ACUTE AND SUBACUTE TOXICITY OF THE MEOH/METHYLENE CHLORIDE BARK EXTRACTFROM *DRYPETES GOSSWEILERI* (EUPHORBIACEAE) IN WISTAR RAT

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SUMMARY

Introduction: *Drypetes gossweileri,* is widely used in the Traditional Medicine as a panacea (cure-all) and has shown antifungal and antibacterial properties.

Method: We evaluated the *Drypetes gossweileri,* bark methanol/methylene chloride extract for its behavioural and pharmaco-toxicological effects after acute and subacute administration through oral route in both males and females rats.

Results: We observed in acute toxicity that single oral doses (4 - 12g/kg) of the extract of bark of *Drypetes gossweileri,* in rats did not produce mortality or significant changes in the general behaviour and biochemical parameters of rats. In subacute toxicological studies in both males and females rats the *Drypetes gossweileri,* bark extract (administered orally at 48 hours doses of 500mg/kg and 1000mg/kg for 4 weeks), and did not cause any changes in biochemical and haematological parameters. No noteworthy signs of toxicity were noted in feeding or body weight with the exception of male rats at

1000mg/kg dose where a light diminution in body weight was observed. Morphological examination of various organs and statistical analysis of relative's organs weight revealed that there were no differences between the control groups which receive respectively distilled water and maize oil and treated rats.

Conclusion: The methanol methylene chloride extract *Drypetes gossweileri* bark*,* appears to be safe at the studied doses.

Key words: *Drypetes gossweileri*, crude extract, acute toxicity, subacute toxicity

Drypetes gossweileri (Euphorbiaceae) is a medicinal plant used in folk African medicine. This plant is widely found in rainforest of Cameroon, Gabon, Congo, Central Africa and Ghana (1)**.** It is known for its numerous beneficial effects as a panacea (cure all), specifically for gastrointestinal disorders, as an anthelmintic or an antirhumatismal (2)**,** an antiseptics and an analgesic (3)**.** It is also used as aphrodisiac or remedy for dysentery, urethral gonorrhoea, intestinal affections, severe pneumonia, bronchitis, and for abortion (3)**.** This plant also has antitumoral and purgative activities (4). Agnaniet et al., (5)**,** extract an essential oil from the bark of *D. gossweileri,* rich in benzyl cyanide, but poor in terpenes. Stem bark extract of *Drypetes gossweileri* have shown an antibacterial activities (6) and antifungal (7).

Notwithstanding the widespread use of *Drypetes gossweileri* plant in traditional medicine and despite the fact that many plants of

this genus exhibit significant toxicity, some as abortion of pregnant women and digestive disorders, no systematic toxicological study has been undertaken with this plant. Therefore, the purpose of the present study was thus to investigate the safety of the methanol/methylene chloride extract of stem bark extract of *Drypetes* g*ossweileri* by determining its behavioural and pharmaco-toxicological effects after acute and subacute administration in wistar rats.

Methods

1.1 Plant material

Stem bark of *Drypetes* g*ossweileri* were collected at Mount Eloundem (Yaoundé, Cameroon) on June 2005 and stored at room temperature in a dry place. The plant was authenticated as *Drypetes* g*ossweileri* S. Moore at the National Herbarium of Cameroon where a voucher specimen are deposited under the identification number 5746/SRF/Cam.

1.2 Preparation of the bark extract of Drypetes gossweileri

The stem bark was cut up slice, then dried and ground to powder. The powder was suspended in methanol/methylene chloride (1/1) mixture (150g powder per 800ml solvent mixture) and kept at laboratory temperature during 48 hours and stirred every 5 hours (this was repeated three times). After maceration, the mixture was filtered with Whatman n°1 filter paper. The obtained filtrates were poured and evaporated under reduce pressure and controlled temperature (60°C) by using a vacuum on rotary evaporator (Büchi

461, Water Bath). The yield of the crude extract was 4.53% (w/w) and the final *Drypetes* go*ssweileri*-extract was stored at −20 ◦C until further use.

1.3. Animals

Wistar albino rats (98–115 g for acute toxicity and sub-acute toxicity) of both sexes were obtained from the animal house of the Laboratory of Microbiology (Applied Microbiology and Molecular Pharmacology Unit) of the Faculty of Science, University of Yaoundé 1. The rats were given food and water *ad libitum*. All the animals were kept under laboratory conditions for an acclimatization period of 7 days prior the experiments. The bioassay was conducted in accordance with the internationally accepted principle guidelines for evaluating the safety and efficacy of herbal medicines (8).

