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PHYTOCHEMICAL STUDY AND CARDIOVASCULAR TOXIC EFFECTS INVESTIGATION OF ROOT BARKS POWDER AND EXTRACTS FROM *CALOTROPIS PROCERA* (AIT.) R.BR.

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ABSTRACT

Calotropis procera (Ait.) R.Br. (Apocynaceae, APGIII) is a small shrub widely used in sub-Saharan Africa and particularly in Burkina Faso in the treatment of various diseases such as sickle cell disease, asthmatic disease, cancer. Like its therapeutic virtues, *Calotropis procera* is also known for its toxicity, especially on the cardiovascular system. The objective of this study was to assess the likely toxic effects of the aqueous extract of the root barks of the plant on the cardiovascular system *in vivo*. For this, a phytochemical screening was carried out on the powder of the plant's root barks. Also, an assessment of cardiovascular toxicity by measuring of blood pressure and heart rate was carried out using tail cuff method in Wistar rats treated daily with the extract for two weeks. The phytochemical study revealed the presence of numerous chemical groups including cardenolides. The assessment of cardiovascular toxicity showed that the aqueous extract of the plant at dose of 20 mg / kg causes a transient elevation in systolic and mean arterial pressure of rats treated during the first 3 days of treatment ($P < 0.001$ versus control) followed by a return to basic values after one week. However there was no effect on the diastolic blood pressure and heart rate. Given these results, the aqueous extract of the plant does not presents major toxic effects on the cardiovascular system *in vivo*.

But, the study of cardiovascular toxic effects should be continued for a safe use of this plant in pharmaceutical formulations.

KEYWORDS: *Calotropis procera*, cardenolides, cardiovascular effects.

1. INTRODUCTION

Calotropis procera (Ait.) R. Br. (Apocynaceae, APG III) (*C. procera*) commonly called "Pomme de Sodome" in french is a small tree, distributed in tropical and subtropical Africa, Asia and America.[1, 2] This plant is widely used in traditional medicine in several African countries for the treatment of various diseases.[3] The whole plant is used for the treatment of fever, rheumatism, indigestion, cold, eczema, diarrohea, boils, and jaundice. The root is used for the treatment of eczema, leprosy, elephantiasis, asthma, cough, rheumatism, diarrhoea, and dysentery. The stem is used for the treatment of skin diseases, intestinal worms.[4, 5] The leaves and root barks of the plant are used in Burkina Faso for the treatment of sickle cell disease.[6]

Several studies have shown that this plant possesses various pharmacological activities such as anti-inflammatory and analgesic activities[4, 5, 7-9], antibacterial activities[10, 11], antifungal[12] and anticancer properties.[13] The investigations conducted on the plant by the laboratories of pharmacology and toxicology of "Unité de Formation et de Recherche en Science de la Santé" (University of Ouagadougou) and the Institute for Research in Health Sciences, Burkina Faso resulted the development of a herbal drug named FACA®.[14] This drug which is a mixture of root barks powder of *Calotropis procera* (Ait.) R. Br. and *Zanthoxylum xanthoxyloïdes* Lam. (RUTACEAE) has revealed clinically some effectiveness for the treatment and prevention of sickle cell crisis in children at the University Hospital Yalgado Ouedraogo in Ouagadougou, Burkina Faso.[15]

However, as the reputation of its therapeutic properties, *C. procera* is also known for its toxicity due to its abundant latex that contains cardiac glycosides.[3] The previous studies of acute oral toxicity of aqueous and alcoholic extracts from root barks of the plant have shown that these extracts have low toxicity. Also, a sub-chronic toxicity study performed on Wistar rats showed that the aqueous extract of root barks of the plant at a dose of 20 mg / kg in daily admministration is tolerated in rats for 6 weeks.[16] In addition, the effects of plant extracts on the activity of diverse muscles including cardiac muscle were investigated.[17] However, no study has focused specifically on the cardiovascular effects *in vivo* of *C. procera*. The aim of

this study was therefore to evaluate the phytochemical constituents of the root barks of the plant and also to assess the possible cardiovascular toxic effect of the aqueous extract of this part of plant on the cardiovascular system *in vivo*.

