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Original Research Article

Toxicological Profile of the Stem Bark Extract of *Cylicodiscus gabunensis* Harms (Fabaceae)

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Abstract

This study aimed at evaluating the toxicity of the methanolic extract of *Cylicodiscus gabunensis* stem bark, a plant traditionally used against female fertility problems. Acute toxicity was evaluated in rats by sequential administration of a single dose of 2000 mg/kg and 5000 mg/kg of body weight of extract according to the method described by protocol 425 of the Organization for Cooperation and economic development. Subacute toxicity was assessed by daily administration of the extract at 100, 200 and 400 mg/kg for 28 days. The weight evolution and the signs of toxicity were observed, the biochemical and haematological parameters of the blood of the animals were collected and analysed. The administration of the extract at 2000 and 5000 mg/kg caused no death and no toxic signs. The weight evolution, the biochemical and haematological parameters of the surviving rats were analysed and compared with those of the controls. Acute oral toxicity data determined that the LD50 of the extract is greater than 5000 mg/kg. Repeated dose administration did not cause any deaths or significant changes in haematological parameters. Some signs of intoxication were however noted and certain biochemical parameters analysed in the rats showed statistically significant differences compared to the controls. A decrease in creatinine was observed on the renal level, an increase in transaminases and a decrease in alkaline phosphatase on the hepatic level. An increase in total proteins at 100 and 200 mg/kg and triglycerides at 100 mg/kg were observed and also a significant drop in LDL-cholesterol at 100, 200 and 400 mg/kg. This study shows that the stem barks of *C. gabunensis* would be moderately toxic in repeated oral administration, additional studies should be carried out to determine the effect of the extract on the tissues of the different organs.

Keywords: *Cylicodiscus gabunensis*, toxicity, LD_{50}, haematological and biochemical parameters, lipid profile.

INTRODUCTION

Cylicodiscus gabunensis Harms is a sun-loving plant of great socio-cultural and economic importance belonging to the Fabaceae family. It is an imposing tree of very large size that can reach 60 m in height and 300 m in diameter at breast height. *C. gabunensis* is not subject to any export restrictions under CITES and is not listed on the IUCN red list, its wood occupies a place of choice in the timber industry and is marketed as Okan or Denga [1-4]. In Cameroon, Okan wood ranked second in log exports in 2010 and 2011. In 2016, Okan was the 6th most produced species in Central Africa [5, 6]. *C. gabunensis* is also traditionally used by rural people. It is considered a fetish tree that occupies a prominent place in the rites and traditions of indigenous communities [7, 8]. Also called soap bark, the bark of *C. gabunensis* is used to treat diseases and symptoms such as stomach aches, stomach aches, prostatitis, rheumatism, diabetes, venereal diseases, malaria, psoriasis, infertility, respiratory infections, chicken pox and measles [4, 9]. Combined with other species, the field of application in traditional pharmacopoeia is even wider [10]. These therapeutic virtues would be the work of phytochemicals such as saponins, tannins, polyphenols, coumarins, sterols, triterpenes, flavonoids and reducing sugars which give *C. gabunensis* medicinal properties [4]. Scientific studies based on

observation, experimentation and analyses based on empirical prescriptions of medicinal plants are increasingly accredited. Paradoxically, certain medicinal plants can be the cause of poisoning causing more or less varied disorders. Because they are natural, plants are wrongly considered non-dangerous, insofar as knowledge, preparation and consumption requirements are not always mastered [11, 12]. It is with a view to enhancing the medicinal flora that this study is part of in order to determine the toxicological profile of the extract of the stem bark of *Cylicodiscus gabunensis.*

MATERIALS AND METHODS

Plant Material

Fresh stem bark of *Cylicodiscus gabunensis* was harvested in Nkong-Keni village, located in Bondjock district, Nyong-Ekelle department, (Centre, Cameroon).

Animal Material

Nulliparous, non-pregnant, 02-month-old female albino Wistar rats weighing between 115 g and 160 g were used in this study. They were raised in the animal facility of the Department of Biology of Animal Organisms of the University of Douala. They were subjected to a 14-day acclimatization period in cages lined with shavings. They received tap water and a standard diet ad libitum throughout the experiment.

