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Cardiotonic potential of aqueous extracts from the leaves and roots of *Balanites aegyptiaca* on the cardiac mechanics of crapauds (*Bufo bufo*)

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ABSTRACT

The aim of the present study was to evaluate the cardiotonic activity and to determine the probable mechanisms of action of aqueous extracts of the leaves (EAFBAE) and roots (EARBAE) of *Balanites aegyptiaca* on isolated toad heart. The cardiotonic activity of the extracts and their mechanisms of action were determined using the Langerdhorff device. In this study, digoxin (0.5 mg/mL) was used as the standard, while adrenaline (10-2 mg/mL) acetylcholine (10-6 mg/mL) and propranolol (10-6 mg/mL), amlodipine (10-6 mg/mL) and atropine (0.1 mg/kg) were used to determine the probable mechanisms of action of the extracts. The study of the cardiotonic activity showed that EAFBAE (3mg/mL) and EARBAE (2mg/mL) significantly (< 0.05) induced positive inotropic and chronotropic effects on the isolated toad heart as compared to digoxin. In the presence of propranolol (β-adrenergic antagonist) and amlodipine (calcium channel blocker), EAFBAE (3mg/mL) and EARBAE (2mg/mL) significantly decreased the cardiac contractility (heart rate, force of contraction and cardiac output), as compared to adrenaline. The results of this study suggest that the extracts tested have beneficial and interesting effects in stimulating cardiac activity via the adrenergic pathway and calcium channels.

Keywords: *Balanites eagyptiaca*, Isolated Heart, Toads, Chronotropic, Inotropic.

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of death in the world, accounting for almost 19 million deaths per year, or around one-third of all deaths worldwide (WHO, 2017). In Africa, 15% of medical conditions are attributable to CVD, and 10-20% of deaths are attributable to them. Among these diseases, the most common are heart failure, coronary heart disease or ischaemic heart disease and cerebrovascular disease (WHO, 2017).

In chad, heart failure accounts for almost 30.84% of cardiovascular diseases hospitalised in the cardiology department

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(Betrand, 1993). his cardiovascular disease is characterised by a reduction in the heart ability to pump enough blood to meet the body's needs. It can be caused by a variety of factors, such as coronary artery disease, high blood pressure, valvular disease or cardiomyopathy. There are several classes of drugs used to treat Cardiac insufficiency including cardiac glycosides (digoxin), adrenergic and dopaminergic receptor agonists (isoproterenol and dobutamine), calcium mobilisers (levosimendan), phosphodiesterase inhibitors and synthetic catecholamines. Although effective, these drugs sometimes have harmful side effects and contra-indications for a certain category of people, which limits their use (Zakaria et al., 2023). It is therefore necessary to explore alternatives, including medicinal plants, which can offer patients a wide range of affordable options (Rouhama, 2018).

A number of plants are known for their cardiotonic effects, due to the active molecles they contain, such as glycosides, alkaloids and phenolic compounds. The aqueous extract of the leaves and seeds of *Datura metel* (Pousset ,2006) and the aqueous extract of *Berberis lyceum* Royle (Berberidaceae) (David et al., 2020). *Balanites aegytiaca* (*BAE),* which is the subject of the present study, is a traditionally plant used in chad to treat various illnesses including chest pain, diabetes and arterial hypertension. This empirical use suggests that this medicinal plant has cardiotonic potential. Hence, suggesting that it would contain certain pharmacologically active compounds, notably, alkaloids, coumarins, phenolic compounds, flavonoids, triterpenes, tannins and saponins (Betrand, 1993; Zakaria et al., 2023).

For this reason, this study was undertaken to determine the cardiotonic effects and the probable mechanisms of actions of aqueous extracts of the leaves and roots of *Balanites aegyptiaca* on cardiac mechanics in toads.

MATERIALS AND METHODS

Reagents and equipment

NaCl (9g/l), KCl (0.42 g/l), $CaCl_2$ (0.24 g/l), dextrose (1.0 g/l) and $NaHCO_3$ (0.5 g/l).

Digoxin, adrenaline, amlodipine, acetylcholine and atropine. These drugs were purchased from the pharmacy store in the town of Maroua. Kymograph and Starling cardiac lever (Orchid Scientific, model SRD-01) used to record cardiac responses.

Cardiac function recording equipment

The various parameters of cardiac contractility were measured using a kymograph from Orchid scientific, model SRD-01, series SRD-01/17-18 /39 made in India (Figure 17). The kymograph is a mechanical device for recording the phases of organ movements (e.g. cardiac tissue). It establishes a graphical representation of spatial position in time, in which one axis represents spatial time (Fenn et al., 2017; Suhaeri and Vitri, 2014).

Plant material

BAE leaves and roots were harvested at the southern entrance to the city of N'Djamena in April 2022. They were dried in the shade at room temperature for 14 days. The taxonomic identification of the plant was carried out by Dr GAIWA Daakreo of the Faculty of Science at the University of Doba and authenticated using the West African dry zone tree, shrub and liana identification manual (Arbonnier, 2009; Ahmad et al., 2012).

Animal material

Common toads *Bufo bufo* of both sexes and weighing between 40 and 65 were used to assess the cardiotonic potential of aqueous extracts of *BAE* leaves and roots. These toads were captured in the water retention pond of the national road equipment company of Cameroon (SNER-CAM) and transported to the artificial pond in the laboratory of the Maroua Protestant College, where they were acclimatised for three weeks before any manipulation. Their diet consisted of insects such as termites and moths. Animal procedures were conducted in strict accordance with the national institutes of Health (NIH) Guide for the care and Use of Laboratory Animals (NIH Publication N° 85-23 Rev.1985).

Preparation of the extract

Aqueous extracts of *BAE* leaves and roots were prepared using the infusion method. A mass of 50 g of leaf and root powder was

dissolved in 450 ml of distilled water heated to 70°C for 1 hour. After cooling, the mixture was filtered through Whatman GF/C n°4 paper (90mm). The filtrate obtained was evaporated in a ventilated oven at 45°C until a powder was obtained.

This powder, representing the aqueous extract of BAE leaves or root, was used for further work. The extraction yield was 10.75% for the aqueous leaf extract and 11.50% for the aqueous root extract.

Phytochemical screening of Aqueous extracts of BAE

Aqueous extracts of leaves and roots were subjected to various qualitative tests based on color reactions and/or differential precipitations: ferric chloride, Shinoda, Mayer, Liebermann-Burchard, Salkowski and sodium hydroxide tests (Bruneton, 2009) .

