HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.04.001

Preliminary studies of acute and sub-chronic toxicity of the aqueous extract of *Guibourtia tessmannii* (Harms) J. Leonard stem barks (Caesalpiniaceae) in mice and rats

Noreen Orianna Koumba Madingou[1,2*], Aristide Traore[3], Alain Souza[4], Marlaine Michelle Boukandou Mounanga[1], Raissa Reine Aworet Samseny[1], Sylvin Ouedraogo[3], Alfred Sababenedyo Traore[2]

[1]*Laboratory of Pharmacology and Toxicology, Pharmacopoeia and Traditional Medicines Institute, BP 1935 Libreville, Gabon*

[2]*Research Center in Biological, Food and Nutritional Sciences, University of Ouagadougou, 03 BP 7129 Ouagadougou, Burkina Faso*

[3]*Department of Medicine and Traditional Pharmacopoeia/Pharmacy, Health Science Research Institute, 03 BP 7192 Ouagadougou 03, Burkina Faso*

[4]*Department of Biology, Faculty of Sciences, University of Sciences and Technique of Masuku (USTM), BP 941 Franceville, Gabon*

ARTICLE INFO

Article history:
Received 10 Sep 2015
Received in revised form 19 Sep, 2nd revised form 19 Oct, 3rd revised form 2 Nov, 4th revised form 9 Nov, 5th revised form 30 Nov 2015
Accepted 20 Dec 2015
Available online 22 Apr 2016

Keywords:
Guibourtia tessmannii
Stem barks
Plant extract
Herbal medicine
Toxicity

ABSTRACT

Objective: To investigate the toxicity of aqueous extract of *Guibourtia tessmannii* (Harms) J. Leonard (*G. tessmannii*) and evaluate its safety.
Methods: NMRI mice were used to determine the acute toxicity of *G. tessmannii*. Increasing concentrations of the plant extracts were administered intraperitoneally or by force-feeding. General behavior and death were monitored and recorded daily for 7 days. In order to determine the sub-acute toxicity of the extract, several doses were administered by oral gavage daily for 28 days in adult Wistar rats. Different parameters were assessed including body weight, food and water intake, biochemical parameters and several vital organ weights.
Results: LD_{50} of 328.78 mg/kg was obtained by *i.p.* route and more than 5000 mg/kg was obtained in acute toxicity by oral route. In sub-acute toxicity, no significant alteration was observed in body weight or vital organs, food and water intake, and biochemical parameters.
Conclusions: The results showed that the aqueous extract of *G. tessmannii* has low toxicity intraperitoneally and no sub-acute toxicity via oral intake.

1. Introduction

It is well known that plants are an important source of drugs worldwide [1–3]. Indeed, over 50% of chemical drugs used for the treatment of various diseases are derived from vegetables [4]. In the case of cardiovascular diseases, drugs such as digitoxin, digoxin, lanatosides A, B, C, are derived from *Digitalis purpurea* and *Digitalis lanata* which are traditionally used by indigenous people as poison [5]. However, the traditional usage of plants is not always a guarantee of the plant safety. In accordance with Ashafa *et al.* [3], it is plausible to assume that a history of a plant usage does not proof its safety.

In Gabon, the use of medicinal plants is claimed to have an important role in health care system. However, several deaths are regularly reported by practitioners using traditional medications due to overdosing. Moreover, in this country, few scientific

*Corresponding author: Noreen Orianna Koumba Madingou, Laboratory of Pharmacology and Toxicology, Pharmacopoeia and Traditional Medicines Institute, BP 1935 Libreville, Gabon.
Tel: +226 241 06 39 16 83
E-mail: madnoreen01@gmail.com

Foundation Project: Supported by the doctoral training funds no. 84722652 USTM/4802 AC-USTM, of the University of Sciences and Technique of Masuku.
Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

studies have been conducted to investigate the potential toxicity or eventual side effects of traditional recipes in experiments. *Guibourtia tessmannii* (Harms) J. Leonard (*G. tessmannii*) is one of the most abundantly used medicinal plant in Central Africa for many purposes such as the treatment of cardiovascular diseases [6–8], and its aphrodisiac effects in Cameroon [9].