2. MATERIAL AND METHODS

2.1. Plant material

Fresh roots of *C. procera* were collected in Roumtenga located to 25 km north-East from Ouagadougou, capital city of Burkina Faso, in July 2010. A sample of harvested plant was identified and authenticated at the "Herbier National du Burkina (HNBU)" located at "Centre National de Recherche Scientifique et Technologique (CNRST)" where the voucher specimen has been deposited under number 8716.

The barks of fresh root of *C. procera* were washed with tap water, dried in shade under ventilation and then powdered using a mechanical grinder (Broyeur à lames, Gladiator Est. 1931 Type BN 1 Mach. 40461 1083). The powder obtained was used for phytochemical characterization and preparation of extracts for biological investigation.

2.2. Animals

The toxic effect of the aqueous extract from root barks of the plant on blood pressure and cardiac function was assessed "*in vivo*" in Wistar Rat using noninvasive methods. Female and Males Wistar rats (average weight 160 ± 42 g), procured from the "Centre International de Recherche-Développement sur l'Elévage en zone Subhumide" (CIRDES), Burkina Faso, were used for the study. All animals were maintained in a controlled temperature room of 22-25°C with a 12 h dark/ light cycle with free access to water and standard laboratory pellet enriched with protein (29%). The protocol of experimentation using animals was carried out in accordance with protocols already validated by the Research Institute of Health Sciences (IRSS, Burkina Faso) and that meet international standards (guiding line set by the European Union on the protection of animals (CEC Council 86/609).[18, 19]

2.3. Phytochemical characterization

Qualitative tests were performed on root barks powder of *C. procera* for the detection of chemical groups using standard methods.[20, 21] The following tests were used for characterization of different chemical groups.

a- Tests for alkaloids

In testing for alkaloids; 10 g of plant powder were macerated in 50 ml sulfuric acid solution (10%) for 24 hours. This mixture was filtered and the volume was adjusted to 50 ml with distilled water. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1 ml portion was treated the same way with Dragendorff's reagent. Turbidity of precipitation with either of those reagents was taken as preliminary evidence for the presence of alkaloids in filtrate. In this case, confirmation of results is to carry out a specific extraction of alkaloids.

A negative test may conclude in to the absence of alkaloid in any form (true alkaloids or quaternary alkaloids).

b- Tests for flavonoids

Shibata's test was used for characterization of flavonoids. 5 g of plant powder were macerated in 100 ml of boiling distilled water for 15 min. The mixture was filtered and the filtrate was adjusted to 100 ml with distilled hot water.

5 ml of hydrochloric alcohol, 1 ml of isoamyl alcohol and some magnesium turnings were added to 5 ml of filtrate. A release of hydrogen and the appearance of a pink-orange color (flavones) or pink-purple (flavanones) or red (flavonones, flavanonols) in isoamyl alcohol supernatant layer indicates the presence of a free flavonoid (genin). The colors are less intense with flavonoid glycosides. The reaction is negative with chalcones, dihydrochalcones, aurones, isoflavones and catechins.

c- Test for anthraquinones

Borntrager's test was used for the detection of anthraquinones. A mixture of 1 g of drug powder and 10 ml of chloroform was heated carefully in a water bath for 3 minutes. After hot filtration, the filtrate was adjusted to 10 ml with the same solvent. 1 ml of diluted ½ NH_4OH solution was added to 1 ml of the filtrate and the mixture was shaken. The appearance of a more or less red coloration in the ammoniacal (lower) phase indicates the presence of free anthraquinones.

For bound anthraquinones, 10 ml of distilled water was added to a part of residue of the powder exhausted with chloroform. The mixture was boiled with 1 ml of concentrated hydrochloric acid in boiling water bath for 15 minutes and then cooled under a stream of water and filtered. The filtrate was adjusted to 10 ml with distilled water.