Isolation and preparation of toad hearts

Isolation of the toad hearts followed the procedure described by Neerati and al in 2014. The toads were decerebrated and demedullated in turn, then placed supine on a cork board. An incision was made along the midline of the animals' bellies, then the sternum and thoracic musculature were split. A triangular cut was made in the thorax to expose the entire heart. The pericardium was then carefully removed from the isolated heart. The aorta was freed and a thread passed underneath, which was then used to attach it to the cannula. A small incision was made in the aorta to insert the cannula filled with physiological solution, which was gently slid towards the heart. The wire was ligated around the cannula then the heart of the animal was extracted and fixed with the wire from the cannula to a hook, located on a rack in order to hold the heart securely and the surrounding tissues cut **(**Neerati et al., 2012)

Study of cardiotonic activity

The cardiotonic potential of *BAE* leaf and root extracts was assessed by comparison with that of digoxin, considered as the reference substance. The parameters recorded were heart rate, contraction force and cardiac output. To do this, the isolated heart was washed for approximately 3 min with normal Ringer's solution after each administration of the reference drug or the extracts tested, until rhythmic cardiac contractions were obtained before it was perfused with the pharmacological substances at different concentrations. Digoxin was tested at a concentration of 0.5 mg/ml. Aqueous leaf extract was prepared at concentrations of 1.5 mg/ml, 3 mg/ml and 6 mg/ml, while aqueous root extract was prepared at concentrations of 1 mg/mL, 2 mg/mL and 4 mg/mL. The contraction force, expressed in millimeters (mm), was determined using a caliper. Heart rate, expressed in beats/minute, was measured by counting the number of peaks obtained in one minute. Cardiac output was obtained as the product of contraction force and heart rate and is expressed in milliliters per minute. A new heart was prepared whenever the previous one no longer showed normal rhythmic contractions (Etou et al., 2005).

Determination of the probable mechanisms of action of the plant extracts

Assessment of the effect of aqueous extracts of the leaves and roots on calcium channels and β-adrenergic receptors.

To determine the effects of each aqueous extract used, 06 isolated toad hearts were used. First, the isolated heart was washed for approximately 5 min with normal Ringer's solution until rhythmic cardiac contractions were obtained before being perfused with adrenaline at a concentration of 10^{-2} mg /mL. After washing, each heart was pre-treated with amlodipine (10^{-6}mg/mL) for 5min before infusion with adrenaline (10^{-2} mg/mL) and the aqueous extract of the leaves at concentrations of 1.5, 3 and 6 mg/mL or the aqueous extract of the roots at concentrations of 1, 2 and 4 mg/mL respectively. In another series of studies, amlodipine was replaced by propranolol (10-6mg/mL), a β-adrenergic receptor blocker (Tang et al., 2016). In both sets of studies, heart rate, force of contraction and cardiac output were determined as previously described.

Evaluation of the effect of aqueous extracts of the leaves and roots on muscarinic receptors

In a first series of experiments, hearts were injected with atropine (0.1 mg/kg), a muscarinic receptor antagonist and then continuously perfused with aqueous extracts of the leaves and roots of BAE at concentrations of 1.5 and 2.5 mg/kg respectively. Approximately five minutes after injection of atropine and extracts respectively, the hearts were subjected to a continuous infusion of acetylcholine at 10-6mg/mL.

In a second series of experiments, the administration of acetylcholine preceded the administration of atropine and the different concentrations of extracts, after which the different parameters were once again recorded (Almança et al., 2016).

Statistical analysis

The data obtained were analysed using graph pad prism software, version 5.00. All results were expressed as Means ± SEM (Standard Error of the Mean). The One Way Analysis of Variance (ANOVA) test was used, followed by Tukey's post-test, to compare batch means with each other. Values of $p < 0.05$ were considered significant.

RESULTS

Screening phytochemical quality of the EARBA and AEFBA

Qualitative phytochemical analysis of aqueous extracts of BAE leaves and roots revealed the presence of several secondary metabolites belonging to different chemical compound families, the most abundant of which include phenolics, flavonoids, tannins and saponosides. However, certain chemical groups such as glucosides, anthraquinones and anthocyanins were also absent.

The results obtained are shown in the Table 1 below

Determination of the cardiotonic potential of aqueous extracts of the leaves and roots of *Balanites aegyptiaca*

Perfusion of isolated toad hearts with aqueous extracts of the leaves and roots BAE resulted in a significant ($p<0.05$) increase in contraction force, heart rate and cardiac output compared with normal ringer hearts. These increase was up to 69.42% and 70.06% respectively for contraction force; 69.98% and 70.42% for heart rate; and 76.94% and 77.33% for cardiac output. Perfusion of the aqueous extracts of the leaves ans roots of BAE was also significantly ($p<0.05$) more efficient on the cardiac contractility parameters assessed in the present study, when compare to digoxin (0.5 mg/mL). The inotropic and chronotropic effects observed were more pronounced at concentrations of 2 mg/mL for EARBE and 3mg/mL for EAFBE. In addition, the aqueous extracts of the leaves and roots BAE induced reversible cardiac arrest at concentrations of 4 and 6 mg/mL respectively (Figures 1 and 2).

The photographs (A, B, C) in figure 3 show the recordings of heart mechanics as a function of different treatments. The figure shows that digoxin caused a progressive increase in contraction amplitude at concentrations of 0.125, 0.25 and 0.5 mg/mL. At 1.25 mg/mL, digoxin induced reversible cardiac arrest. On the other hand, when the hearts were treated with aqueous extracts of BEA roots and leaves, an increase in contraction amplitude was observed at concentrations of 1.5 mg/mL (leaves) and 1 mg/mL (roots) compared with the normal control. This increase in amplitude became even greater at concentrations of 3 mg/mL and 2 mg/mL of leaf and root extracts respectively. However, at higher concentrations (10 and 15 mg/mL), the root and leaf extracts tested led to a decrease in contraction amplitude and even reversible cardiac arrest.

Determination of possible mechanisms of action

Comparison with a calcium channel blocker

Perfusion of isolated hearts with adrenaline (10^{-2} mg/ml) or aqueous extracts the leaves (3mg/mL) and roots (2mg/mL) of BAE resulted in a significant increase (17.57%, 17.48%, and 16.89% respectively) in heart rate compared with the normal control group.

In contrast, there was a significant decrease ($P < 0.001$), about 45.68%, 45.05% and 45.09% in heart rate when the hearts were treated with the following combinations:

amlodipine (10^{-6} mg/mL)-adrenaline (10^{-2} mg/mL) and amlodipine (10^{-6} mg/mL)-aqueous extract of the roots (2 mg/mL) or leaves of BAE (3 mg//mL) combinations, respectively (Figures 4 and 5).