Extraction

The freshly harvested bark was first cleared of all dead parts, then washed with water to remove any form of impurity. The fresh bark was weighed, then dried away from light and humidity for three weeks. The dried barks were ground, then macerated in methanol for 72 hours. The solution obtained was filtered with Whatman paper and then concentrated using a rotary evaporator.

Evaluation of the acute toxicity of the methanolic extract of *Cylicodiscus gabunensis* bark

Acute toxicity was determined using the method described in protocol 425 of the Organization for Economic Cooperation and Development [13]. To determine the oral toxicity of *Cylicodiscus gabunensis*, nine female rats of the Wistar strain, divided into 3 batches of 3 rats were used (2 test batches and 1 control batch). The rats were fasted for 8 to 12 hours before the start of the manipulations. The rats of the control batch received distilled water and the rats of the experimental batches, a single dose of the methanolic extract of *C. gabunensis* (2000 mg/kg and 5000 mg/kg) orally using a gastric tube. A time interval of 48 hours was observed between the first and the second test. Rats were deprived of food and water for 4 hours after treatment. After treatment, the rats were observed regularly during the first 24 hours and with particular attention during the first 4 hours in order to detect any behavioral disorders. The number of dead rats per batch was noted after 48 hours. Survivors were followed for 14 days. The LD50 was determined using the Globally Harmonized Classification System (GHS), as provided by the OECD. This represents the single dose of a test substance obtained by statistical calculation, likely to cause the death of 50% of the rats of a group during a treatment.

Evaluation of the subacute toxicity of the methanolic extract of *Cylicodiscus gabunensis* bark

Subacute toxicity was determined using the method described in protocol 407 of the Organization for Economic Cooperation and Development [14]. The extract was administered daily orally to 20 female rats of the Wistar strain divided into 4 batches of 5 rats each: Batch A: receiving distilled water at 10 ml/kg of body weight (control batch); Batches B, C, and D receiving an extract solution at the rate of 100, 200 and 400 mg/kg of body weight respectively. The extract solution was administered daily by gavage through a gastric tube over a period of 28 days. The rats were fed and hydrated ad libitum weighed before the start of treatment and then once every week. At the end of the treatment, the rats were fasted for 24 hours, then a blood sample was taken followed by dissection using ketamine, at a rate of 50 mg/kg. The organs were then removed (liver, kidney, lungs, heart and spleen) and cleaned of fat, then rinsed in salt water and weighed for estimation of relative weight and preserved in formalin.

$$Rw = \frac{Ow}{Bw} * 100$$

Rw: relative weight of the organ (g/100 g)
Ow: organ weight (g)
Bw: rat body weight (g)

Evaluation of the effects of *Cylicodiscus gabunensis* stem bark extract on haematological parameters

The blood samples taken were collected in tubes with anticoagulant (EDTA). The haematological parameters for the digital blood count (white blood cells, lymphocytes, monocytes, granulocytes, red blood cells, haemoglobin, haematocrit, platelets, VGM, TCMH, CCMH) were carried out using a haemocytometer for automatic counter blood cells from uncoagulated blood sample.

Evaluation of the effect of the methanolic extract of *Cylicodiscus gabunensis* stem bark on biochemical parameters

Several biochemical parameters were assayed including transaminases, serum creatinine, alkaline phosphatase, total bilirubin, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and total proteins on blood taken from rats using SGMitalia and Biotec kits.

Statistical analyses

Data were presented as mean ± standard deviation (sd) and analysed using Graphpad Prism version 8.1 software. The effect of the treatment on the quantified parameters was evaluated with the two-factor ANOVA test (analysis of variances) (case of toxicity parameters in rats). When the ANOVA result was significant, the Dunett and Bonferroni tests were used to locate the origin of the significance. The mean values of the parameters of the test groups were compared with those of the various controls. A probability of less than 5% was considered significant.