5 ml of filtrate were stirred with 5 ml of chloroform and organic phase was then removed and introduced in a tube. A diluted ½ NH_4OH solution was added in tube and the whole shaken. The presence of anthraquinones is revealed by the more or less intense red coloration in the ammoniacal lower phase.

d- Test for anthocyanosides

1-2 sodium hydroxide pellets were added to 1 ml of extract (aqueous phase of the hydrolyzate) and the appearance of a blue color indicates the presence of anthocyanins in the extract.

e- Test for tannins

Ferric chloride reagent (1ml of 1%) was added to 5 ml of plant extract (infused of plant powder in water). The formation of dark blue or greenish black indicates the presence of tannins.

f- Test for coumarins

5 ml of etheric extract was evaporated to dryness and the residue was dissolved in 2 ml of hot water. After cooling, 0.5 ml of NH_4OH (10%) was added followed by observation under an ultraviolet lamp (λ = 366 nm). The appearance of an intense greenish blue or violet fluorescence indicates the presence of coumarin.

g- Test for saponins

Distilled water (2ml) was added to 2 ml of aqueous decoction (1%) and shaken in graduated cylinder lengthwise for 15 minutes. Development of foam on the surface of the mixture, lasting for 10 minutes indicates the presence of saponins.

h- Test for cardiac glycosides

A mixture of 1 g of plant powder, 10 ml of ethanol (60 °) and 5 ml of a neutral lead acetate (10%) solution was heated in a boiling water bath for 10 min and filtered. The filtrate is then stirred with 10 ml of chloroform and the chloroformic phase is removed and shared between 3 test tubes and evaporated. The residues were taken up in 0.4 ml of isopropanol. The extracts were then treated with reagents of Baljet, Kedde or Raymond Marthoud. In the presence of KOH (5%) the appearance of orange coloration (Baljet's test), purplish red (Kedde's test) and purple fleeting (Raymond-Marthoud's test) indicate the presence of cardiac glycosides.

i- Test for mucilages

5 mL of absolute alcohol was added to 1 ml of aqueous decoction (10%) in a tube and allowed to stand for 10 minutes. Obtaining a flocculent precipitate after gentle agitation indicates the presence of mucilage.

j- Test for steroids and triterpenoids

Libermann-Burchard's test was used. 10 ml of chloroform extract was evaporated to dryness and the residue was dissolved with 2 ml of chloroform and acetic anhydride (1: 1 v / v). 1 ml of concentrated H_2SO_4 was added to the top of the liquid. At the interface, the appearance of brownish purple or red ring and a bluish green supernatant indicates the presence of steroids and triterpenoids.

2.4. Determination of water content, total ash and acid-insoluble ash of root bark powder

The determination of water content, total ash, and acid-insoluble ash of root barks powder from *C. procera* was carried out according to quality control methods for medicinal plant materials described by World Health Organisation.[22]

2.5. Cardenolides quantification

Ten (10) g of root barks powder of *C. procera* are macerated in 100 ml of solvent (50% ethanol or distilled water) for 24 h followed by percolation with the same solvent until exhaustion. After filtration and centrifugation, 100 ml of solution of lead acetate 5% was added to the supernatant of each extract and boiled for 2 minutes. The mixture is then centrifuged and the clear supernatant was collected in a flask and extracted with chloroform (3x15 ml). The chloroform phase was dried with anhydrous sodium sulfate, filtered and evaporated. The residue of each extract (aqueous and hydroethanolic) was taken up in 2 ml of absolute ethanol.[23]

The dosage of cardenolides was then carried out through UV spectrophotometry. Absorbance of each extract obtained was measured at 550 nm wavelength after addition of a 3,5-dinitrobenzoic acid solution and KOH. Simultaneously a digoxin solution (digoxine® injection 0.5mg / 2ml) was used to prepare series of dilution. The absorbance of different dilutions has served to establish the calibration curve. The cardenolide content is then calculated from the absorbance of each extract at the same wavelength (550 nm) and the equation of the line.

2.6. Cardiovascular toxic effects evaluation

The evaluation of cardiovascular toxic effects of extract was carried out *in vivo* on Wistar rats using the tail cuff method (blood pressure recorder, model 58500; UGO BASILE biological research apparatus, Comerio Varese, Italy).