Adrenaline, the aqueous extract of the leaves and the aqueous extract of the roots of BAE induced a significant increase (44.10% and 33.17%, and 33.16% respectively) in contraction force when compared to control hearts.

When the aqueous extracts of the leaves and roots of BAE or adrenaline were combined with amlodipine, the results showed a significant decrease ($p<0.001$) in contraction force of around 71.2%, 71.65% and 71.65% respectively, as compared with the normal control group (Figure 5).

Under the same experimental conditions, a significant decrease ($P < 0.001$) of 92.94%, 92.37% and 92.37% in cardiac output was recorded in hearts treated with the same combinations, respectively, as compared to adrenaline treated hearts (10^{-2} mg/mL).

Perfusion with adrenaline (10^{-2} mg/mL) or aqueous extracts of the leaves (3mg/mL) or roots (2 mg/mL) induced an increase in the amplitude of contraction of isolated hearts around 70% and 68% respectively. However, in the presence of amlodipine, the above-mentioned substances caused a slight reduction of 60% and 57% respectively in the amplitude of cardiac contraction.

Comparison with a *β-adrenergic* receptor blocker

Hearts perfused with adrenaline and those perfused with the 2mg/mL and 3mg/mL concentration of aqueous extract of BAE roots and leaves, induced a significant ($p<0.05$) increase in heart rate of 16.85% and 22.97% respectively, as compared to hearts perfused with normal ringer. However, when compared with hearts perfused with adrenaline alone, hearts perfused with aqueous extracts of the leaves and roots of BAE or with adrenaline preceded by propranolol resulted in a significant ($p<0.001$) decrease in heart rate. This decrease accounted for about of 44.71% and 45.43% when the hearts were perfused with the extracts and 40.07% when they were perfused with adrenaline (Figures 7 and 8).

Concerning the force of contraction, perfusion of isolated toad hearts with aqueous extracts of the leaves and roots of BAE or adrenalin respectively induced a significant increase ($p<0.001$) in contraction force compared to hearts perfused with normal Ringer. This increase was up to 55. 12% and 57.14% for the roots and the leaves extracts, respectively.

However, compared with hearts perfused with adrenaline alone, hearts perfused with aqueous extracts of leaves and roots or with adrenaline preceded by propranolol resulted in a significant ($p<0.001$) decrease in contraction force of 67.55% and 70.44% for the aqueous extract of roots and leaves respectively and 69.48% when perfused with adrenaline.

Perfusion of hearts with aqueous extracts of the roots and leaves or adrenaline caused a significant increase ($p<0.001$) in cardiac output, when compared with hearts perfused with normal ringer (normal control), i.e. an increase of 63.78% and 64.79% for aqueous extract of the roots and leaf extracts and 61.82% for adrenaline. In the presence of propranolol, there was a significant decrease ($p<0.001$) in the force of contraction of hearts perfused with both aqueous extracts and adrenaline, compared with those perfused with adrenaline alone: 88.14% and 89.15% for BAE aqueous leaves extracts respectively and 78.57% for adrenaline.

The photographs in Figure 6 show recordings of heart activity after different treatments (amlodipine-adrenaline (A) and amlodipine-aqueous extract of BEA leaves (B) and roots (C)). These photographs show that perfusion with adrenaline (10-2 mg/mL) or aqueous leaf extract (3 mg/mL) or root extract (2 mg/mL) induced an increase in the amplitude of contraction of isolated hearts. However, in the presence of amlodipine, the above substances induced a slight decrease in the amplitude of cardiac contraction.

Comparison with atropine (muscarinic receptor blocker)

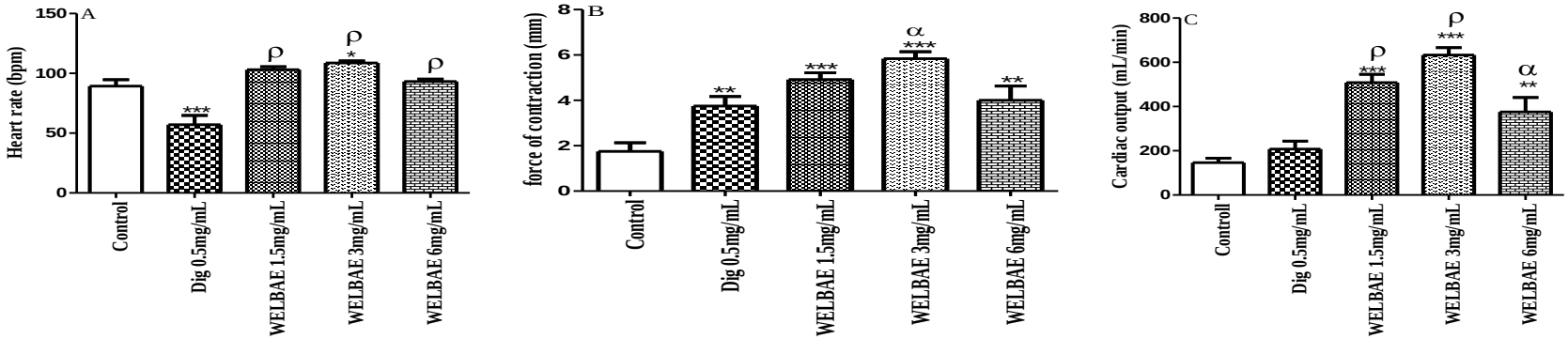
Figures (10 and 11) show the influence of atropine (0.1mg/kg) on heart rate and contraction force in hearts treated with 10-6M acetylcholine and aqueous leaf and root extracts. Thus, compared with control hearts perfused with ringer, acetylcholine-perfused hearts showed a significant ($p<0.01$) decrease in heart rate of the order of 56.25%. Under the same experimental conditions, perfusion of hearts with aqueous leaf extract (2 mg/ml) or root extract (3mg/ml) in the presence or absence of atropine (0.1 mg/ml), resulted in a significant increase in heart rate of 22.71% and 22.70% respectively compared to the normal control group. Hearts perfused with atropine alone or with the atropine-acetylcholine combination resulted in a significant ($p<0.001$) increase in contraction force. Compared with hearts perfused with acetylcholine (10-6M), the increase in contraction force was 45.60% for hearts treated with atropine alone, and 40.35% for those treated concomitantly with atropine and acetylcholine. The study also showed that aqueous extract alone or in combination with acetylcholine induced an increase in contraction force. This increase was respectively 46.70% and 46.67% for the aqueous leaf extract (2 mg/ml) and 52.50% and 52.47% for the aqueous root extract (3 mg/ml) (Fig. 10-11 A). Under the same experimental conditions (Fig. 10-11 B), hearts perfused with aqueous leaf or root extract in the presence or absence of atropine showed a significant ($p<0.001$) increase in contraction force. This increase was respectively of the order of 30.78% and 30.75 for extracts alone and 47.76% and 47.70 for extracts combined with atropine. Finally, when hearts were perfused with acetylcholine alone, the results showed a significant ($p<0.001$) decrease in cardiac output of 50.16% compared with hearts perfused with normal ringer. However, aqueous extracts of leaves and roots alone or combined with atropine (Figures 10-11C) resulted in an increase in cardiac output compared with hearts perfused with normal ringer. This increase was 54.06% and 54.07% respectively for leaf and root extracts alone, and 62.54% and 62.55% for said extracts combined with atropine.