1.4. Acute toxicity

In order to study any possible toxic effect or changes in normal behaviour, 5 groups of 10 rats (5 males and 5 females) were used in this experiment. The acute toxicity of the plant was studied by preparing three different concentrations of the extract (4, 8, and 12 g/kg b.w.), and administered orally (single dose) to three groups of animals. The first and second groups were taken as controls and received respectively distilled water and maize oil. Animals were kept without food for 12h prior to dosing and were monitored continuously for 3h after dosing for any sign of toxicity. The symptoms, motor activity, posture and mortality were checked. Animals were kept under observation for 7 days and were monitored daily for changes in body weight, food and water consumption and for any sign of toxicity. At the end of observation animal were

sacrificed and the blood and selected organs was collected for further biochemical and anatomo-pathological analysis

1.5 Subacute toxicity studies in rat

Forty rats were divided into four groups of 5 animals per sex. They were kept under the same conditions as described above. The first two groups were given orally respectively distilled water and the vehicle of the extract (maize oil) and taken as controls. The remaining two groups were given orally 500 and 1000mg/kg b.w. of *Drypetes gossweileri* stem bark crude extract once every two days for 4 weeks. During the 4week dosing period, all animals were observed daily for clinical signs and mortality patterns once before dosing, and during dosing.

1.4.1 Weekly body weight

The body weight of each rat was assessed during the acclimatization period, once every 7 days during the dosing period and once on the day of sacrifice. The relative body weight (RBW) of each animal was then calculated as follows:

RBW = absolute body weight of one time interval (g)/body weight of water control rat on the start of dosing day (g)×100.

1.4.2 Food and water consumption

The amounts of food and water consumed were measured daily from the quantity of feed and water supplied and the amount remaining after 24 h.

1.4.3 Hematology and Preparation of serum samples

On day 28 of the dosing period, all the animals were euthanized by decapitation under ether anesthesia and blood samples were drawn from the jugular vein of each sacrificed animal. The samples were collected in two different tubes. The dry test tubes and allowed to stand for complete clotting; the clotted blood samples were centrifuged at 3000 rpm for 15 min and serum samples were aspirated off and frozen. The blood in heparin test tube served for hematological analysis. Red blood cell, white blood cell and platelet counts, hemoglobin concentration (g/l) and hematocrit estimation were carried out using an automated globular counter, HYCEL Diagnostics (Celly, type CA 4001 series no.: CA40D 1975).

1.4.4 Relative organ weight and preparation of homogenate samples

After taking the blood, the abdominal cavity of each animal was opened and organs namely the heart, liver, lungs, pancreas and kidneys were quickly removed, cleaned with ice-cold saline, weighed and stored at −80 ◦C. Apart of the liver and kidney tissues were thawed and homogenized 20 times (w/v) by homogenizer in ice-cold Tris–HCl 50mMKCl 50mM buffer (pH 7.4). The homogenates were centrifuged at 6000 rpm for 30 min and the supernatant was then used for biochemical parameter assays.

The relative organ weight of each animal was then calculated as follows: absolute organ weight (g)/body weight of rat on day of sacrifice (g) × 100.

1.4.5 Microscopic examination

After sacrificing the animals, small pieces of liver and kidney were fixed in 10% formal saline for histopathological studies before storing the organs at −80 ◦C. Tissues were processed by conventional techniques using an automatic tissue processor (Shandon, Sakura Fine Technical Co.; Ltd. Model 4634). The paraffin embedded sections of 4–5μm thickness were prepared with microtome, stained with hematoxylin and eosin for microscopic examination using optical microscope (Leica DM LB 2 series no.: 1151200).

1.5 Serum and Hepatic Biochemical parameters

The biochemical parameters, glucose (kit glucoPlus, using Glucometer), serum and hepatic protein (9), serum and hepatic aspartate aminotransferase and alanine aminotransferase **(**10), serum creatinine (11)**,** hepatic alkaline phosphatase (12), serum conjugate and non conjugate bilirubine (13), total cholesterol (14), hepatic malone dialdéhyde (15) and hepatic glutathione (16), were determined and optical density was measured at the corresponding wavelength with a spectrophotometer (Ciba Corning 550 Express, England).

1.6 Phytochemical evaluation of the crude extract

Phytochemical screening of the crude extract for its presence of primary and secondary metabolites (lipids, triterpenes, steroids, essentials oils, saponins, polyphenols, anthocyanes, anthraquinones,

flavonoïds, alkaloids, Tannins) was done using Harbone (17) and Odebeyi and Sofowora (18) protocol.

1.7 Statistical analysis

The values were expressed as mean±standard error of the mean (S.E.M.). The statistical analysis of data was by analysis of variance (ANOVA) using 5% level of significance. The statistical package used was Graph Pad Instat 3.0. A one-way ANOVA enabled us to observe the significant differences between the values using Tukey test.