RESULTS AND DISCUSSION

Subacute toxicity

Determination of LD50, evaluation of behavioural parameters and weight gain

After 14 days of observation, no death was observed in the treated rats, which did not allow the determination of the LD50 which is thus greater than 5000 mg/kg. Oral administration of doses of 2000 mg/kg and 5000 mg/kg did not cause any change or signs of toxicity in rats (Table I).

Table I: Effect of *Cylicodiscus gabunensis* stem bark extract on the behaviour of rats

Behavioural signs	Control	2000 mg/kg	5000 mg/kg
Mortality (%)	00	00	00
Appearance of coat	N	N	N
Eye appearance	N	N	N
Excessive restlessness	A	A	A
Reaction to sound	N	N	N
Reaction to light	N	N	N
Appearance of secretion or excretion	A	A	A
Weird behaviour	A	A	A
Trembling	A	A	A

N: Normal; **A:** Aggressive

A non-significant increase (p>0.05) in weight mass in the different groups of rats treated with the extract compared to the control group was observed (Table II).

Table II: Effect of *Cylicodiscus gabunensis* stem bark extract on weight change

	Distilled water	2000 mg/kg	5000 mg/kg
Week 1	114.87 ± 9.67	105.33 ± 6.47	114.33 ± 6.08
Week 2	139.67 ± 6.20	127 ± 3.57	129.66 ± 9.16

Subacute toxicity

Effect on weight change

The effects of daily oral administration at repeated doses of Cylicodiscus gabunensis stem bark extract caused a non-significant increase (p>0.05) in the masses of the treated rats, compared to the control batch (Table III).

Table III: Effect of Cylicodiscus gabunensis stem bark extract on weight change for 28 days

Treatments	D1	D7	D14	D21	D28
Distilled water	144.6 ± 5.84	156.2 ± 6.44	156.8 ± 4.45	163 ± 3.83	165.6 ± 3.07
Cg 100	148.8 ± 6.74	156.6 ± 7.87	157 ± 7.81	164.6 ± 6.56	163.6 ± 6.17
Cg 200	149 ± 7.56	154.8 ± 6.41	156 ± 5.90	164.8 ± 4.14	165.6 ± 3.64
Cg 400	138 ± 4.76	150.6 ± 5.50	152.8 ± 4.68	161.8 ± 4.27	163 ± 4.48

Effect of *Cylicodiscus gabunensis* bark extract on organ mass

No statistically significant variation was observed between the control and the different doses of the extract on the organs (Figure 1).

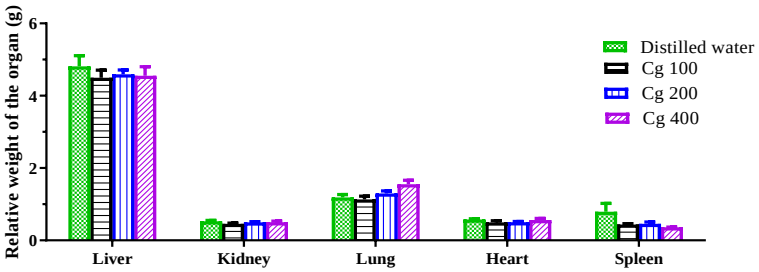

Figure 1: Effect of the extract on the average mass of the organs

Effect of *Cylicodiscus gabunensis* stem bark extract on haematological parameters

The complete blood count revealed no significant variation (p> 0.05) in the haematological parameters between the different batches treated with the extract and the control batch (Table IV).

Table IV: Effect of Cylicodiscus gabunensis stem bark extract on haematological parameters