The tail cuff method is a non-invasive measurement of blood pressure based on the same principle as the plethysmographic method used in clinic for the measurement of blood pressure in the brachial artery. The cuff is inflated gradually. When the cuff pressure becomes higher than the systolic pressure, the blood flow in the tail artery is interrupted. The sleeve is then progressively deflated. Systolic pressure is measured when the blood can flow back into the artery, that is to say when the pressure imposed by the sleeve becomes equal or less than the systolic blood pressure.

The method has been previously validated in the laboratory of toxicology, health and environment of health doctoral school of the University of Ouagadougou. The validation of the method was adapted from the method previously described by Gerold and Tschirky[24] and was consisted to a daily measurement (n = 5) and at different times (three time a day) during one week of systolic blood pressure, diastolic and heart rate in untreated rats to verify the reproducibility of the values obtained.

a-Drug and treatment

The cardiovascular toxic effects were assessed with the aqueous extract. The aqueous extract was prepared by macerating of 250 g of powder in 2,5 l of distilled water during 24 h at room temperature. This procedure was repeated two times with the residues of extraction. After that, the extracts were filtered through cotton wool and the filtrates were then centrifuged at 3000 rpm for 10 min. The collected supernatant of aqueous extract was then lyophilized, packaged in a bottle and stored in desiccators. The lyophilizate obtained was used for the test.

The rats were randomly divided into 2 groups of 10 (5 males and 5 females) each and kept in separate polypropylene cages two weeks before the use.

The first group served as control and received a daily oral administration of vehicle (distilled water) and the second group received a daily oral administration of plant extract at the dose of 20 mg / kg for two weeks. Measurements of blood pressure and heart rate were then performed every two hours after administration of vehicle or extract and at d0, d1, d3, d7 and d14 using an indirect measuring and recording of bloodless pressure in unanaesthetized rats

by tail cuff method (Model Blood Pressure Recorder 58500, UGO BASILE biological research apparatus, ITALY).

b-Experimental procedure to record blood pressure and Pulse rate

Basically, rats were placed into suitable harnesses from which the tail protrudes.

In order to familiarize the animals to the conditions of restraining they were kept 3-4 min in the restrainer, their tails fitted with cuff and transducer. The exercise was repeated 2-3 times a day during a week.

Before the tests, animals were pre-warmed in hot box at about 29-30°C for at least 30 min, in order to cause a sufficient vasodilatation in the caudal artery. After this, cuff and the pulse transducer have been properly fit to the tail of the animals. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the unanaesthetized, prewarmed, restrained rats by tail-cuff through the pneumatic connection.Mean arterial pressure (MAP) was then calculated using the following formula

$$MAP = \frac{2}{3} DBP + \frac{1}{3} SBP$$ [25, 26]

The Heart rate (HR) of the animals is automatically assessed in real time by a heart rate counter which picks the signal from the pulse transducer.

Each value represents the average of 4 consecutive measurements per animal.

2.7. Data analysis

The results of *in vivo* test are expressed as means ± SEM (Standars Error of the Mean). Statistical difference between the control and the treated groups were analyzed using one-way analysis of variance (ANOVA), followed by Dunett's multiple comparison tests through the Graph Pad Prism 5.0 program. Differences were considered to be statically significant at $p < 0.05$.

3. RESULTS

3.1. Phytochemical characterization: The results of qualitative phytochemical analysis of root barks powder from *C. procera* are summarized in Table 1.

Table 1: Qualitative phytochemical analysis of root barks powder from *C. procera*

Phytochemical test	Results
Alkaloids	-
Flavonoids	-
Tannins	-
Anthraquinones	-
Anthocyanosides	-
steroids and triterpenoids	+
Saponins	+
Mucilages	+
cardiac glycosides	+
Coumarins	+

Key: + = Present; - = Absent.

3.2. Water content, total ash and acid-insoluble ash of root barks powder from C. procera

The results of water content, total ash and acid-insoluble ash of root barks powder from *C. procera* are presented in table 2.

Table 2: Water content, total ash and acid-insoluble ash of root barks powder from *C. procera*

substances	Content (%)
Water (gravimetric determination)	8.54%
Water (Azeotropic method)	8.00%
Total ash	6.50%
acid-insoluble ash	3.93%

3.3. Cardenolides content: Table 3 shows the cardenolide content in aqueous and hydroethanolic extracts of root barks powder from *C. procera*.