2 Results

2.1 Acute toxicity

2.1.1 Behavioural observations and mortality patterns

The animals showed behavioural changes 5 min after the administration of the extract up to 12 g/kg (b.w.). The changes which lasted 2–3 hours, included prostration, motionlessness, slow response to external stimuli and slow breathing. All male and female rats treated with dose of 4, 8 and 12 g/kg of the extract of *D.* go*ssweileri* remained alive during the 7 days of observation.

2.1.2 Consumption and body weight trends

The relative body weight gain of the rats treated with extract was not different compared to that of the control groups (figure 1&2). Water and food consumptions of dosed (4, 8 and 12 g/kg) and control groups were similar (data note shown).

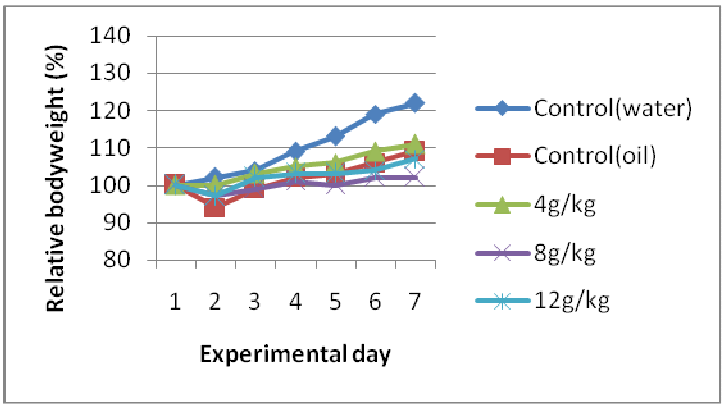

Figure 1: Relative body weight trend for male Wistar rats dosed once with stem bark of *D. gossweileri* extract of at 0, 4, 8, and 12 g/kg b.w. Each data point represents the mean± S.E.M. (n = 5).

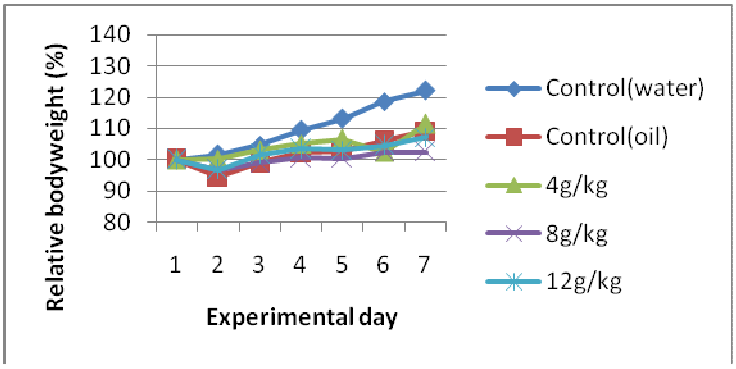

Figure 2: Relative body weight trend for female Wistar rats dosed once with stem bark of *D. gossweileri* extract of at 0, 4, 8, and 12 g/kg b.w. Each data point represents the mean±S.E.M. (n = 5).

2.1.3 Selected organs examination and serum biochemical parameters

The pathological examination (macroscopic) of internal organs revealed that there were no signs of abnormalities. In fact,

there were no difference in the weight and colour or texture of the heart, pancreas, lung, kidney and liver (data not shown) or serum biochemical parameters (Table 1) of the rats in the control and treated groups in both sexes. The no-observed-adverse-effect level (NOAEL) was above 12 g/kg for both sexes (19).

Table 1: Mean serum biochemical parameters in rats treated with stem bark extract of *Drypetes gossweileri* (0, 4, 8 and 12 g/kg b.w.) after 7 days dosing. Each data column represents the mean±S.E.M. (*n* = 5). Data column in the same parameter with * sign are significantly different ($p < 0.05$).