Phytochemical studies performed on *G. tessmannii* revealed the presence of bioactive compounds such as tannins, phenolic, triterpenoids and alkaloids [6,7]. In order to study the biosafety of this extract in the present study, we determined the acute and sub-acute toxicity effect of the extract of this plant.

2. Materials and methods

2.1. Plant material

Stem barks of *G. tessmannii* were collected in the south of Gabon in August 2010. The plant was authenticated in the Gabon National Herbarium, the Institute of Pharmacopeia and Traditional Medicine, Libreville (Gabon) where a voucher specimen (SRFG 879 LBV) was deposited.

2.2. Aqueous extract

The stem barks of the plant were sun-dried and crushed into powder using mortar and Culatti micro-crusher. The powder obtained (1 kg) was macerated in 2000 mL of water during 48 h at room temperature and filtered using a Whatman millipore filter. The filtrate was lyophilized at −40 °C. The powder obtained (67.7 g) was stored at 5 °C until further use.

2.3. Animals

NMRI mice weighing 19–30 g were used in the acute toxicity test. Animals were provided by the Health Science Research Institute (IRSS), Ouagadougou (Burkina Faso). For the sub-acute toxicity, albino Wistar rats weighing 180–300 g were used. These animals were provided by the Institute of Pharmacopoeia and Traditional Medicine, Libreville, Gabon. All animals were housed under standard laboratory conditions [(25 ± 1) °C] with free access to food and water. Experimental protocols were carried out and followed the Guide for the Care and Use of Laboratory Animals of Gabon.

2.4. Acute toxicity tests

The oral acute toxicity test and the intraperitoneal acute toxicity test were performed. Male and female NMRI mice were randomly distributed into two control groups and 8 treated groups with 10 animals in each group. Among the 8 treated groups, 5 groups of animals were subjected to the intraperitoneal acute toxicity test (5 males and 5 females), and 3 groups of animals were subjected to the oral acute toxicity test, in the same proportions. The two control groups received the water orally or by *i.p.* (0.5 mL vehicle). For the oral acute toxicity test, the treated groups received increasing doses of plant extract (2000, 3000 and 5000 mg/kg weight). Regarding the intraperitoneal acute toxicity test, increasing doses of the plant extract (150, 250, 300, 500 and 600 mg/kg weight) were administered.

Animals were deprived of food and water overnight prior to the drug administration. The mice were observed at 0, 30, 60 and 120 min after treatment. The animals were observed for morbidity and mortality once a day, for up to 14 days, with food and water provided. The number of survivors after 7 days period was recorded [10–12]. The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50} [13].

2.5. Sub-chronic toxicity

Wistar rats (180–250 g) of both gender were divided into four groups of 6 animals each (3 males and 3 females) and were housed under standard conditions and room temperature [(25 ± 1) °C].

The control group received the vehicle (0.5 mL) and the others received increasing oral doses of the plant extract (150, 1500 and 3000 mg/kg weight) by gavage.

Sub-chronic toxicity was evaluated after a single daily administration of extract *per os* for a period of 28 days. Animals were observed daily. Clinical signs, behavioral pattern, food and water intake, and body weight were monitored. At the end of the 28 days period, animals were deprived of food and water for 15 h and then sacrificed for serum biochemical analyses and organs weighing.

For serum biochemical analyses, blood samples collected from the heart were dispensed into plain tubes and were centrifuged at 3500 r/min for 10 min. The serum samples obtained were then used for biochemical parameters analysis such as: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), total protein, creatinine, urea, total cholesterol, using an automated biochemistry analyzer (Selectra XL Vital Scientific, Elitech Group Company).

Vital organs such as heart, lungs, liver, kidneys, spleen, testes, ovary and uterus were carefully dissected, washed with normal buffer, weighed and examined macroscopically.