Table 3: Cardenolide content (expressed as digoxin equivalents) of extracts of root barks powder from *C. procera*

Type of extract	Cardenolide content (expressed as digoxin equivalents %)
Aqueous extract	0,01
hydroethanolic extract	0,43

This result indicates that the cardenolides content is low in aqueous extract compared to the hydroethanolic extract.

3.4. Cardiovascular toxic effects of aqueous extract from the root barks of C. procera

a- Effect of the aqueous extract from the root barks of C. procera on systolic blood pressure in rats

Fig. 1 shows the changes in systolic blood pressure (SBP) of male and female control rats and treated daily with the aqueous extract from root barks of *C. procera* at the dose of 20 mg/kg b.w. for 2 weeks.

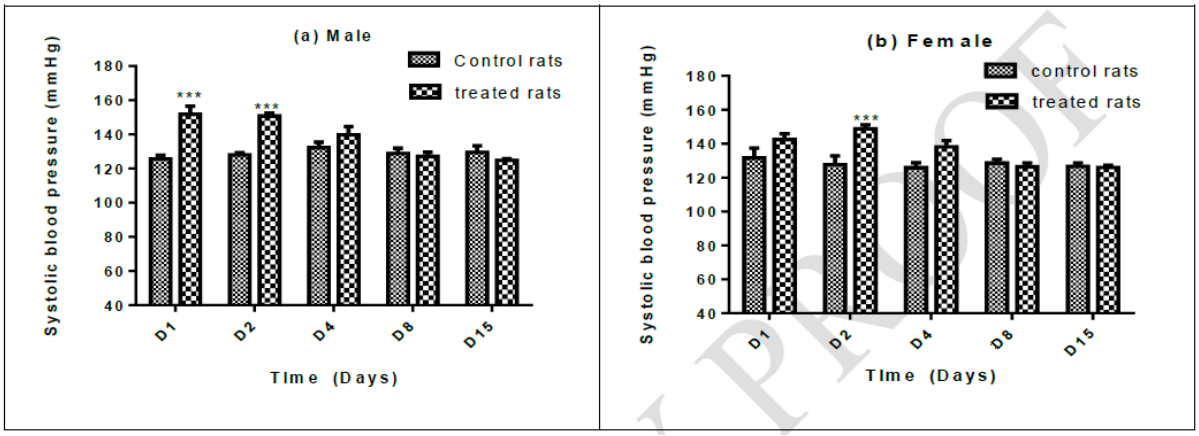

Fig. 1: Changes in Systolic blood pressure for male (a) and female (b) control rats and treated with the aqueous extract of *C. procera* during the time.

Values are expressed as mean ± SEM for 5 Wistar rats in each group. P value: *** P <0.001 compared to controls.

As showed in this figure, the daily administration of aqueous extract from plant at dose of 20 mg/kg in male and female rats induces a transient elevation of SBP during the first three (3) days of treatment followed by a return to the basic values after a week of treatment. This increment was statistically significant at D1 and D2 in male rats (P <0.001 versus control group) (Fig.1a) and D2 in treated female rats (P <0.001 versus control group) (Fig.1b).

b- Effect of the aqueous extract from the root barks of C. procera on Diastolic blood pressure in rats

Fig. 2 illustrate change in diastolic blood pressure (DBP) of male and female control rats and treated daily with the aqueous extract of *C. procera*'s root barks at the dose of 20 mg/kg b.w. for 2 weeks.