The photographs in Figure 12 show records of the activity of hearts treated respectively with different combinations of atropine-acetylcholine (A, B); aqueous extracts of the leaves of *BAE*-acetylcholine (C, D) and aqueous extracts of the roots of *BAE*-acetylcholine (E, F). These photographs show that the amplitude of contraction highly varied when the hearts were treated respectively with atropine alone or when it preceded the administration of acetylcholine at 10^{-6}M concentration. Moreover, perfusion of the hearts with the different extracts preceded or not by acetylcholine did not modify the activity of the different extracts, characterized by an increase in the amplitude of cardiac contraction.

Table 1 : Results of the qualitative phytochemical test.

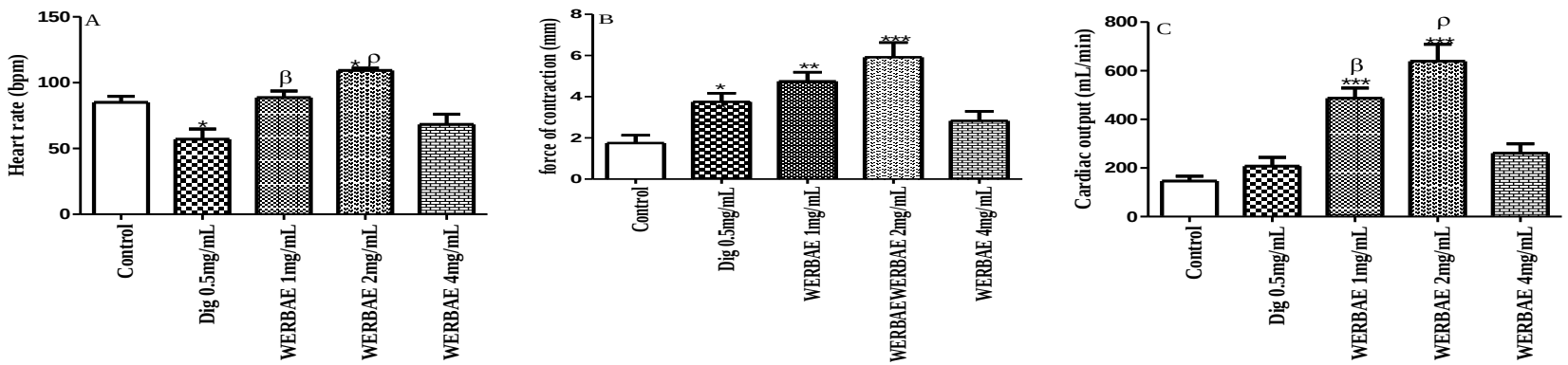
Composé classes	EARBA	AEFBA
Alkaloids	+	+
phenolic compounds	+++	+++
Flavonoids	+++	+++
Triterpenes	+	+
Steroids	-	-
Tannins	+++	+++
Glucosides	-	-
Anthraquinones	-	-
Coumarines	-	+
Anthocyanes	-	-
Saponosides	+++	-

Legend ; - : Ausente, + : present, ++ : moderate presence, + ++ : very abundant.



ESM, n=6. ***P<0.001;**P<0.01, *P<0.05: significant difference from normal control. αp<0.05; βp< 0.01; ρp< 0.001: significant difference from digoxin.

Figure 1: Activity of the aqueous extract of BAE leaves on parameters of cardiac contractility in isolated toad hearts. Each value represents the mean.



ESM, n=6. ***P<0.001; **P<0.01, *P<0.05: significant difference from normal control.
αp< 0.05; βp< 0.01; ρp< 0.001: significant difference from digoxin .

Figure 2: Activity of the aqueous extract of BAE roots on parameters of cardiac contractility in isolated toad hearts. Each value represents the mean.

