
Hematocrit (%)	52.13 ± 1.26	47.83 ± 1.09	52.43 ± 1.10
MCV (fl)	61.83 ± 0.49	59.97 ± 0.73	61.23 ± 0.51
MCH (pg)	16.93 ± 0.43	17.43 ± 0.13	16.97 ± 0.12
MCHC (%)	27.40 ± 0.66	29.20 ± 0.50	27.73 ± 0.03
Platelets (10 <sup>3</sup> μl)	787.33 ± 18.52	823.00 ± 70.38	800.67 ± 27.72
Neutrophils (%)	41.00 ± 0.58	35.67 ± 5.05	33.33 ± 3.53
Lymphocytes (%)	53.00 ± 0.58	58.67 ± 0.67*	60.67 ± 2.41*
Eosinophil (%)	1.00 ± 0.58	0.00 ± 0.00	0.67 ± 0.67
Monocytes (%)	5.00 ± 0.58	3.33 ± 0.67	5.33 ± 0.67

Values represent the mean ± SEM (n = 3/group); \*P < 0.05 vs. control. WBC: White blood cells; RBC: Red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration

**Table 6.** Effects of hydroethanolic stem bark extract of *Pithecellobium dulce* on female Wistar rat hematological parameters.

Parameters	Control	Extract dose (g/kg)	
		0.5	1
WBC ( $10^3 \mu\text{L}$ )	7.17 $\pm$ 0.38	7.33 $\pm$ 0.73	7.13 $\pm$ 0.39
RBC ( $10^6 \mu\text{L}$ )	8.00 $\pm$ 0.12	7.93 $\pm$ 0.39	8.60 $\pm$ 0.03
Hemoglobin (g/dL)	14.13 $\pm$ 0.29	14.07 $\pm$ 0.45	14.53 $\pm$ 0.18
Hematocrit (%)	50.93 $\pm$ 1.18	49.30 $\pm$ 2.37	51.77 $\pm$ 1.20
MCV (fL)	63.67 $\pm$ 1.15	62.23 $\pm$ 0.59	60.20 $\pm$ 1.58
MCH (pg)	17.67 $\pm$ 0.12	17.77 $\pm$ 0.38	16.90 $\pm$ 0.23
MCHC (%)	27.77 $\pm$ 0.63	28.60 $\pm$ 0.72	28.13 $\pm$ 0.77
Platelets ( $10^3 \mu\text{L}$ )	835.33 $\pm$ 32.34	857.00 $\pm$ 81.55	792.00 $\pm$ 42.30
Neutrophils (%)	31.67 $\pm$ 4.85	37.00 $\pm$ 4.59	38.67 $\pm$ 3.53
Lymphocytes (%)	64.33 $\pm$ 4.85	58.67 $\pm$ 5.70	57.33 $\pm$ 3.39
Eosinophils (%)	0.33 $\pm$ 0.33	1.00 $\pm$ 0.58	1.00 $\pm$ 0.58
Monocytes (%)	3.67 $\pm$ 0.33	3.33 $\pm$ 0.67	3.00 $\pm$ 0.58

Values represent the mean  $\pm$  SEM (n = 3/group). MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell; RBC: Red blood cells.

## DISCUSSION

Medicinal plants and their derivatives have been used as an alternative to allopathic medicines in many countries (Silva et al., 2011). Despite the wide usage, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies (Graça et al., 2007). *Pithecellobium dulce* fruit and leaves have been studied for their pharmacological properties in accordance with the high use of these organs (Hosmani, 1995). In Hawaii and India, it has a reputation as a pest in grass pastures and still in use as food (sweet pods) (Katekhaye and Kale, 2012; Shweta and Metha, 2013). The present study was designed to evaluate the toxicity of the bark extract on Wistar rats. In the traditional pharmacopoeia, *Pithecellobium dulce's* stem bark reduced into powder percolated in alcohol or water is used to handle fever and dysentery, wounds chancres, pains and convulsions (Nagmoti et al., 2012). As reported by Pithayanukul et al. (2005), PD inhibited *Naja kaouthia* venom activities by precipitating venom proteins due to condensed tannins. In fact, the hydroethanolic stem bark extract contains high amounts of phenolic compounds including, flavonoids, proan-

thocyanidins. The bark yields 37% tannins of the catechol type while the leaves yield quercetin, kaempferol, dulcitol and afezilin (Zapesochnaya et al., 1980).