2.6. Statistical analysis

Data were expressed as mean ± SEM. They were analyzed by GraphPad Prism version 5.0 for Windows. Data were assessed by One-way ANOVA followed by Dunnett's multiple comparison test. *P* values less than 0.05 were considered as statistically significant.

3. Results

3.1. Acute toxicity study in mice

Oral administration of increasing doses of the aqueous extract of *G. tessmannii* (2000, 3000 and 5000 mg/kg) did not produce any abnormal behavioral responses in male and female mice during the 14 days of observation. No mortality was recorded at all dose levels. Orally, the LD_{50} appeared to be > 5000 mg/kg.

When administered intraperitoneally, the aqueous extract of *G. tessmannii* (150 mg/kg body weight to 600 mg/kg body weight) showed a LD_{50} at 328.78 mg/kg body weight. Table 1 summarizes treatment-related responses observed in acute intraperitoneal toxicity study.

3.2. Sub-chronic toxicity study in rats

3.2.1. Mortality and general behavior

Oral ingestion of the aqueous extract of *G. tessmannii* (150 mg/kg body weight to 3000 mg/kg body weight) for 28

Table 1

Acute toxicity of aqueous extract of *G. tessmannii* stem barks, administered by the oral or intraperitoneal route to mice.

Route	Dose (mg/kg)	Mortality (%)	Toxic symptoms
Oral	0	0.00	None
	2000	0.00	None
	3000	0.00	None
	5000	0.00	None
Intraperitoneal	0	0.00	Abdominal contraction
	150	3.00	Abdominal contraction
	250	16.67	Abdominal contraction, catatonia
	300	50.00	Abdominal contraction, catatonia
	500	66.67	Abdominal contraction, catatonia, tachypnea, sedation
	600	98.40	Abdominal contraction, catatonia, tachypnea, sedation

Mice were observed daily for signs of toxicity for 14 days.

Table 2

Sub-chronic toxicity of aqueous extract of *G. tessmannii* administered by oral route to rats for 28 days.

Doses (mg/kg)	Toxic symptoms
0	None
150	None
1500	Irritability (+), piloerection
3000	Irritability (+++), diarrhea, piloerection

+: Level of irritability.

days did not induce any mortality in rats of both genders. No significant difference in food and water intake was recorded in treated and control groups (Table 2). However, abnormal behaviors, such as irritability, diarrhea and piloerection were observed at the 3rd week of the treatment when compared to the control group.

3.2.2. Body weight alteration in rats

Regarding the body weight, the plant extract (150–3000 mg/kg) did not induce any significant change in the body weight both in males and females (Table 3).

3.2.3. Biochemical analyses

Table 4 shows the effects of *G. tessmannii* on biochemical parameters of the serum of rats. A significant decrease in AST ($P < 0.05$ at 3000 mg/kg) and glucose ($P < 0.01$ at 150 mg/kg and 1500 mg/kg) was observed in treated rats. Whereas, no significant change in serum concentration of ALT, ALP, cholesterol, urea, total protein and creatinine was induced by increasing dose of *G. tessmannii* in both male and female rats.

3.2.4. Effect of G. tessmannii on the weight of body organs

Table 5 shows the effect of *G. tessmannii* on the weight of body organs during 28 days. No significant change in vital organs weight was induced by the oral administration of increasing doses of *G. tessmannii* (150–3000 mg/kg).

Table 3

Effect of aqueous extract of *G. tessmannii* stem barks on the weight of rats during 28 days. mg.