Sex	Parameters	Control (water)	Control (oil)	4g/kg	8g/kg	12g/kg
Males	Total protein (g/l)	4.230±0.640	4.610±1.150	4.550±2.232	4.333±1.205	6.403±1.919
	AST (UI/l)	164.203±4.663	172.607±5.341	175.567±19.552	162.263±14.265	163.877±7.405
	ALT (UI/l)	21.980±6.808	21.333±10.797	23.27± 2.910	21.330± 1.816	25.21±15.637
	Creatinine (mg/l)	0.123±0.008	0.129±0.018	0.133±0.030	0.123±0.020	0.13±0.014
	Cholesterol (g/l)	0.043±0.003	0.053±0.003	0.063±0.023	0.057±0.007	0.047±0.006
	Conjugate bilirubine (mg/l)	16.560±0.610	18.027±0.559	17.707±0.367	18.980±0.244	18.477±0.145
	Totale bilirubine (mg/l)	31.500 ±1.085	31.297±0.203	32.183±0.378	32.183±0.532	31.567±0.903
Females	Total protein (g/l)	5.990±0.359	4.893±0.702	6.003±0.346	6.527±0.647	6.617±0.345
	AST (UI/l)	164.203±4.663	172.607±5.341	179.390±25.810	120.240±4.362*	173.580±8.726
	ALT (UI/l)	21.980±3.931	21.333±6.234	23.270±3.920	21.007±2.647	21.657±4.348
	Creatinine (mg/l)	0.113±0.004	0.120±0.000	0.153±0.049	0.153±0.040	0.130±0.010
	Cholesterol (g/l)	0.053±0.019	0.093±0.041	0.070±0.020	0.060±0.006	0.050±0.006
	Conjugate bilirubine (mg/l)	18.663±0.809	17.333±1.383	17.660±0.420	16.270±0.666	16.430±0.554
	Totale bilirubine (mg/l)	30.957±0.246	34.977±2.185	32.527±1.637	32.183±0.854	31.773±0.957

Caption: Control (water): group which received distilled water, Control (oil): group which received maize oil. AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase).

2.2 Subacute toxicity studies in rats

2.2.1 Clinical signs and mortality patterns

Animals resumed normal behaviour and activity immediately after each administration of the extract. The bark extract of *D. gossweileri* at doses of 500 and 1000mg/kg, given orally for 28 days, did not produce death of rats.

2.2.2 Weekly body weight, feed and water consumption patterns

There were no variable changes in food and water consumption patterns during the 28 days treatment period (Fig. 3, Fig. 4, Fig. 5 and Fig. 6). The body weight gains of the treated males and female rats (Fig. 7&8) were globally similar to those of control groups, except the group of male rats at the 1000mg/kg dose level which showed a light decrease in weight gain (Fig. 8) to suggest a toxicity effect of the extract at this level.

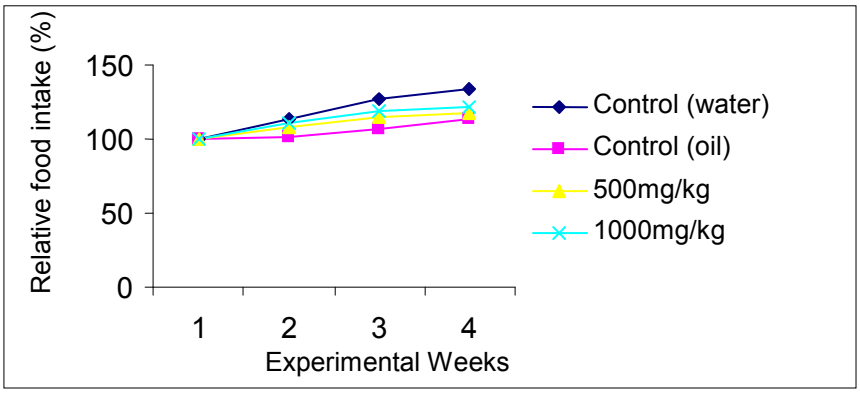

Figure 3: Food consumption trends of male Wistar rats fed with extract of *D. gossweileri* at 0, 500, and1000m g/kg b.w. for 4 weeks. Values for consumption are based on total intake and average relative body weight of the preceding time interval.

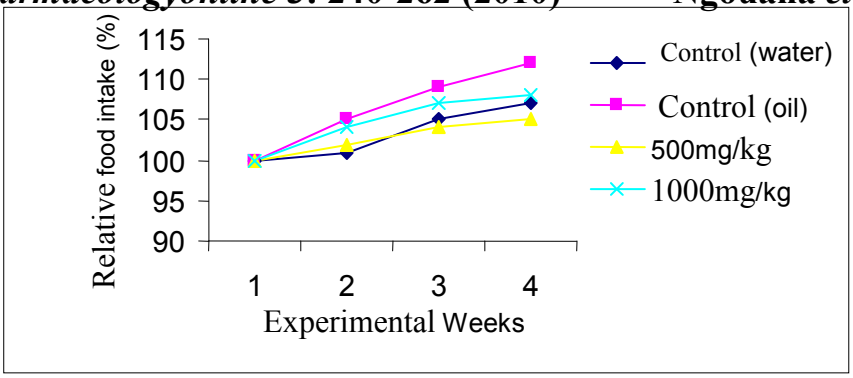

Figure 4: Food consumption trends of female Wistar rats fed with extract of *D. gossweileri* at 0, 500, and 1000m g/kg b.w. for 4 weeks. Values for consumption are based on total intake and average relative body weight of the preceding time interval.