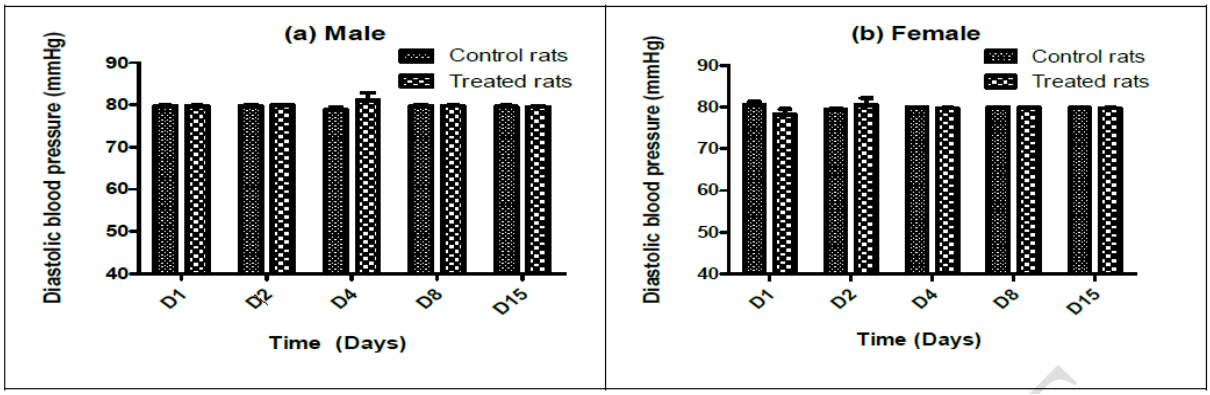

Fig. 2: Changes in Diastolic blood pressure for male (a) and female (b) control rats and treated with the aqueous extract of *C. procera* during the time.

Values are expressed as mean ± SEM for 5 Wistar rats in each group.

Analysis of this figure shows that daily administration of plant extract in male and female rats does not cause a change of DBP in treated group versus control group.

c- Effect of the aqueous extract from the roots barks of C. procera on mean arterial pressure in rats

The change in mean arterial pressure (MAP) of male and female control rats and treated daily with the aqueous extract of the plant is shown in Fig.3.

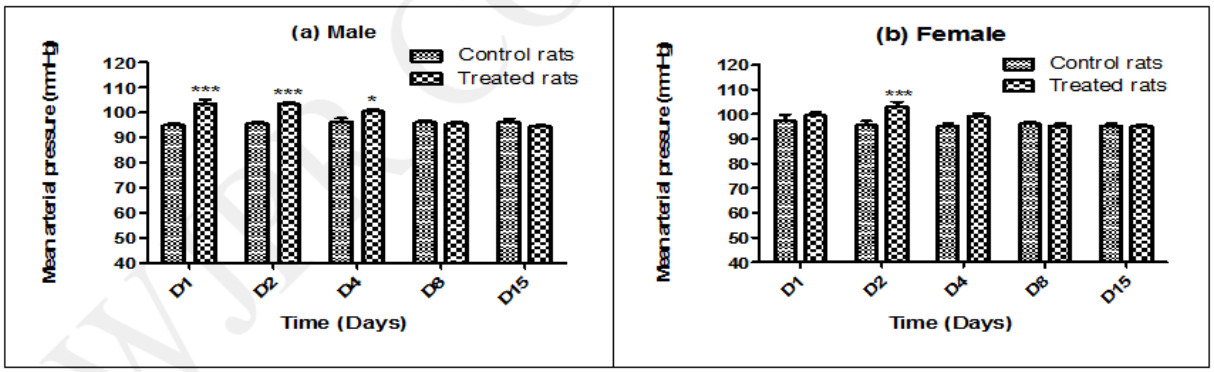

Fig. 3: Changes in mean arterial pressure for male (a) and female (b) control rats and treated with the aqueous extract of *C. procera* during the time.

Values are expressed as mean ± SEM for 5 Wistar rats in each group. P values: * p <0.05; *** P <0.001 compared to controls.

As shown in Fig. 3, the daily administration of the plant extract causes a significant elevation of MAP during the first three days of treatment in male rats (fig. 3a) and after 24 hours in female rats (Fig. 3b) compared to control rats, followed by a return to basic values.

d- Effect of the aqueous extract from the root barks of C. procera on heart rate in rats

Fig. 4 shows the variation in heart rate (HR) of male and female control rats and treated daily with the aqueous extract from the root barks of *C. procera* at a dose of 20 mg / kg b.w. for two weeks.