Doses (mg/kg)	Day 0		Day 7		Day 14		Day 21		Day 28	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
0	181.00 ± 2.08	159.67 ± 5.45	196.67 ± 7.51	178.67 ± 10.03	231.33 ± 12.86	198.67 ± 2.96	223.33 ± 17.16	228.33 ± 6.00	240.67 ± 11.68	230.33 ± 5.17
150	146.33 ± 6.56	190.67 ± 10.33	201.00 ± 12.71	162.33 ± 10.72	223.00 ± 7.63	182.67 ± 6.36	241.33 ± 10.97	196.67 ± 5.45	239.67 ± 8.25	190.00 ± 4.00
1500	176.67 ± 14.44	205.00 ± 15.88	203.33 ± 9.33	211.00 ± 9.50	211.67 ± 696	202.50 ± 12.50	255.00 ± 8.08	219.50 ± 10.50	241.33 ± 14.94	221.50 ± 12.50
3000	222.00 ± 9.00	183.33 ± 16.67	224.00 ± 9.00	190.33 ± 15.89	247.00 ± 4.58	202.67 ± 16.33	258.67 ± 9.28	230.67 ± 18.34	257.67 ± 7.53	232.33 ± 17.02

Table 4

The biochemical parameters of male and female rats orally administered the aqueous extract of *G. tessmannii* stem barks for 28 days.

Gender	Parameters	Control	Aqueous extract of *G. tessmannii* (mg/kg)		
			150	1500	3000
Male	AST (IU/L)	196.00 ± 5.60	139.97 ± 4.48[a]	132.2 ± 3.30[a]	107.00 ± 2.10[a]
	ALT (IU/L)	55.45 ± 3.80	51.50 ± 5.55	46.57 ± 0.84	51.95 ± 4.05
	ALP (IU/L)	492.85 ± 2.36	386.40 ± 7.34[a]	341.20 ± 9.36[a]	355.5 ± 6.5[a]
	Cholesterol (mmol/L)	1.90 ± 0.10	1.70 ± 0.10	1.90 ± 0.17	2.25 ± 0.05
	Urea (mmol/L)	9.50 ± 0.30	7.97 ± 0.43	8.23 ± 0.86	8.25 ± 0.15
	Glucose (mmol/L)	5.70 ± 1.06	3.57 ± 0.67[b]	3.07 ± 0.57[b]	2.23 ± 1.45[b]
	Total protein (g/L)	57.70 ± 0.40	54.47 ± 2.87	57.30 ± 1.05	61.50 ± 2.00
	Creatinine (μmol/L)	92.10 ± 0.32	90.43 ± 8.66	91.47 ± 4.14	93.00 ± 3.20
Female	AST (IU/L)	199.80 ± 8.30	172.80 ± 4.90	135.35 ± 6.05[a]	123.85 ± 4.85[a]
	ALT (IU/L)	61.50 ± 9.15	62.00 ± 5.78	51.55 ± 6.65	57.13 ± 1.13
	ALP (IU/L)	241.15 ± 5.05	263.90 ± 5.73	243.90 ± 9.03	245.20 ± 4.40
	Cholesterol (mmol/L)	1.85 ± 0.05	1.90 ± 0.15	1.95 ± 0.15	2.00 ± 0.11
	Urea (mmol/L)	9.25 ± 0.25	9.17 ± 0.76	10.20 ± 0.50	8.55 ± 0.15
	Glucose (mmol/L)	3.00 ± 0.50	2.60 ± 0.45[b]	1.30 ± 0.30[b]	1.20 ± 0.30[b]
	Total protein (g/L)	55.80 ± 0.30	62.13 ± 3.87	62.80 ± 4.30	66.85 ± 3.35
	Creatinine (μmol/L)	84.60 ± 4.40	88.43 ± 5.89	99.95 ± 2.55	98.80 ± 1.40

Values are mean ± SEM ($n = 3$). ANOVA with Dunnett tests showed [a]: $P < 0.05$; [b]: $P < 0.01$ compared with control group.

Table 5
Organ weights of male and female rats orally administered the aqueous extract of *G. tessmannii* stem barks for 28 days. g.