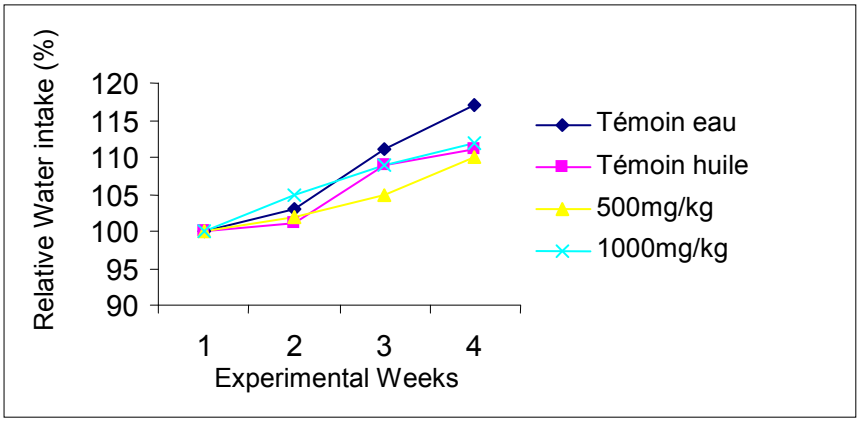

Figure 5 : Water consumption trends of male Wistar rats fed with stem bark extract of *D. gossweileri* at 0, 500, and1000m g/kg b.w. for 4 weeks. Values for consumption are based on total intake and average relative body weight of the preceding time interval.

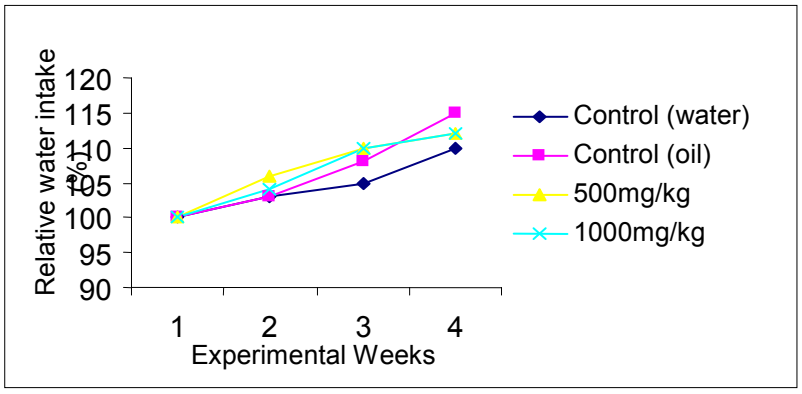

Figure 6: Water consumption trends of female Wistar rats fed with stem bark extract of *D. gossweileri* at 0, 500, and1000m g/kg b.w. for 4 weeks. Values for consumption are based on total intake and average relative body weight of the preceding time interval.

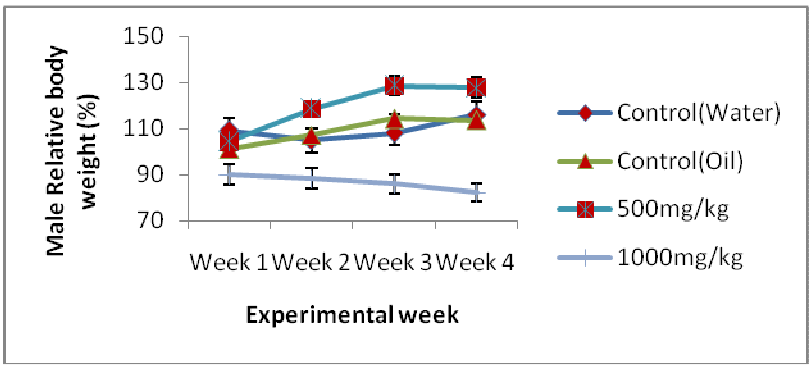

Figure 7: Relative body weight pattern in male Wistar rats fed with stembark extract of *Drypetes gossweileri* at 0, 500, and 1000m g/kg b.w. for 4 weeks. Each data point represents the mean±S.E.M. (n = 5). Data points in the same week with different color are significantly different ($p < 0.05$).

