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ORIGINAL RESEARCH

Cardiotonic Activity of the Aqueous Extract of *Euphorbia Poissonii* on Isolated Frogs' Hearts

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ABSTRACT

Aim: The effects of the water extract of the leaves of *Euphorbia Poissonii* (Euphorbiaceae) on frogs hearts contraction and its possible mechanism action were investigated.

Method: Using the Langerdhorff model of the isolated frog heart preparation, the strength and frequency of heart contractions as well as cardiac output (CO) were measured under perfusion with test drugs.

Results and conclusion: When assessed with digoxin and adrenaline as positive controls, the water extract (3 mg/ml) exhibited significant positive inotropic (70% improvement compared to baseline) and chronotropic (17.79% improvement) effects. Additionally, heart contractility was inhibited in the presence of propranolol and amlodipine, suggesting that the testing extract is having adrenergic properties. Reversible heart arrest was noticed at the dose of 6 mg/ml.

KEYWORDS:
Euphorbia poissonii; frog heart, inotropic and chronotropic effects

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INTRODUCTION

Cardiovascular disease is considered as a major health and socioeconomic burden in the world. Sub-Saharan Africa is considered as an epidemiological transition zone.[1,2] A recent study conducted in the South-West Region of Cameroon showed that heart failure, an important form of cardiovascular disease in Africa, represented 3.9% of all admissions in the medical unit and it predominantly occurs at a younger age.[3] Cardiac glycosides are the drugs of choice to treat heart failure but their low therapeutic index and severe side effects limit their clinical usefulness.[4] In addition to their unavailability, dangers associated with their intoxication have also been a challenge.[5] It is, therefore, necessary to explore alternative measurements including medicinal plants that may provide patients with a wider range of affordable options.[6] In this regard, several plants have also been studied for cardiotonic activity,[7,8] where positive inotropic and chronotropic effects were shown on the isolated frogs' heart preparations. The genus *Euphorbia* is largely spread across the world.[9] Their derived products such as extracts and compounds are known to be active in a broad range of medicinal properties. These include diuretic and laxative activities, cytotoxic, mitochondrial respiratory chain inhibition, viral and bacterial infection inhibition, anti-inflammatory, and multidrug resistance modulation.[9,10] Its medicinal application

also includes the treatment of lumbago, viral diseases such as HIV-1, and diabetes.[11–13] *Euphorbia poissonii (Ep)* is a plant widely grown in sub-Saharan Africa and generally found in rocky places and sometimes in botanical gardens. Preliminary phytochemical screening of the aqueous extract of *E. poissonii* leaves revealed the presence of reputed cardiotonic compounds including families like glycosides, flavonoids, polyphenols, and alkaloids.[11] Moreover, the use of the plant as a hunting poison in Africa could have a direct impact on the heart's activity.[14] The present study was, therefore, carried out to determine the cardiotonic activity of the aqueous extract of *E. poissonii* leaves on frogs' hearts.

MATERIAL AND METHODS

PLANT MATERIAL

The leaves of *E. poissonii* were harvested the Garoua Wildlife School (Benoue Division, North Region, Cameroon) in June 2018. Taxonomic identification was done at the National Herbarium, where the voucher specimen was deposited under N°42092/HNC in June 2018.

ANIMAL MATERIAL

Frogs (*Bufo bufo*) weighing between 40 and 65 g were kept in an artificial pond located at the Maroua Protestant College for 3 weeks. The frogs were given free access to food which was consisted of insects like termites and small locusts; a vitamin complement was also added. Animal procedures were conducted with strict adherence to the National Institutes of Health (NIH) Guide for the care and use of Laboratory Animals (NIH Publication #85-23 Rev. 1985).

PREPARATION OF THE AQUEOUS EXTRACT OF EP LEAVES

To 50 g of powdered plant material, 450 ml of distilled water was added and heated at 70 °C for 1 hour. After cooling, the mixture was filtered using the No. 4 Whatman GF/C paper (90 mm). The filtrate solution obtained was evaporated in a ventilated oven at 45 °C until a paste was collected. This paste served as an aqueous extract for the rest of the work and yield 7.44 %.