Gender	Organs		Control	Aqueous extract of *G. tessmannii* (mg/kg)		
				150	1 500	3 000
Male	Heart		0.37 ± 0.01	0.30 ± 0.02	0.33 ± 0.01	0.38 ± 0.02
	Lungs		0.67 ± 0.06	0.79 ± 0.03	0.62 ± 0.02	0.71 ± 0.03
	Liver		2.76 ± 0.16	2.64 ± 0.06	2.46 ± 0.13	2.70 ± 0.09
	Kidneys	Left	0.30 ± 0.00	0.31 ± 0.01	0.30 ± 0.01	0.32 ± 0.00
		Right	0.33 ± 0.00	0.32 ± 0.00	0.31 ± 0.01	0.33 ± 0.01
	Spleen		0.26 ± 0.02	0.21 ± 0.01	0.25 ± 0.03	0.23 ± 0.02
	Testis		1.15 ± 0.15	1.38 ± 0.02	1.18 ± 0.03	1.27 ± 0.09
Female	Heart		0.37 ± 0.00	0.41 ± 0.03	0.34 ± 0.01	0.37 ± 0.03
	Lungs		0.86 ± 0.01	0.77 ± 0.07	0.99 ± 0.12	0.93 ± 0.16
	Liver		2.85 ± 0.01	2.74 ± 0.22	2.63 ± 0.02	2.72 ± 0.16
	Kidneys	Left	0.31 ± 0.00	0.33 ± 0.01	0.32 ± 0.02	0.35 ± 0.01
		Right	0.32 ± 0.01	0.35 ± 0.01	0.31 ± 0.01	0.34 ± 0.01
	Spleen		0.28 ± 0.01	0.29 ± 0.01	0.27 ± 0.02	0.25 ± 0.01
	Ovary and uterus		0.54 ± 0.02	0.64 ± 0.09	0.43 ± 0.05	0.44 ± 0.01

4. Discussion

The results of the present acute toxicity study indicated, under our experimental conditions, a wide safety range of aqueous extract concentrations of *G. tessmannii* stem barks. This plant extract at 1 000–5 000 mg/kg in oral administration exhibited an oral LD_{50} value above 5 000 mg/kg and did not induce any mortality. Indeed, based on LD_{50} value and according to the classification of Ouédraogo *et al.* [14], the chemical labeling and classification of acute systemic toxicity from Organization for Economic Cooperation and Development [15] and from World Health Organization [16], the plant extract could be assigned as a Class 5 drug and then, recognized as low toxic product. This statement was strengthened by the results obtained in intraperitoneal administration. Furthermore, we observed quickly reversible signs of toxicity, and a LD_{50} estimated at 328.78 mg/kg which suggest a low toxicity of the plant extract according to the classification of Mezui *et al.* [17]. The difference observed between the LD_{50} values of the oral and intraperitoneal routes may be explained by the low bioavailability of the components that might cause toxicity, the poor absorption from the gastrointestinal tract, or as a result of a high first-pass effect and rapid metabolism to non-toxic metabolites [18].

In the sub-acute toxicity study in rats, the aqueous extract of *G. tessmannii* (150–3 000 mg/kg body weight) did not induce any change in animal behavior, food and water intake, and vital organs weight as well as the body weight gain. It is well known that decrease in body weight gain as well as internal organ weights is a sensitive index of toxicity after exposure to toxic substances [14,19]. In fact, increase or decrease in body weight could be due to adverse effects of drugs [19]. The loss of appetite caused by stress or physiological adaptation to a drug's intake leads to the reduction of caloric intake when the body weight reduces [14] or the body fat accumulation during body weight gain [20].

On the basis of these results, under the same conditions, it has been suggested that the aqueous extract of *G. tessmannii* did not induce any acute toxicity.

The liver and kidneys are two crucial organs that play a key role in detoxification [21]. The effect of the plant extract was studied on the serum level of ALT, AST, ALP, which are essential for assessing the hepatic function [22] and on urea and creatinine levels in the blood which are usually used to evaluate the kidney function [23]. While a rise of transaminases reflects the liver inflammation or damage [24], any rise in creatinine level suggests damages of nephrons function [25].