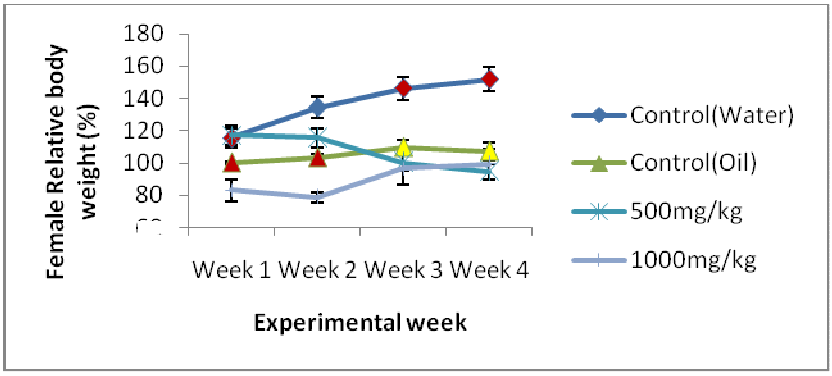

Figure 8: Relative body weight pattern in female Wistar rats fed with stembark extract of *Drypetes gossweileri* at 0, 500, and 1000m

2.2.3. Relative organ weight and Histopathological studies

There were no significant changes in the relative weights of the liver, heart, kidneys, spleen and lungs of the treated male and female rats in relation to control groups (Table 2).

For the Histopathological studies, the macroscopic analysis of the target organs of the treated groups (lung, liver, pancreas, heart and kidney) did not show significant changes in colour and texture when compared with control groups in both sex. The microscopic analysis of liver and kidney did not change when compared to control groups (Fig. 9).

Table 2: Effect of stem bark extract of *Drypetes gossweileri* on the relative organ weights of male and female rats after 4 weeks oral dosing. Each data column represents the mean±S.E.M. (n = 5). Relative organ weight = (organ weight/body weight) ×100.

Relative organs weight						
Sex	**Dose**	**pancreas**	**kidney**	**liver**	**lung**	**heart**
Males	Control (water)	0.410±0.040	0.767±0.064	4.317±0.361	1.010±0.103	0.337±0.032
	Control (oil)	0.613±0.015	0.793±0.107	4.767±0.517	1.107±0.176	0.407±0.027
	500mg/kg	0.423±0.047	0.773±0.078	4.020±0.358	0.783±0.059	0.430±0.055

	1000mg/kg	0.673±0.270	0.900±0.179	5.333±1.040	0.887±0,127	0.470±0.079
Females	Control (water)	0.503±0.012	0.813±0.012	4.267±0.186	0.890±0.105	0.48±0.052
	Control (oil)	0.640±0.281	0.703±0.081	3.923±0.438	0.597±0.199	0.370±0.030
	500mg/kg	0.430±0.038	0.900±0.025	5.457±0.327	1.063±0.182	0.430±0.008
	1000mg/kg	0.610±0.064	0.880±0.080	5.443±0.950	1.240±0.070	0.360±0.064

Caption: Control (water): group which received distilled water, Control (oil): group which received maize oil.

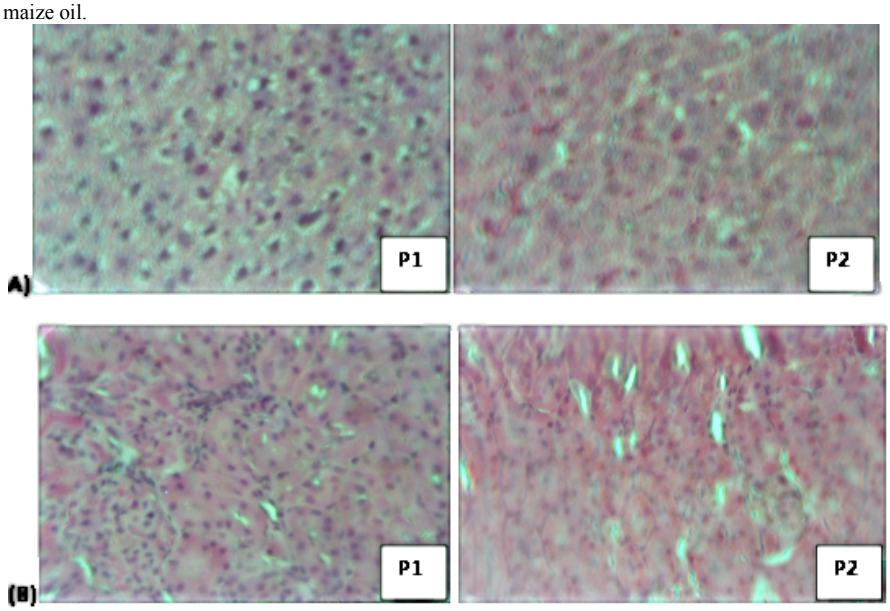

Figure 9: (A) Photomicrographs of the sections of the liver showing normal features in control (maize oil) rats (P1), and the liver of rats treated orally with 1000m g/kg of stem bark extract of *D. gossweileri* for 4 weeks showing no alteration (P2). (B) Photomicrographs of the sections of the kidney showing normal features in control (maize oil) rats (P1), and the kidney of rats treated orally with 1000m g/kg of stem bark extract of *D. gossweileri* for 4 weeks showing no alteration (P2).