ISOLATION OF THE FROGS' HEARTS AND MEASUREMENT OF CARDIAC CONTRACTILITY PARAMETERS

The isolation of the frogs' hearts was done according to the standard procedure. Briefly, a common frog decerebrated and demedulated was fixed supine on a board. The exposure of the heart was done by removing it from the skin, the thoracic muscle, and the pericardium. A small incision in the venous sinus was made to introduce the cannula filled with batrachian ringer solution. The cannula was gently moved to slide into the ventricle. Subsequently, a ligature was made around the cannula while lifting the tip of the ventricle and the isolated heart was rid of surrounding tissue.[15] The aqueous extract solutions of the leaves of *E. poissonii* and the reference substances used were prepared by dissolving them in the ringer solution (g / L: CaCl 2: 0.24; NaCl: 9; KCl: 0.42; NaHCO3: 0.5; dextrose: 1.0).

The parameters of the cardiac contractility were measured according to the Langerdhorfftype device. Indeed, the heart was connected to the Starling lever and adjustments needed to record the heart's responses were performed. The level of the Ringer solution in the Syme cannula was maintained by attaching a glass tube in the cap to the reservoir (Mariott bottle). The isolated heart was washed for about 5 min with Ringer's solution until a regular cardiogram was recorded on a kymograph paper wrapped around a rotating drum driven by a motor at the speed of rotation of 1.25 mm/s before infusion containing test drugs was introduced. Infusion with each drug concentration was preceded by washing the heart with Ringer's solution[15] and the parameters of cardiac contractility were recorded.

STUDY OF THE CARDIOTONIC ACTIVITY

Cardiotonic potential on normal isolated heart

Cardiotonic potential was evaluated using the aqueous extracts of the leaves of *E. poissonii* and compared to that of digoxin. The responses of digoxin and the tested plant extracts at various concentrations were recorded and their cardiac activity in terms of heart rate (HR), height of force of contraction (FC), and cardiac output (CO) was noted. The frogs' hearts were rinsed for about 2 min with Ringer solution after every administration of reference drug or tested extract till it was brought to the normal state. Digoxin was tested at concentrations of 0.125, 0.25, and 0.5 mg/ml. The aqueous extract of the leaves of *E. poissonii* was prepared at 2, 3, and 4 mg/ml. The basal cardiac contraction was recorded after the administration of the Ringer solution. A new heart was prepared each time the previous one no longer had normal rhythmic contractions.

DETERMINATION OF THE POSSIBLE MECHANISM OF ACTION

Comparison with a β-blocker

Once the basal cardiac contraction was recorded, adrenaline was administrated at 10^{-2} mg/ml. After 4 min, this was followed by propranolol (10^{-4} mg/ml) or the aqueous extract of the leaves of *E. poissonii* (3 mg/ml) infusion.

Comparison with calcium channel blocker

In this study, the isolated frogs' hearts were infused with adrenaline (10^{-2} mg/ml) or the aqueous extract of the leaves of *E. poissonii* (3 mg/ml), preceded by amlodipine (10^{-6} mg/ml) injection.

Statistical analysis

Data are presented as mean ± Standard error on the mean (SEM) for a number (n) of six scores. The statistical analysis of the results was done using the Graph Pad prism software version 5.0. After analyzing variances by a one-way ANOVA test, the intergroup averages were compared using a non-parametric turkey test. The significance limit was set at $p < 0.05$.