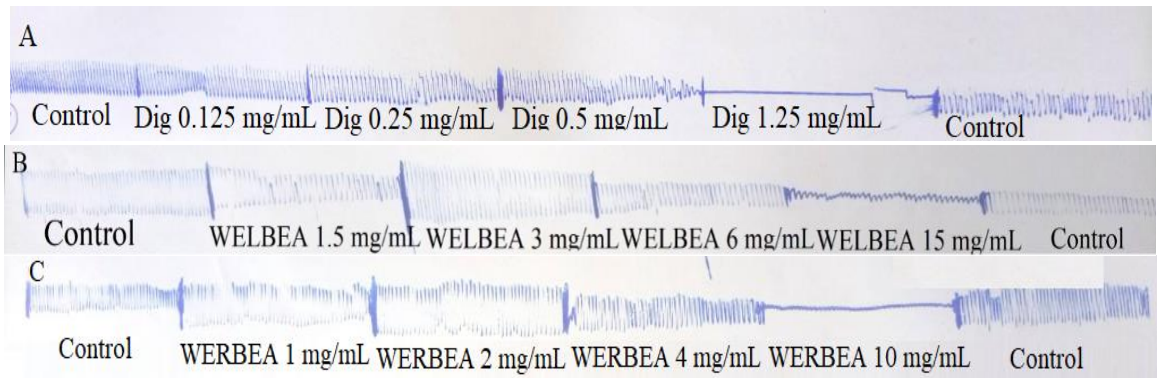
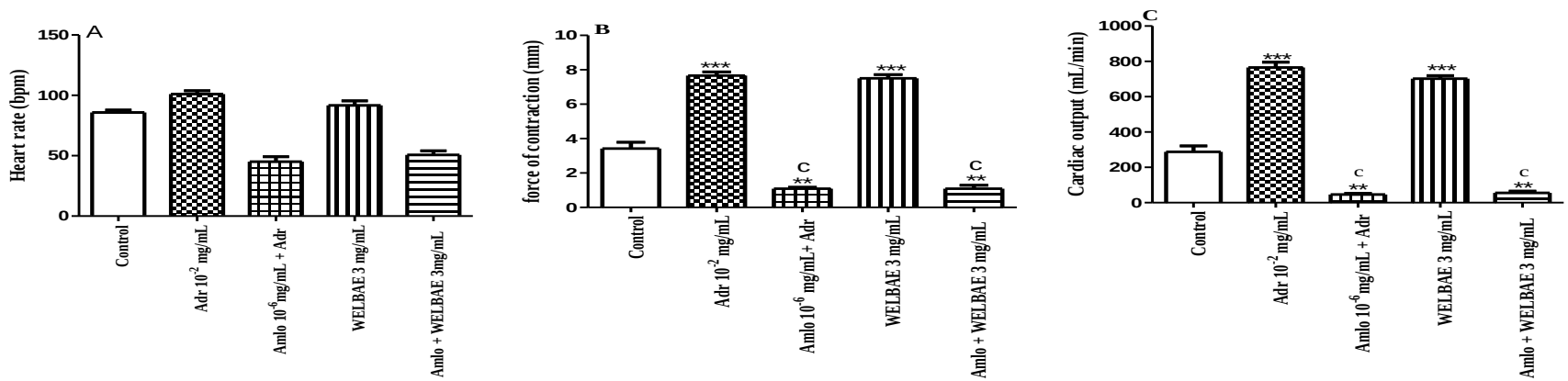
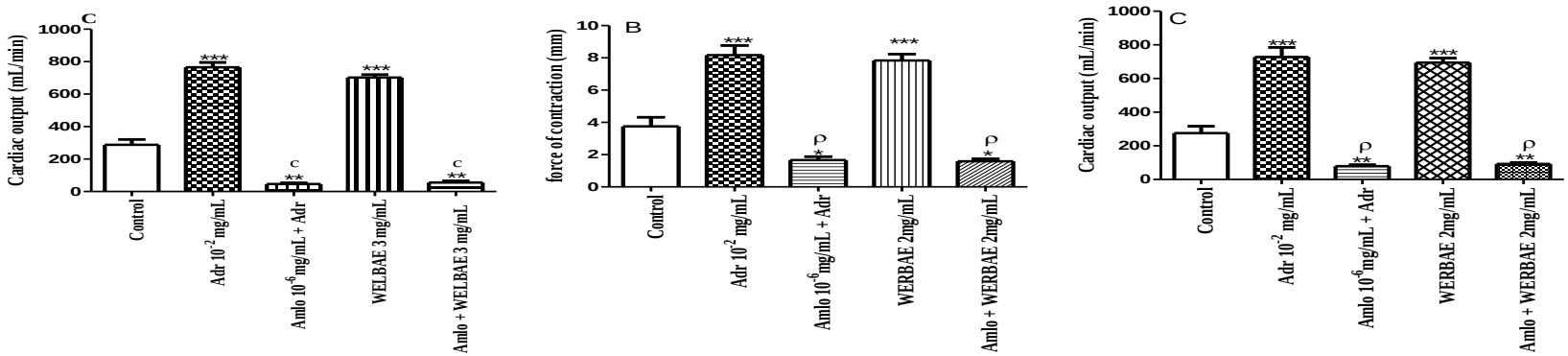


Figure 3: Kymograms of hearts treated with different concentrations of digoxin (A) and aqueous extracts of Balanites aegyptiaca leaves (B) and roots (C).



ESM, n=6. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$: statistically significant compared with the normal control. cp < 0.001: statistically significant compared with the positive control group (Adrenalin 102 mg/mL).

Figure 4: Study of the interaction between amlodipine and the aqueous extract of BAE leaves on cardiac contraction force (A), heart rate (B) and cardiac output (C). Each value represents the mean



ESM, n=6. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$: statistically significant compared with the normal control. cp < 0.001: statistically significant compared with the positive control group (Adrenalin 10^{-2} mg/mL).

Figure 5: Study of the interaction between amlodipine and the aqueous extract of BAE roots on cardiac contraction force (A), heart rate (B) and cardiac output (C). Each value represents the mean