Regarding the hepatic function monitoring, the plant extract did not produce deleterious changes in ALT, AST or ALP levels since no increase of these parameters was observed, suggesting no hepatotoxicity effect of this plant extract. However, we observed a decrease in AST level in both males and females treated with the plant extract. The ALP level also significantly decreased in the male group, but not in female group receiving *G. tessmannii* extract. This result corroborates the ability of the extract of *G. tessmannii* to restore functional status of liver [7].

No change in creatinine or urea level was obtained suggesting that the plant extract does not affect the renal function.

Regarding other biochemical parameters such as total cholesterol and total protein, no significant difference was observed compared to the control group. Whereas, a decrease in glucose level was recorded indicating a hypoglycemic effect of the plant extract.

Overall, the aqueous extract of *G. tessmannii* appeared to be low or non-toxic. Studies including hematopoietic system and histology are undertaken to further support the safety of the herbal medicine from *G. tessmannii*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors wish to thank Professor Bopassa Jean Chrisostome, Dr Mewono Mewono Ludovick and Bading Bayissi for their kind help regarding the translation of the manuscript in English. We also wish to thank the staff of Health Science Research Institute and Institute of Pharmacopeia and Traditional Medicine and their laboratory personnel, especially Mayinda Ekagba Erica Lorlei, Ada Nguema Sandra Marquise, Wabekota Dagmar Corène for their assistance. This work was supported by the doctoral training funds no. 84722652 USTM/4802 AC-USTM, of the University of Sciences and Technique of Masuku.

References

[1] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol* 2014; **4**: 177.

[2] Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. *Metabolites* 2012; **2**: 303-36.

[3] Ashafa AO, Orekoya LO, Yakubu MT. Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats. *Asian Pac J Trop Biomed* 2012; **2**: 811-7.

[4] Tabassum N, Hamdani M. Plants used to treat skin diseases. *Pharmacogn Rev* 2014; **8**: 52-60.

[5] Obeng EA. *Guibourtia tessmannii* (Harms) J. Léonard. In: Lemmens RHM, Louppe D, Oteng-Amoako AA, editors. *Plant resources of tropical Africa*. Wageningen: Wageningen University; 2011.

[6] Madingou NOK, Souza A, Lamidi M, Mengome LE, Mba CEM, Bayissi B, et al. Study of medicinal plants used in the management of cardiovascular diseases at Libreville (Gabon): an ethnopharmacological approach. *Int J Pharm Sci Res* 2012; **3**: 111-9.

[7] Nyangono BCF, Chakokam Ngangoum RM, Kuate D, Ngondi JL, Enyong Oben J. Effect of *Guibourtia tessmannii* extracts on blood lipids and oxidative stress markers in triton WR 1339 and high fat diet induced hyperlipidemic rats. *Biol Med* 2012; **4**: 1-9.

[8] Fernande NBC, Marthe T, Laure NJ, Enyong OJ. *In vitro* antioxidant activity of *Guibourtia tessmannii* Harms, J. Leonard (Cesalpinoidae). *J Med Plant Res* 2013; **7**: 3081-8.

[9] Watcho P, Defo PBD, Wankeu-Nya M, Carro-Juarez M, Nguelefack TB, Kamanyi A. *Mondia whitei* (Periplocaceae) prevents and *Guibourtia tessmannii* (Caesalpiniaceae) facilitates fictive ejaculation in spinal male rats. *BMC Complement Altern Med* 2013; **13**: 4.

[10] El-Said Gad MM. Acute and repeated-doses (28 days) toxicity of thymol formulation in male albino rats. *Aust J Basic Appl Sci* 2012; **7**: 915-22.

[11] World Health Organization. WHO Expert Committee on specifications for pharmaceutical preparations-WHO technical report series, No. 863, thirty-fourth report. Geneva: World Health Organisation; 1996. [Online] Available from: http://apps.who.int/medicinedocs/en/d/Js5516e/ [Accessed on 25th August, 2015]

[12] Ntchapda F, Abakar D, Kom B, Nana P, Hamadjida A, Dimo T. Acute and sub-chronic oral toxicity assessment of the aqueous extract leaves of *Ficus glumosa* Del. (Moraceae) in rodents. *J Intercult Ethnopharmacol* 2014; **3**: 206-13.