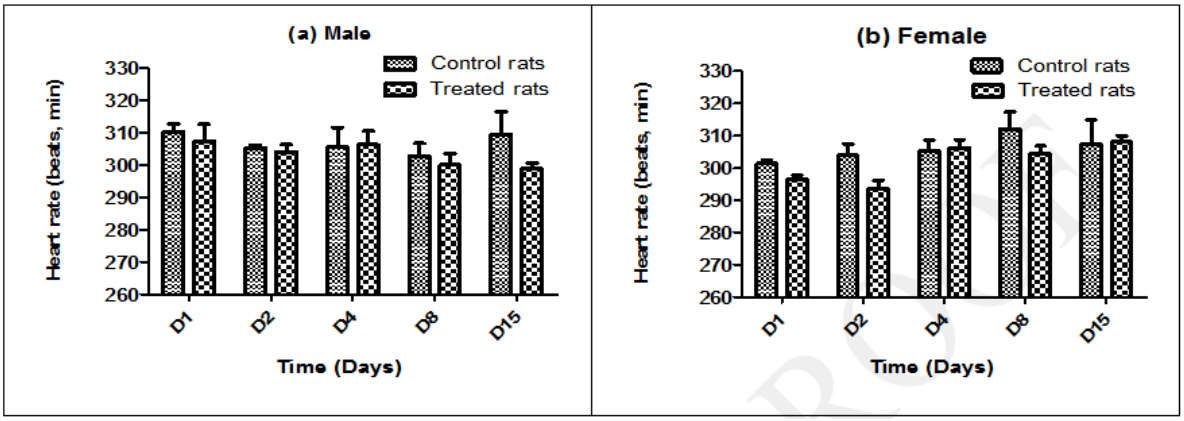

Fig. 4: Changes in heart rate for male (a) and female (b) control rats and treated with the aqueous extract of *C. procera* during the time.

Values are expressed as mean ± SEM for 5 Wistar rats in each group.

As shown in Fig. 4, the daily administration of aqueous extract of *C. procera* root barks at a dose of 20 mg/kg b.w. has no statistically significant effect on the heart rate in treated male and female rats compared to control rats.

4. DISCUSSION

4.1. Phytochemical investigation

In the present study, the water and ashes contents of the powder from the root barks of the plant were determined. The result show that the water content is less than ten (10) % indicating that the powder of the plant can be stored for a long time in good conditions without major risk of deterioration of chemical principles.[27] In fact, an excess of water in medicinal plant materials will favour microbial growth, the presence of fungi or insects and alteration of the therapeutic properties of the drug by degradation of the active ingredients over time.[22, 28] However, powder should be kept in a desiccator to avoid the effect of humidity.

The total ash contents and acid-insoluble ashes of the root barks powder of the plant were 6.5 and 3.93 respectively, reflecting a wealth of plant powder in mineral element. Some authors were found values of 10.17 and 2; respectively for total and acid-insoluble ashes of root barks

powder of *C. procera* of Mali.[29] This difference with our results could be explained by the soil factors i.e. the middle or the soil in which the roots were harvested; the mineralogical composition of the soil being different from a medium to another.

The phytochemical characterization was allowed to highlight the presence of compounds such as coumarins, saponin, sterols and triterpenes and cardiotonics glycosides in the powder of root barks of *C. procera*. However, the absence of some compounds such as alkaloids, phenolic and polyphenolic compounds in the powder of the plant was noted. These results corroborate those of other authors which showed the presence of carotenoids, steroids, cardiac glycosides, triterpene glycosides, saponins, monosaccharides, and holosides and polyuronides (mucilage) in extracts from roots barks of *C. procera* from Mali.[29] Some authors have revealed the presence of alkaloids in extracts from root barks of the plant.[10] Also, the presence of tannins, flavonoids in all parts of the plant has been reported.[30-32] The lack of certain compounds (alkaloids, tannins) in the root bark powder of the plant could be explained by climate and soil factors. These factors are of paramount importance in plant biosynthesis.[33]

The main chemical groups found in the powder from the root barks of the plant has various pharmacological properties but in certain conditions can lead to toxic effects. The saponins for example are cellular irritants on different organs, expectorants on the lung parenchyma, haemolytic on the red cells by direct contact.[34] The cardiac glycosides have an effect on the heart and blood vessels, causing a loss of intracellular potassium by inhibition of membrane ATPase-dependent sodium-potassium. Cardiotonic glycosides also cause contraction of smooth muscle of gastrointestinal tract, bronchus and uterus at toxic doses.[35] Coumarins and derivatives are hypothermisants, antihypertensives, anticoagulants and antispasmodic.[36]