Haematological parameters	DW	Cg 100	Cg 200	Cg 400
White blood cells (x10^3/µL)	3 ± 0.28	3.8 ± 0.26	5.4 ± 0.23	4.4 ± 0.27
Lymphocytes (x10^3/µL)	0.42 ± 0.26	0.46 ± 0.28	0.62 ± 0.32	0.48 ± 0.37
Monocytes (x10^3/µL)	0.58 ± 0.10	0.5 ± 0.09	0.4 ± 0.10	0.28 ± 0.05
Granulocytes (x103/µL)	3.6 ± 0.28	5.2 ± 0.22	5 ± 0.26	5.2 ± 0.16
Red blood cells (x10^6/µL)	4.83 ± 0.18	2.83 ± 0.22	5.03 ± 0.22	4.63 ± 0.28
Haemoglobin (g/dL)	15.2 ± 024	14.4 ± 0.23	15.2 ± 0.26	15.4 0.23
Haematocrit (%)	51.96 0.28	47.56 0.50	49.56 0.39	47.96 ± 0. 49
Platelets (x10^3/µL)	412.2 ± 1.17	272.8 ± 2.17	365.6 ± 1.88	290.8 ± 2.29
VGM (fL)	87 ± 0.46	93.2 ± 0.45	91 ± 0.41	86.6 ± 0.46
TCMH (pg)	30.4 ± 0.24	29 ± 0.16	28.6 ± 0.24	30.2 ± 0.27
CCMH (g/dL)	33.2 ± 0.20	32.4 ± 0.24	32 ± 0.28	32.8 ± 0.25

Effects of *Cylicodiscus gabunensis* extract on biochemical parameters

Effect of the methanolic extract of *Cylicodiscus gabunensis* on transaminases

A significant difference was observed between the control batch and the batches treated with different doses of the extract. A significant increase in ASAT ($p<0.01$) and ALAT ($p<0.001$) was observed at 200 mg/kg of extract (Figure 2).

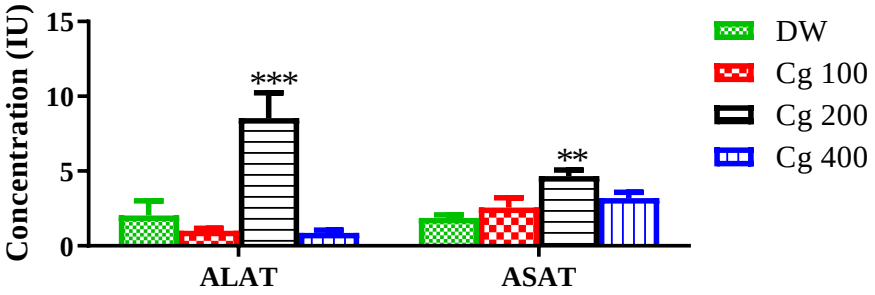

Figure 2: Effect of the extract on serum transaminase levels

Effect of *Cylicodiscus gabunensis* extract on alkaline phosphatase

A significant difference was observed between the control batch and the batches treated with the different doses of the extract. A significant decrease in alkaline phosphatase was observed at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg (Figure 3).

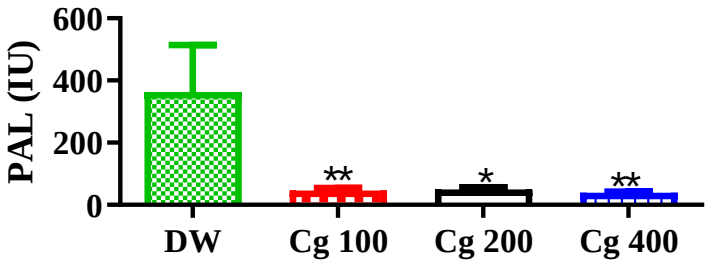

Figure 3: Effect of the extract on alkaline phosphatase

Effect of *C. gabunensis* extract on serum bilirubin level

No significant variation was observed between the control batch and the batches treated with the different doses of the extract (Figure 4).

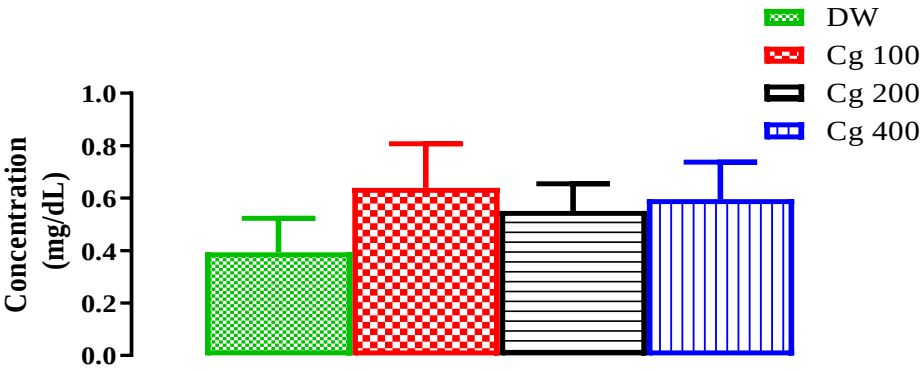

Figure 4: Effect of serum total bilirubin extract

Effect of *Cylicodiscus gabunensis* extract on serum urea level

No significant variation was observed between the control batch and the batches treated with the different doses of the extract (Figure 5).