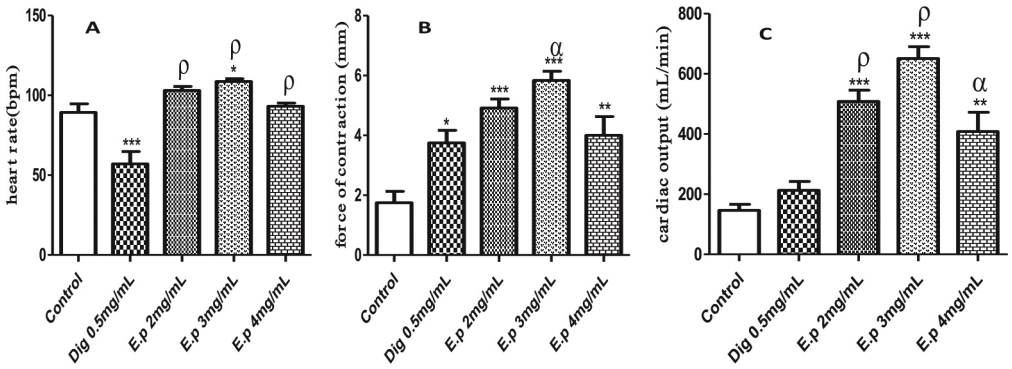


Figure 1 Activity of aqueous extract of the leaves of *EP* on Heart rate (A), FC (B), and CO (C) of isolated frog hearts. Each value represents the mean ± SEM, $n = 6$. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$: statistically significant when compared to control. $^{\alpha}p < 0.05$; $^{\beta}p < 0.05$; $^{\rho}p < 0,001$: statistically significant when compared to digoxin (dig).

RESULTS

CARDIOTONIC POTENTIAL ON NORMAL ISOLATED HEART

Heart rate, height of contraction force, and CO of the isolated frog hearts infused with the aqueous extract of the leaves of *E. poissonii* and digoxin are depicted in Figure 1. It appears that, when compared with Ringer perfused hearts (neutral control), digoxin significantly decreased the HR by 41.43% ($p < 0.001$) and caused an increase in force of contraction (FC) as well as CO of 59.09% and 25.20%, respectively. Aqueous extract of the leaves of *E. poissonii* perfused at a concentration of 3 mg/ml showed a significant increase ($p < 0.001$) in heart contractility. HR, CF, and CO were 17.79%, 64.40%, and 52.56%, respectively, higher than normal control. Notwithstanding this increase, there was a progressive decrease in the same parameters at the concentration of 4 mg/ml for the aqueous extract of *E. poissonii*. Heart recovered its contractility after extract administration at tested concentrations (Photography 1).

DETERMINATION OF THE POSSIBLE MECHANISM OF ACTION

Comparison with a β-blocker

Infusion of the heart of the frogs with the aqueous extract of the leaves of *E. poissonii* (3 mg/ml) or adrenaline (10^{-2} mg/mL) showed a significant increase ($p < 0.05$) of the cardiac contractility (Figure 2). The improvement in HR, CF, and CO by the plant extract was 20.07%, 54.08%, and 59.30%, respectively; while that of adrenaline account for 16.85%, 54.08%, and 61.82%, respectively. In the presence of propranolol (10^{-4} mg/mL), infusion of the heart with the aqueous extract of the leaves of *E. poissonii* or adrenaline significantly decreased ($p < 0.05$) heart contractility (Photography 2). The reduction of HR, CF, and CO was about 68%, 77.56%, and 88.48%, respectively, when using the aqueous extract of the leaves of *E. poissonii* and 40.07%, 79.59%, and 77.37%, respectively, for hearts infused with adrenaline (Figure 2).

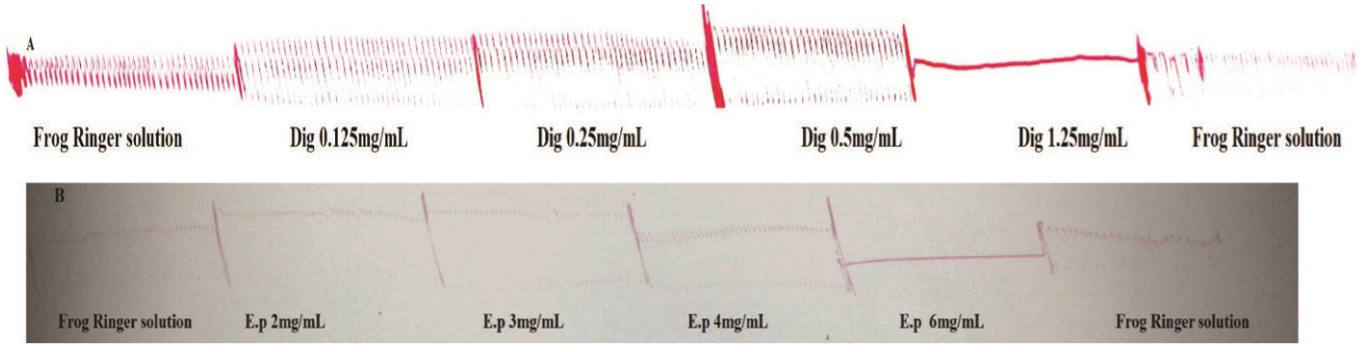
COMPARISON WITH CALCIUM CHANNEL BLOCKER

Results of the study on the influence of Amlodipine (10^{-6} mg/ml) on the frogs' heart contractility of adrenaline and the aqueous extract of the leaves of *E. poissonii* are presented in Figure 3 and Photography 3.