2.2.4. Haematology

Haematological parameters are shown in Table 3. There were no significant changes in the red blood cell, white blood cell and platelet

counts and in haemoglobin concentration and hematocrit for the treated groups compared with the control groups.

Table 3: Mean haematological parameters. Red blood cell count, white blood cell count , platelets count, hematocrit level and hemoglobin concentration in rats treated with stem bark extract of *D. gossweileri* (0, 500 and 1000m g/kg b.w.) after 4 weeks dosing. Each data column represents the mean±S.E.M. (n = 5). Data column in the same parameter with different superscript letters are significantly different ($p < 0.05$).

	Hematological parameters					
Sex	Dose	WBC ($10^3/l^{-1}$)	RBC ($10^6/l^{-1}$)	Hemoglobin (g/dl)	Hematocrit (vol. %)	Platelet ($10^4/l^{-1}$)
Male	Control (water)	5.500± 0.076	5.657±0.083	11.300±0.416	35.967±0.516	7.700±0.529
	Control (oil)	6.033±0.034	5.567±0.395	11.800±0.600	36.267±1.322	7.733±0.145
	500 mg/kg	5.597±0.126	6.380±1.153	12.033±0.233	37.200±2.676	7.547±0.226
	1000 mg/kg	6.097±0.636	5.580±1.588	11.800±1.021	35.700±3.132	7.653±0.085
Female	Control (water)	5.340±0.112	5.780±0.187	11.433±0.491	35.700±0.173	7.647±0.234
	Control (oil)	5.367±0.255	5.723±0.465	11.737±0.148	35.533±1.800	7.423±0.284
	500 mg/kg	5.470±0.403	6.147±1.028	11.833±0.338	37.66±2.404	7.600±0.153
	1000 mg/kg	5.647±0.187	5.747±1.383	11.467±1.450	35.333± 4.842	7.733±0.088

Caption: Red blood cell (RBC), white blood cell (WBC), Control (water): group which received distilled water, Control (oil): group which received maize oil.

2.2.5. Serum and hepatic biochemical findings

The serum and hepatic biochemical profiles are shown in Table 4 and Table 5 respectively. Subacute oral administration *D. gossweileri* extract (up to a dose of 1000mg/kg) did not show changes in serum creatinine, cholesterol, conjugate and total bilirubine, total proteins, AST, ALT, PAL and blood glucose level in both sex. On the other

hand hepatic level of total proteins, AST, ALT, MDA and glutathione did not varied when compared to the control group which received oil.

Table 4: Serum biochemical changes in rats following oral treatment with *D. gossweileri* for 4 weeks

Sex	Parameters	Control (water)	Control (oil)	500mg/kg	1000mg/kg
Male	Total protein (g/l)	6.870±0.547	6.437±0.212	5.507±0.377	5.717±0.124
	AST (UI/l)	70.580±3.637	69.820±19.634	52.460±30.424	65.940±0.547
	ALT (UI/l)	64.970±0.970	54.947±15.193	56.997±7.195	70.467±5.323
	creatinine (mg/l)	0.207±0.043	0.207±0.043	0.163±0.043	0.203±0.083
	Total cholesterol (g/l)	0.127±0.027	0.187±0.037	0.173±0.007	0.167±0.013
	conjugate bilirubine (mg/l)	7.180±0.593	8.930±1.136	7.660±1.077	10.370±2.392
	Totale bilirubine (mg/l)	7.567±0.716	11.793±0.887	11.250±0.718	11.523±2.365
	Glucose (mg/ dl)	85.333±2.667	66.000±8.718	71.667±5.207	80.667±10.105
	ALP (UI/l)	4.370±0.098	4.470±0.116	4,390±0.026	4.490±0.067
Female	Total protein (g/l)	4.497±2.139	4.910±0.777	4.063±1.443	3.887±1.350
	AST (UI/l)	73.370±7.457	80.487±8.727	67.877±4.598	80.150±8.896
	ALT (UI/l)	82.420±19.635	92.120±0.971	80.810±4.968	90.830±3.757
	creatinine (mg/l)	0.333±0.083	0.373±0.072	0.330±0.150	0.163±0.043
	cholesterol (g/l)	0.180±0.080	0.190±0.175	0.160±0.138	0.190±0.183
	conjugate bilirubine (mg/l)	9.090±0.423	9.467±0.743	9.730±2.950	10.583±0.615
	Totale bilirubine (mg/l)	12.137±1.164	12.820±1.910	12.273±2.639	13.363±1.263
	Glucose (mg/ dl)	103.000±6.500	78.000±0.000	84.000±3.000	103.000±7.125
	ALP (UI/l)	4.393±0.067	4.517±0.015	4.440±0.087	4.300±0.051

Caption: AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), and protein (total protein). Each value represents the mean±S.E.M. (n = 5). Values in the same column with * are significantly different ($p < 0.05$). Control (water): group which received distilled water, Control (oil): group which received maize oil.