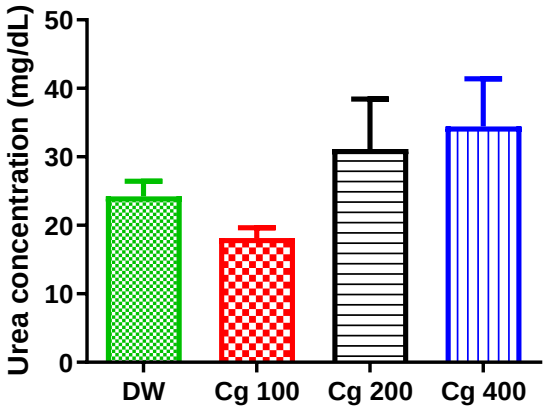

Figure 5: Effect of extract on serum urea level

Effect of *Cylicodiscus gabunensis* bark extract on serum creatinine level

A significant difference was observed between the control batch and the batches treated with the different doses of the extract. A significant drop in the serum creatinine level is observed in the batch receiving the extract at 200 mg/kg compared to the control batch (Figure 6).

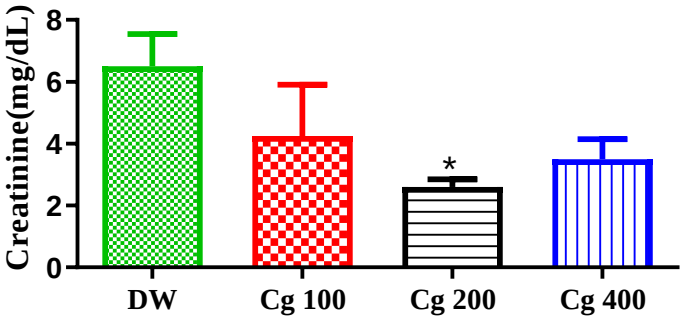

Figure 6: Effect of the extract on serum creatinine level

Effect of *Cylicodiscus gabunensis* extract on serum proteins

A significant difference was observed between the control batch and the batches treated with different doses of the extract. A significant increase in serum proteins at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg (Figure 7).

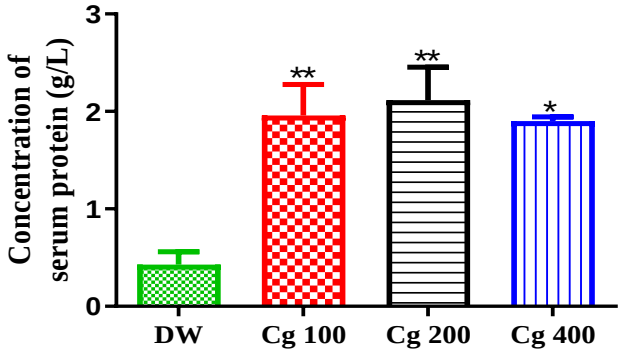

Figure 7: Effect of extract on serum protein level

Effect of the extract on the lipid profile

A significant difference was observed between the control batch and the batches treated with different doses of the extract. The figure above shows that the total cholesterol did not undergo any variation between the different batches. A significant increase ($p<0.05$) in triglycerides of 316.99% at the dose of 100 mg/kg was observed and a significant dose-dependent decrease in LDL-cholesterol at 100, 200 and 400 mg/kg was noted (Figure 8).