Amlodipine significantly prevented ($p < 0.05$) plant extract and adrenaline-induced heart contractility. The reduction in HR, FC, and CO was of 34.83%, 80.62%, and 87.14%, respectively, for the aqueous extract of the leaves of *E. poissonii* and 45.18%, 79.59%, and 88.68%, respectively for adrenaline.

DISCUSSION

The present study was designed to evaluate the cardiotonic potential of *E. poissonii* (Euphorbiaceae) and determine the possible mechanism of action.



Photography 1 Record showing the cardiotonic activity of the aqueous extract of the leaves of digoxin (dig) (A) and *E. poissonii* (EP) (B) on the functioning of the isolated frogs' hearts.

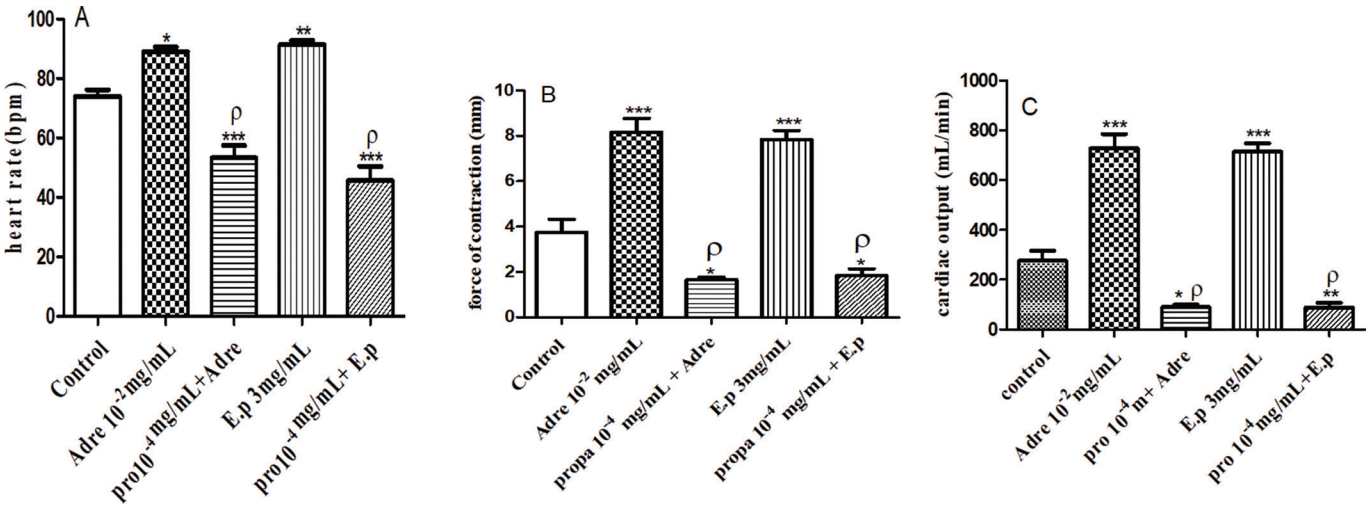
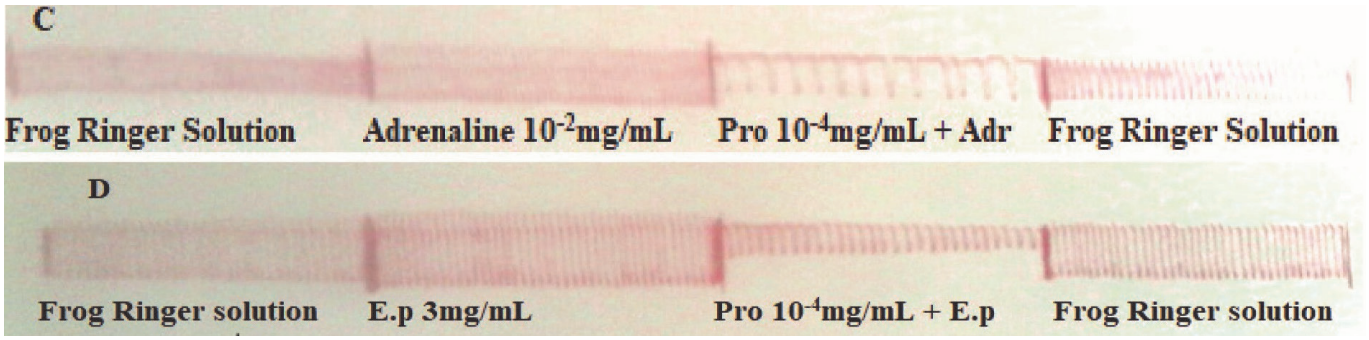