Table 5: Effect of oral administration of *D. gossweileri* bark extract on hepatic biochemical parameters in rats

Sex	Parameters	Control (water)	Control (oil)	500mg/kg	1000mg/kg
Male	Total proteins (g/l)	4.420±0.322	4.200±0.469	4.860±0.022	8.090±2.021

	AST (UI / l)	171.640±3.633	128.970±36.727	192.970±3.637	203.640±0.364
	ALT (UI / l)	78.543±6.357	58.180±2.019	49.477±5.359	50.100±9.539
	GSH (mmol/ mg)	20.653±0.954	15.800±2.528	21.373±6.527	17.843±8.285
	MDA (Mol/mg)	0.200±0.019	0.170±0.004	0.180±0.023	0.210±0.030
Female	Total proteins (g/l)	7.803±1.065	6.400±0.514	4.570±0.282	6.477±0.702
	AST (UI / l)	139.960±5.986	135.113±28.155	142.323±29.419	140.283±19.021
	ALT (UI / l)	54.710±3.269	73.760±17.820	64.970±7.272	51.390±0.727
	GSH (mmol/ mg)	22.160±2.563	20.443±3.645	21.083±5.521	22.230±1.135
	MDA (mol/mg)	0.113±0.009	0.167±0.009*	0.171±0.009*	0.160±0.010*

Caption: AST (aspartate aminotransferase), ALT (alanine aminotransferase), protein (total protein), GSH (glutathion) et MDA (malone dialdehyde). Each value represents the mean±S.E.M. (n = 5). Values in the same column * are significantly different from control (water) ($p < 0.05$).

2.2.6 Phytochemical screening

Phytochemical screening was positive for alkaloids, polyphenol, flavonoids, tannins, essential oils, lipids, saponins and triterpenoids.

3 Discussion

3.1 Acute toxicity

Even though, toxic plants are ubiquitous, herbal medicine is used by up to 80% of the population in the developing countries. Despite the widespread used, few scientific studies have been undertaken to a certain safety and efficacy of traditional remedies. The investigation of single administration MeOH/methylene chloride shows that crude extract of *D. gossweileri*, relatively nontoxic (LD_{50}>5000) (23) via the oral route in rats, at least up to the maximum doses of 12g/kg (NOAEL).

3.2. Subacute toxicity

In the Subacute study in rats given orally at doses of 500 and 1000mg/kg, there was no change in animal behaviour, food and water intake, and the body weight gains did not varied significantly to suggest an effect of stem bark extract of *D. gossweileri* on the animal on each of these aspects, although, there were a slight decrease in body weight gain of the male rats at the dose of 1000g/kg. This weight loss may result from disturbances in carbohydrate, protein or fat metabolism (20) associated with *D. gossweileri* which could prevent absorption or nutrient metabolism.

The absence of alteration of haematological parameters, show that the extract did not affect blood element or bone marrow which is the place of their synthesis.

In biochemical parameters, PAL, cholesterol, total and conjugate bilirubine did not vary to suggest cholestasis (21&22), as they are indirect indicator of liver function. Since there were no effect on the level of transaminases (ALAT, ASAT) and creatinine, which are good indicators of liver and kidney functions respectively, it is reasonable to deduce that the *D. gossweileri* extract did not induce any damage to the liver and the kidney functions (24). This is further confirmed by the histopathological assessment (macroscopic or microscopic) of liver and kidney which show no change in their anatomy.

The absence of variation of blood glucose level shows that *D. gossweileri* extract did not affect glucose metabolism.

The presence of class compound such as triterpenoids previously report to contain a cucurbitacine like compound isolated from *D.*

gossweileri known for their high toxicity in Euphobiacae family, should have shown more toxicity activity as reported in other genus of the same family (4). This relatively less toxicity observed could be explained by antagonistic action pro toxic substances (alkaloids and triterpenoids) and protective substances (tannins and flavonoids) found in the crude extract of *D. gossweileri.*

In conclusion, the fact that no substantial toxic effect occurred in animals that were orally administered *D. gossweileri* at a dose of 1000mg/kg b.w., suggest that the margin of safety of the extract is high at dosages used clinically. However, additional long term studies with graded doses of *D. gossweileri* extract are needed to rule out any long term adverse effects and effects which require accumulation.

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