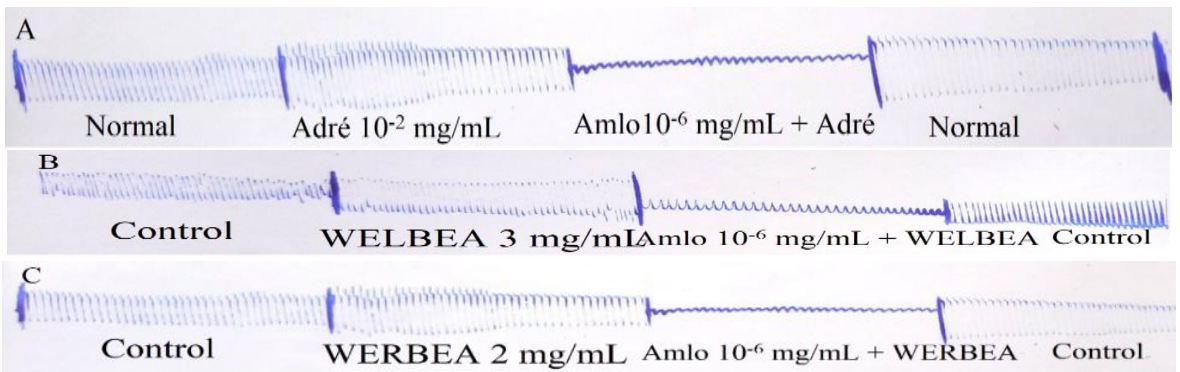
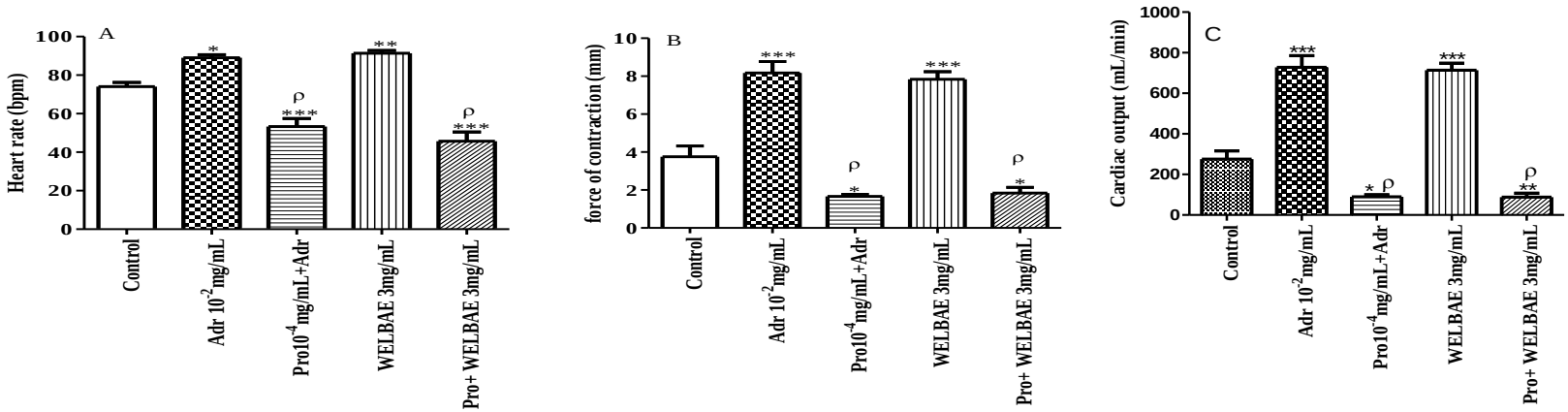
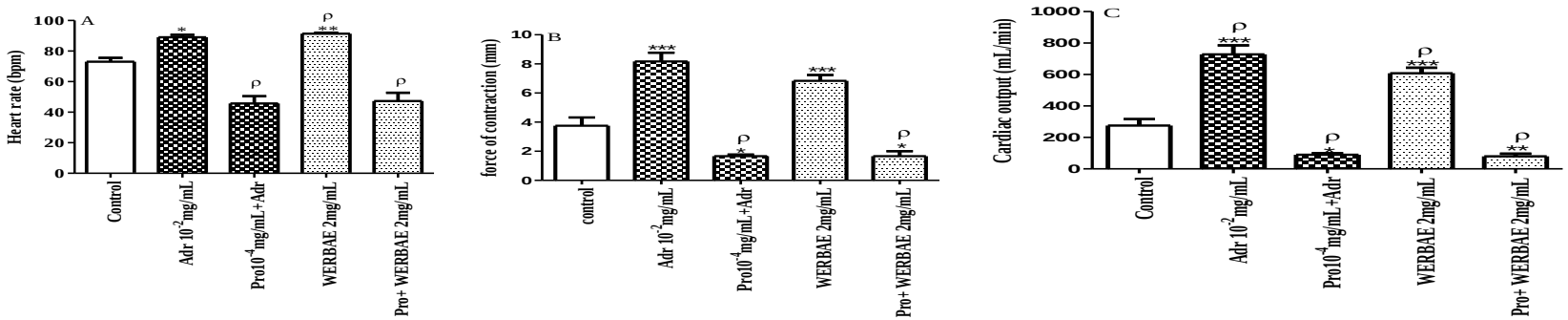


Figure 6: Kymograms of hearts subjected to adrenaline alone and in the presence of amlodipine (A) and to aqueous extracts of BEA leaves (B) and roots (C) alone and in the presence of amlodipine



Each value represents the mean of the ESM, n=6. ***P<0.001; **P<0.01; *P<0.05: significant difference from control (CN); αp< 0.05; βp< 0.01; ρp< 0.001: significant difference from adrenaline.

Figure 7: Interaction between propranolol (pro) - adrenaline (Adr) / aqueous extract of Banalites aegyptiaca (Es) leaves on cardiac contractility parameters.



Each value represents the mean of the ESM, n=6. ***P<0.001; **P<0.01; *P<0.05: significant difference from control (CN); αp< 0.05; βp< 0.01; ρp< 0.001: significant difference from adrenaline

Figure 8: Interaction between propranolol (pro) - adrenaline (Adr) / aqueous extract of Banalites eagyptiaca (Es) roots on cardiac contractility parameters.

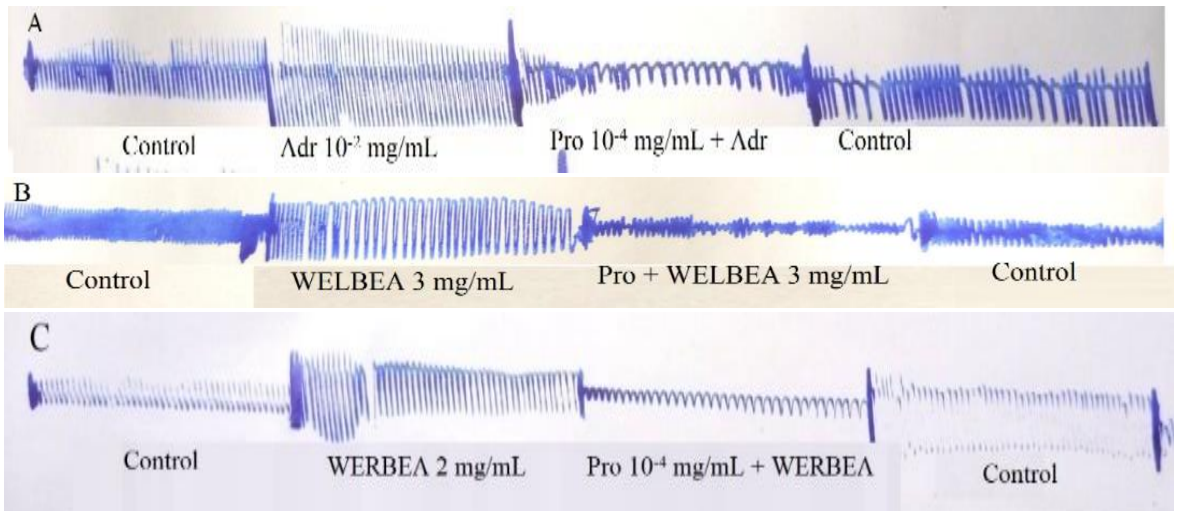
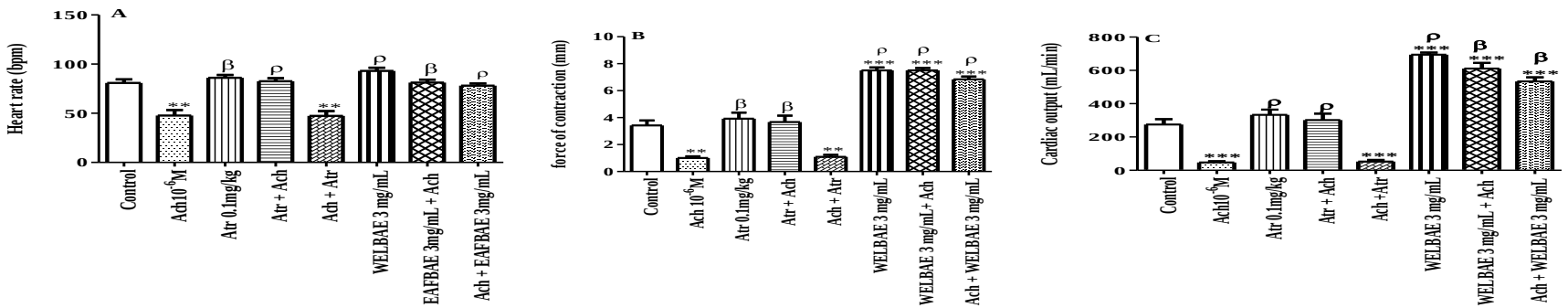


Figure 9: Recording showing the interaction of propranolol (prop) - adrenaline (Adr) or leaf and root extracts on cardiac contractility in toad isolates.