Figure 2 Interaction propranolol (propa) – adrenaline (adr) /aqueous extract of the leaves of (Ep) on HR (A), force of contraction (B), and CO (C). Each value represents the mean ± SEM, $n = 6$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$: statistically significant when compared to control. $^{\alpha}p < 0.05$; $^{\beta}p < 0.01$; $^{\rho}p < 0.001$: statistically significant when compared to the positive control (Adrenaline).



Photography 2 Recording showing the interaction propranolol (prop) – adrenaline (adr) (C) or aqueous extract of the leaves of (Ep) isolated frogs' hearts contractility.

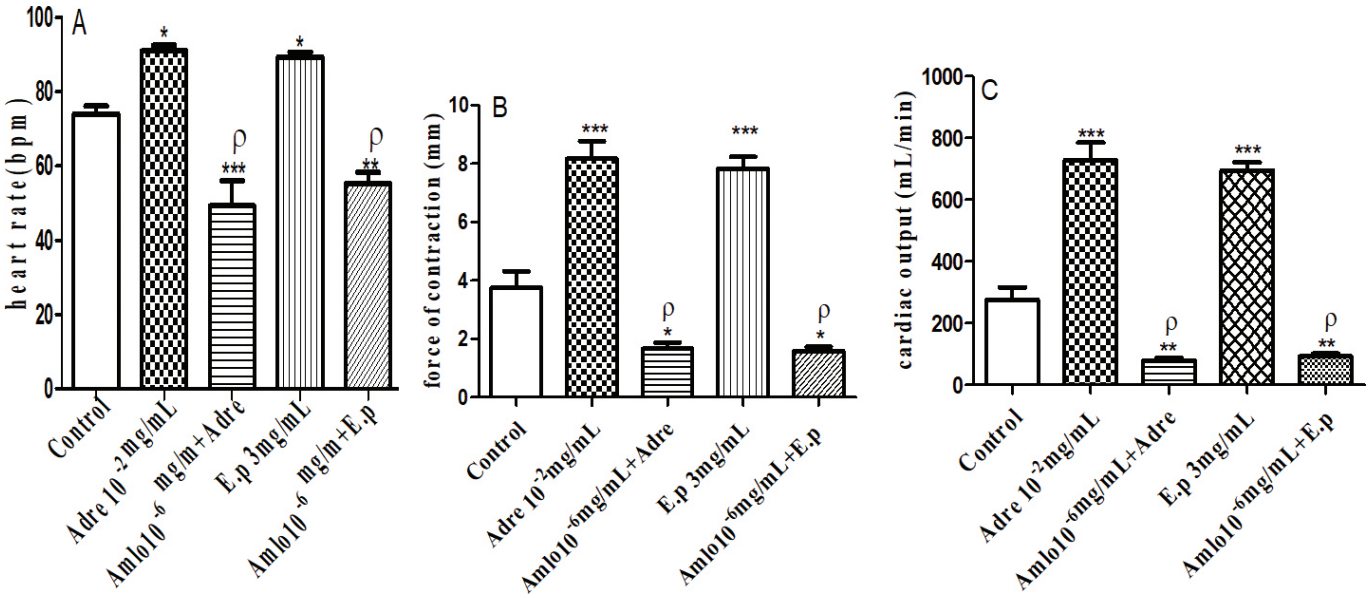
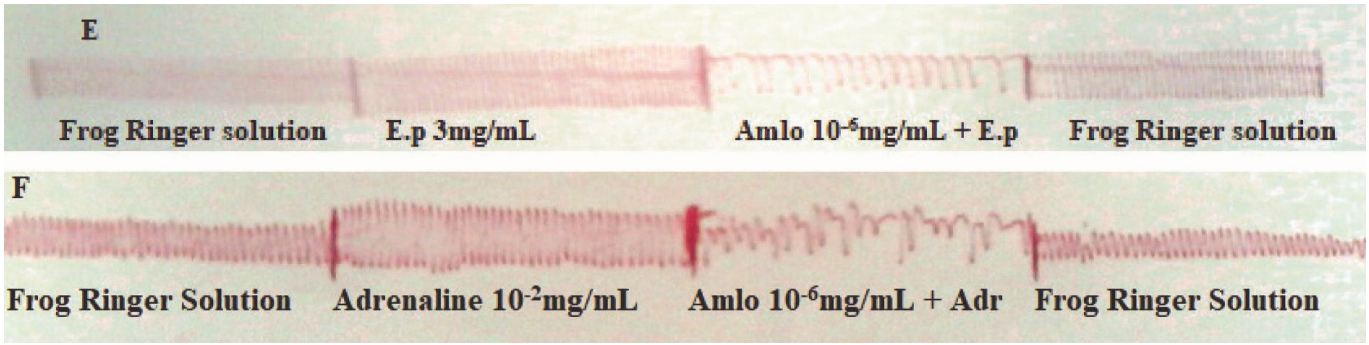