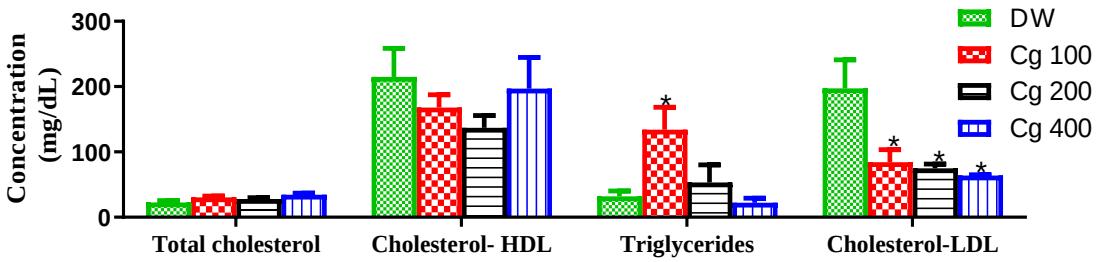

Figure 8: Effect of the extract on the lipid profile

DISCUSSION

Acute and subacute toxicity studies of *Cylicodiscus gabunensis* stem bark extract did not result in any case of rat mortality during the experiment. Behavioural signs did not reveal any toxic effect. In the absence of death, the LD_{50} of the methanolic extract of *C. gabunensis* for female rats would be greater than 5000 mg/kg. These results are in agreement with those obtained by Kouitcheu *et al*. who revealed that the ethylacetate extract of the bark of *C. gabunensis* has an LD_{50} of 11 g/kg which is well and truly higher than 5000 mg/kg [15]. Dongmo *et al* also found that the methanolic extract of the bark of *Tetrapleura tetraptera*, a species of the Fabaceae family, has an

LD_{50} probably greater than 5000 mg/kg [16]. The subacute toxicity study showed no deaths after oral administration of the extract solutions at doses of 100, 200 or 400 mg/kg for 28 days. Studies of the subacute toxicity of *C. gabunensis* carried out by Kouitcheu *et al*., showed lethality from 3 g/kg, this dose being higher than the maximum dose used for this study, this result confirms the absence of deaths in the treated groups [15]. The liver and kidneys are major targets of xenobiotic action, the liver being the main organ of xenobiotic biotransformation, while the kidney serves as the excretory organ of xenobiotics. The results of the renal parameters revealed a significant drop ($p< 0.05$) in the level of creatinine at the dose of 200 mg/kg compared to the control group. The serum urea level showed no significant variation ($p>0.05$) compared to the control. The bark of *C. gabunensis* would contain bioactive substances which would exert protective effects on the kidney by preventing the peroxidation of tissue lipids, thanks to their antioxidant and free radical scavenging activities. These results reveal a nephroprotective effect of *C. gabunensis* bark on the kidney [15]. ALAT and ASAT liver enzymes were significantly ($p<0.05$) elevated in serum at 200 mg/kg after 28 days of treatment with *C. gabunensis*, indicating liver injury and disruption of hepatocyte membranes. This could be due to the presence of bioactive substances that possess cytotoxic activity such as flavonoids. As for the alkaline phosphatases, they decreased significantly in the treated batches compared to the control batch. These results are similar to those of Kouitcheu *et al*., in whom an increase in transaminases without alterations in alkaline phosphatase was observed [15]. The relationship between dyslipidemia and the development of chronic diseases such as diabetes and hypertension is well established [17]. A significant increase in triglycerides at the dose of 100 mg/kg was observed. Triglycerides have a storage role and provide an important energy pool; high levels of triglycerides represent an independent risk factor for cardiovascular disease [18]. A significant decrease in the increasing dose of LDL cholesterol was also observed, which could reduce the likelihood of the onset of atherosclerosis. The drop in LDL-cholesterol could be due to the presence of saponins in the extract, which are known to possess cholesterol-lowering effects [19].

CONCLUSION

The toxicological study of the extract from the stem bark of *Cylicodiscus gabunensis* has shown that the extract administered orally does not present any acute toxicity for doses less than or equal to 5000 mg/kg. The biochemical parameters revealed harmful effects, in repeated oral administration on the liver, which could be liver damage and disruption of the hepatocyte membranes. *C. gabunensis* extract has moderate toxicity with however a margin of safety, extensive studies should be conducted on organ tissues.

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