Each value represents the mean ESM, n=6. ***P<0.001; **P<0.01; *P<0.05: significant difference from control (CN); αp< 0.05; βp< 0.01; ρp< 0.001: significant difference from positive control group (Acetylcholine 10-6 M).

Figure 10: Study of atropine-acetylcholine and aqueous extract of BAE-acetylcholine leaves interactions on cardiac contractility parameters

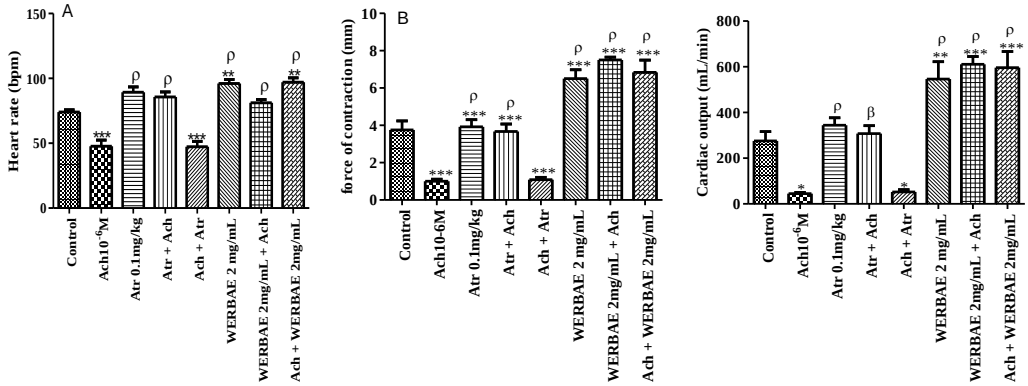
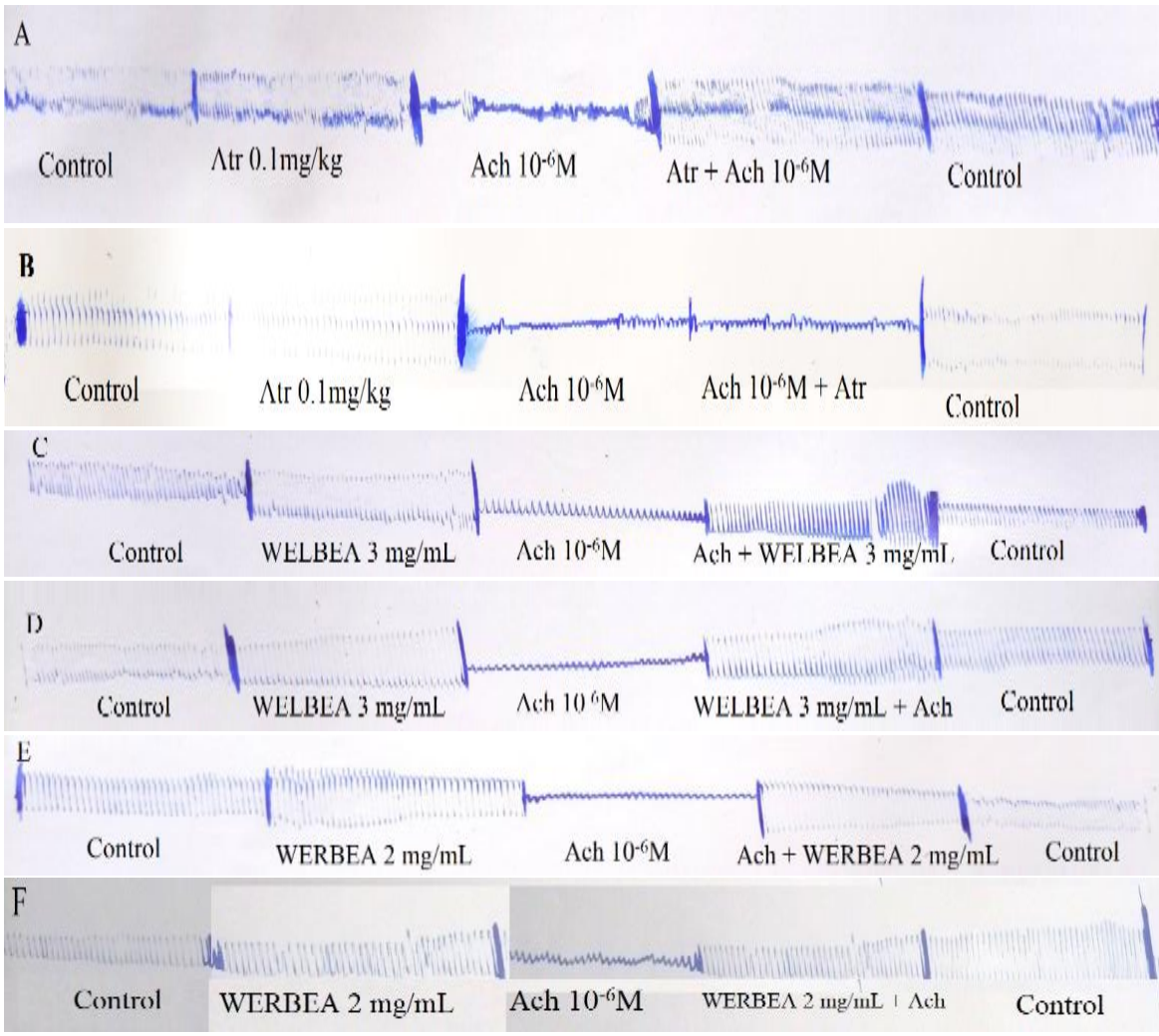


Figure 11: Study of atropine-acetylcholine and aqueous extract of BAE-acetylcholine roots interactions on cardiac contractility parameters

Figure 12: Kymograms of hearts subjected to acetylcholine alone and in the presence of atropine (A, B) and to the aqueous extract of BAE leaves (C, D) and roots (E, F) alone and in the presence of acetylcholine.