The results of the acute toxicity study indicate that the LD<sub>50</sub> of *Pithecellobium dulce* stem bark extract is higher than 5 g/kg. According to Kennedy et al. (1986) who stated that substances with LD<sub>50</sub> higher than 5.0 g/kg by oral route may be considered practically non-toxic, *Pithecellobium dulce* stem bark is non-toxic. The limit test is primarily used in situations where the investigator has information indicating that the test material is likely to be non-toxic or of low toxicity (OECD, 2002). According to previous reports, *Pithecellobium dulce* fruit extract had a LD<sub>50</sub> value of 3916 mg/kg (Megala and Geetha, 2012a; Shweta and Mehta, 2013), suggesting that the stem bark could be less toxic than the fruit.

In the sub-acute toxicity study with female groups, the decrease of body weight observed at the beginning of the experiment could be explained by stress caused by gavage needle daily introduced into their esophagus. This stress has also been observed in male groups during the first week of treatment but there was no weight loss due prob-

ably to their physiology. The major observation was that at the end of treatment, there was no weight gain in female groups. This finding can further be confirmed and let to use the plant extract as to moderate body weight gain especially in female. More research program will be needed for clarification of enzymes involved in those mechanisms.

Analysis of blood parameters is relevant for risk evaluation, as any changes in the hematological and biochemical systems have a higher predictive value for toxicity (Olson et al., 2000). Since there was no effect on the levels of transaminases and creatinine, which are good indicators of liver and kidney functions respectively; it is reasonable to suggest that the *Pithecellobium dulce* stem bark hydroethanolic extract did not induce any damage to the liver and the kidneys and the fact that there was no significant effect on plasma cholesterol levels (Giknis and Clifford, 2008), suggesting a normal function of the liver (Hilaly et al., 2004). Data from Sugumaran and Vetrichelvan, (2008) study on *Pithecellobium dulce*'s fruit extract has shown a significant decrease in the cholesterol, phospholipids and triglyceride levels in the serum. The present results suggested that there was no dose-dependent significant effect of *Pithecellobium dulce* stem bark hydroethanolic extract on those biochemical parameters.

Increasing of kidney weight observed in male Wistar rat group with PD 0.5 g/kg was not dose dependent since the group that was treated with a higher dose did not show such a thing. The same remarks were made on total cholesterol in male treated group. This situation may be due to slight dysfunction of total cholesterol regulation system induced by low dose of the extract leading to kidney's weight gain. The high extract dose corrected that dysfunction by a specific retro-control mechanism not yet explained. The opposite situation has been observed in female lung weight in group treated with 0.5 g/kg. Dose-dependent variation values were obviously related in UOT weight where it decreased suggesting that *Pithecellobium dulce* hydroethanolic stem bark extract might have an atrophic effect on reproductive organs on female Wistar rats.

Urea values diminished in female and male group but more in female. The extract has no harmful effect on kidney and more can be useful by help-

ing these cleansing organs to rid the blood of wastes that are urea and creatinine.

Macroscopic examination of organs from animals treated with the highest dose and control animals showed normal architecture, suggesting no detrimental changes and morphological disorder (Silva et al., 2011) induced by oral administration of the *Pithecellobium dulce* hydroethanolic stem bark extract for 28 days. The oral dose of 1 g/kg/day of this plant extract administered for 28 consecutive days was the highest dose used in sub-acute studies and it did not induce any biochemical and hematological toxicity because those parameters were within the normal reference range as stated by Giknis and Clifford (2008); no remarkable anatomical signs of toxicity were observed, it can be defined as the no-observed-adverse-effect level (NOAEL) for male Wistar rats under experimental conditions used.

Increasing data were recorded in male Wistar rat treated group concerning lymphocytes cells, but not in female animals. Extract concentrations used in this study were not toxic to rat lymphocytes (Singh et al., 2015). Lymphocytes cells proliferation is obviously benefit to immune system. They differentiated in B cells and T cells. B cells by turning into plasmocytes and so to produce antibodies against microbes and can ameliorate the depressed immunity (Manosroi et al., 2006).

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## CONCLUSIONS

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This study has contributed to a better understanding of the sub-acute and acute toxicity of hydroethanolic stem bark extract of *Pithecellobium dulce* used in traditional medicine for several diseases. The data suggested that the oral administration of stem bark hydroalcoholic extract of *Pithecellobium dulce* is relatively safe and has no significant toxic effects in Wistar rats.

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## CONFLICT OF INTEREST

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The authors declare no conflict of interest.

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**Author contribution:**

Contribution	Toudji GA	Dosseh K	Karou SD	Adjrah Y	Anani K	Ameyapoh Y	Simpore J
Concepts or ideas	X		X			X	X
Design	X	X	X			X	X
Definition of intellectual content	X		X	X	X		X
Literature search	X	X	X	X	X		
Experimental studies	X	X	X	X	X		
Data acquisition	X	X	X	X	X		
Data analysis	X		X			X	X
Statistical analysis	X	X	X				
Manuscript preparation	X		X	X	X		
Manuscript editing	X		X	X	X		
Manuscript review	X		X			X	X

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