Figure 3 The influence of Amlodipine (Amlo) on (Adr) and the aqueous extract of the leaves of (Ep)-induced the HR (A), FC (B), and CO in isolated frog heart. Each value represents the SEM average, $n = 6$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$: statistically significant with respect to normal. $^{\alpha}p < 0.05$; $^{\beta}p < 0.01$; $^{\rho}p < 0.001$: statistically significant with respect to the positive control (Adrenaline).



Photography 3 Recording showing the influence of Amlodipine on adrenaline (adr) (E) and aqueous extract of the leaves of (Ep) (F) on isolated frogs' hearts contractility obtained from a kymograph.

The study was conducted by comparing the effect of the aqueous extract of the leaves to that of digoxin, a sympathetic agonist and its antagonist. The extract used induced a positive inotropic effect which was similar to that of digoxin, and maximal contractility was noticed at the concentration of 3 mg/ml. This extract can, therefore, be an alternative to sole drugs used in cardiac failure. This cardiotonic effect was attributed to the chemicals content of the tested plant since the preliminary phytochemical screening of the aqueous extract of the leaves of this plant revealed the presence of chemicals belonging to various families such as glycosides, flavonoids, polyphenols, and alkaloids. These compounds among others are reputed for their cardiotonic potential.[16–18] The aqueous extract of *E. poissonii* also produced positive chronotropic and inotropic effects similar to that of adrenaline. These actions were blocked in the presence of propranolol, a wellknown β-antagonist,[19] suggesting the presence of β1-adrenergic agonist compound(s) in the tested extract. Cardiac contractility induced by the extract of the leaves of *E. poissonii* was also inhibited by amlodipine, a slow-acting membrane calcium channels competitor.[20,21] The corollary of the sympathetic stimulation is the influx of calcium ions from the extracellular source into the myocardial cell.[21,22] Accordingly, the positive inotropic and chronotropic actions of the extract of the leaves of *E. poissonii* are likely to be associated with the β-adrenergic pathway, directly through β1-adrenergic receptors or calcium channel opening.

CONCLUSION

The results of the present study suggested that the aqueous extracts of the leaves of *E. poissonii* caused positive inotropic and chronotropic effects on the isolated preparation of the frog heart. The probable mechanism of action involved in the β1-adrenergic pathway. Further studies are required to identify the active components.

DISCLAIMER

The authors declare no conflict of interest.

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