The dosage of cardenolides was performed by UV spectrophotometry and allowed to rate their content in aqueous and alcoholic extracts of root barks of *C. procera*. The result shows that the cardenolides content in the aqueous extract is low compared to the alcoholic extract. This difference could be explained by the difference of the extraction solvents used and the physicochemical nature of cardenolides in the plant. Indeed, the cardiac glycosides are moderately polar compounds, therefore much more extractable with moderately polar solvents such as ethanol more or less diluted.[23]

4.2. Cardiovascular toxic effects evaluation

The assessment of cardiovascular toxicity of the aqueous extract from the root barks of *C. procera* was conducted using tail cuff method. This noninvasive method has been previously validated in our laboratory starting from that described by Gerold and Tschirky.[24] This method, unlike other measurement techniques such as the measurement of blood pressure and heart rate by the bloody method has some advantages. Indeed, it avoids the disadvantages that could cause anesthetic and operative trauma in the installation of catheters. Also it allows to study the cardiovascular chronic toxicity following repeated administration of a product; blood pressure and heart rate can be measured regularly in the same animal during the study period. Thus, the effects of products that could affect blood pressure and heart rate in the short, medium or long term can be detected.

During the study about the cardiovascular toxic effects of aqueous extract from the root barks of the plant, a single dose of 20 mg / kg of the extract was administered daily to the rats by oral route for two (2) weeks. The choice of this dose was based on the fact that a preliminary study of sub-chronic toxicity revealed that the daily oral administration of aqueous extract from the root barks of the plant is tolerated in Wistar rats for 6 weeks at the same dose.[16]

The result shows that the aqueous extract of the plant causes a significant transient elevation of systolic and mean arterial pressure in rats treated during the first three days of treatment followed by a return to basic values at the end of a week of treatment. The values of systolic and mean arterial pressures of control were almost constant during the study period. The values of systolic blood pressure obtained in Wistar control rats are comparable to those of other authors who used similar methods (bloodless methods). Indeed, Ratsimbason et al.[37] showed that normal systolic blood pressure of Wistar rats is between 120 and 140 mm Hg using the noninvasive method. Also, Joshi et al.[38] have obtained the values of SBP of about 139 mmHg in Wistar rats using the technique of measuring the blood pressure by the tail cuff on unanesthetized rats.

The significant increase in systolic blood pressure can be explained by the presence of cardenolides in the aqueous extract of the plant. Indeed, cardenolides are known for their action on the cardiovascular system. They would act at the membrane level through inhibition of the Na^+/ K^+ ATPase which would result in an increase in intracellular concentration of ionized calcium.[23] This accumulation of calcium ion Ca^{2+} in the intracellular medium led to a strengthening of muscle contraction, including myocardial and a rise in blood pressure.[39-41]

Cardenolides also isolated from the plant such as calotropin, calotoxin and uscharin have a pharmacodynamic action similar to that of ouabain.[42] The presence of this type of cardenolides in extract of the plant could explain the increase in blood pressure obtained. During the evaluation of cardiovascular toxic effect of the extract from the plant, it was not observed a statistical significant difference between the values of the diastolic blood pressure of treated rats compared to controls. Similarly, there was no significant change in heart rate in treated rats with the same dose of the extract (20 mg / kg) compared to the control rats. These results could be explained by the dose of the extract used, the dose is low to induce a negative chronotropic effect in the treated rats.

5. CONCLUSION

This study has allowed to identify several secondary metabolites in the root barks of the plant such as coumarins, saponins, sterols and triterpenes and cardiac glycosides. The presence of these metabolites would be responsible of various medicinal properties of the plant. The evaluation of cardiovascular toxic effect of the plant extract in rats showed that the aqueous extract of the plant does not present major toxic effects on the cardiovascular system in vivo. However, it is necessary to continue these investigations for a safe use of this plant in pharmaceutical formulations.

6. ACKNOWLEDGEMENT

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