[13] Dellai A, Mansour HB, Clary-Laroche A, Deghrigue M, Bouraoui A. Anticonvulsant and analgesic activities of crude extract and its fractions of the defensive secretion from the Mediterranean sponge. *Spongia Off Cancer Cell Int* 2012; **12**: 15.

[14] Ouédraogo S, Somé N, Ouattara S, Kini FB, Traore A, Bucher B, et al. Acute toxicity and vascular properties of seed of *Parkia biglobosa* (JACQ) R. Br Gift (Mimosaceae) on rat aorta. *Afr J Tradit Complement Altern Med* 2011; **9**(2): 260-5.

[15] Organization for Economic Cooperation and Development. OECD guidelines for the testing of chemicals. Paris: Organization for Economic Cooperation and Development; 2008. [Online] Available from: http://www.oecd.org/document/40/0%2C2340%2Cen_2649_34377_37051368_1_1_1_1%2C00.html [Accessed on 25th August, 2015]

[16] World Health Organization. The WHO recommended classification of pesticides by hazard, and guidelines to classification. Geneva: World Health Organization; 2009. [Online] Available from: http://www.who.int/ipcs/publications/pesticides_hazard_2009.pdf [Accessed on 25th August, 2015]

[17] Mezui C, Longo F, Nkenfou C, Sando Z, Ndeme E, Vernyuy Tan P. Evaluation of acute and sub acute toxicity of stem bark aqueous extract of *Anthocleista schweinfurthii* (Loganiaceae). *World J Pharm Pharm Sci* 2015; **4**(3): 197-208.

[18] Ahmad F, Tabassum N. Preliminary phytochemical, acute oral toxicity and antihepatotoxic study of roots of *Paeonia officinalis* Linn. *Asian Pac J Trop Biomed* 2013; **3**: 64-8.

[19] Hayelom K, Mekbeb A, Eyasu M, Wondwossen E, Kelbesa U. Methanolic effect of *Clerodendrum myricoides* root extract on blood, liver and kidney tissues of mice. *Afr Health Sci* 2012; **12**: 489-97.

[20] Gautam MK, Goel RK. Toxicological study of *Ocimum sanctum* Linn leaves: hematological, biochemical, and histopathological studies. *J Toxicol* 2014; **2014**: 135654.

[21] Harizal SN, Mansor SM, Hasnan J, Tharakan JK, Abdullah J. Acute toxicity study of the standardized methanolic extract of *Mitragyna speciosa* Korth in rodent. *J Ethnopharmacol* 2010; **131**: 404-9.

[22] Pandith AA, Lateef A, Shahnawaz S, Hussain A, Malla TM, Azad N, et al. *GSTP1* gene Ile105Val polymorphism causes an elevated risk for bladder carcinogenesis in smokers. *Asian Pac J Cancer Prev* 2013; **14**: 6375-8.

[23] Chang CJ, Tzeng TF, Liou SS, Chang YS, Liu IM. Acute and 28-day subchronic oral toxicity of an ethanol extract of *Zingiber zerumbet* (L.) Smith in rodents. *Evid Based Complement Altern Med* 2012; **2012**: 608284.

[24] Konaté K, Bassolé IHN, Hilou A, Aworet-Samseny RRR, Souza A, Barro N, et al. Toxicity assessment and analgesic activity investigation of aqueous acetone extracts of *Sida acuta* Burn f. and *Sida cordifolia* L. (Malvaceae), medicinal plants of Burkina Faso. *BMC Complement Altern Med* 2012; **12**: 120.

[25] Mariappan G, Saha BP, Sutharson L, Singh A, Garg S, Pandey L, et al. Analgesic, anti-inflammatory, antipyretic and toxicological evaluation of some newer 3-methyl pyrazolone derivatives. *Saudi Pharm J* 2011; **19**(2): 115-22.