DISCUSSION

Preliminary phytochemical screening of the aqueous extract of BAE leaves and roots revealed the presence of chemical compounds belonging to various families such as saponosides, flavonoids, phenolic compounds, triterpenes and alkaloids. This study corroborates that carried out by Hosakatte Niranjana Murthy et al on BAE aqueous, who found the same secondary metabolites. The abundance of certain bioactive compounds in the aqueous extract of leaves and roots can be explained by the fact that the quantity of compounds depends essentially on the variety, growing season, harvesting season, climatic conditions, geographical location (Rama, 2014), the various diseases that can affect the plant, maturity (Melero et al., 2000) and shelf life (Nesher et al., 2007**)**. The compounds found in the aqueous extracts of BAE leaves and roots used in this study are bioactive substances whose therapeutic potential predicts cardiotonic activity. This cardiotonic effect was attributed to the chemical content of the plants tested, since preliminary phytochemical screening of the various extracts revealed the presence of chemical compounds belonging to various families such as glycosides, flavonoids, polyphenols and alkaloids known for their cardiotonic potential (Quenum et al., 2014 ; Lingrel, 2010; Alché, 2003 ; Islam et al., 2005).

Heart failure is a chronic disease complicated by acute episodes leading to hospitalization. It corresponds to the heart's inability to ensure sufficient blood flow, initially during exercise and subsequently under all conditions of daily life. In the present study, the cardiotonic effects of aqueous extracts of leaves and roots of BAE were evaluated on isolated toad hearts. These two preparations therefore produce inotropic (more pronounced) and chronotropic positive effects. These effects could be due mainly to the presence of steroids in this plant (Shin et al., 2005).

When compared with digoxin-perfused hearts, aqueous extracts of the leaves and roots significantly increased contraction force, heart rate and cardiac output. Such a result was previously reported by those by Tsala et al. (2020) who demonstrated that the aqueous extracts of the leaves and seed of *Datura metel* were endowed with the same cardiotonic properties (David et al., 2020). Digoxin is a tonicardiac drug that has been used for many years in the treatment of heart failure (Melero et al., 2000). In the case of cardiac insufficiency, this reference molecule promotes the increase in cardiac output through its inotrope effect and extracardiac effects (reduction in sympathetictone), resulting in a reduction in pre- and postload. The inotropic and chronotropic effects induced by the aqueous extracts of the *leaves* and roots of BAE in this work suggested that these extracts contain bioactive compounds known for their cardiotonic potential (Nesher et al., 2007; Quenum et al., 2014). Indeed, it has been reported that glycosides act at cardiac level by blocking Na/K ATPase Pumps, leading to an increase in intracellular sodium concentration, which would cause a slowing down or even reversal of the Na^+/Ca^{2+} exchanger, responsible for an increase in Ca^{2+} concentration in the cytosol (Melero et al., 2000). This increase in intracellular Ca^{2+} concentration would activate the contractile elements responsible for the positive inotropic effect.

To determine the possible mechanism of action by which aqueous extracts of the leaves and roots of BAE produce their cardiotonic effect, these extracts were administered to isolated hearts in the presence of adrenergic and muscarinic receptor antagonists. The results of the study showed that administration of adrenaline and aqueous extracts of the leaves and roots of BAE induced an increase in the contraction force and performance of isolated toad hearts (Adi et al., 2000). In fact, adrenaline induces positive inotropic and chronotropic effects by binding to cardiac adrenergic receptors, resulting in the influx of calcium ions and the opening of slow-type calcium channels (Alché, 2003). Like digoxin and adrenaline, our extracts are likely to exert their cardiotonic effect via a mechanism that interferes with the opening of slow-type calcium channels in myocardial cells. This hypothesis was confirmed in our

study by the inhibition of the effects induced by aqueous extracts of the leaves and roots of BAE in the presence of amlodipine. Amlodipine is a calcium receptor antagonist, which binds to slow-type cardiac calcium channels, blocking them (Islam et al., 2005 ; Shin et al., 2005).

The various extracts tested also produced positive chronotropic and inotropic effects, similar to those of adrenaline. These actions were blocked in the presence of propranolol, a well-known β-antagonist (Atul, et al., 2013 ; Rochette et al., 2015) suggesting the presence of β1-adrenergic agonist compounds in the tested extracts. This cardiotonic effects is attribuable to the chemical content of the plants extract tested, given that the preliminary phytochemical screening of the same extracts revealed the presence of chemical compounds belonging to various families such as glycosides, flavonoids, polyphenols and alkaloids renowned for their cardiotonic potential (Melero et al., 2000 ; Betrand, 1993). Moreover, glycosides are known to act by blocking Na^+/K^+- ATPase pumps, leading to an increase in intracellular sodium concentration, which in turn slows down or even reverses the Na^+ /Ca^{2+} exchanger, responsible for an increase in Ca^{2+} concentration in the cytosol (Nesher et al., 2007). Consequently, the positive inotropic and chronotropic actions of the various extracts are likely to be associated with the β-adrenergic pathway, directly through β1-adrenergic receptors or the opening of calcium channels (Witt et al., 2004).

Under physiological conditions, the heart receives numerous signals from the sympathetic nervous system, resulting in a decrease in heart rate (Maciel et al., 1986). This phenomenon is pharmacologically very important, as antagonists such as atropine can increase heart rate (Thomas et al., 1966). In view of the above, the interaction of *D. metel* extracts with muscarinic receptors was also evaluated on the isolated batrachian heart. After the sensitivity of the receptors had been tested by using atropine to block the effect of acetylcholine, the plant extracts used also proved to be antimuscarinic. Atropine is an alkaloid extracted from the leaves of Atropa belladona (Belladonna), a member of the Solanaceae family, like the plant tested. This substance opposes the effects of acetylcholine at muscarinic receptors. It binds to these receptors and prevents acetylcholine from accessing them (Rama et al., 2014). Thus, the aqueous extracts of BAE leaves and roots, because of their positive inotropic and chronotropic effects (including increased cardiac output), are thought to contain antimuscarinic substances.

Conclusion

in conclusion, the aqueous extracts of the leaves end roots of *BAE* contain bioactive substances that can increase contraction force, frequency and cardiac output. These compounds therefore have positive inotropic and chronotropic effects on isolated toad heart and are likely to act through the adrenaline pathway and could be of a good interest in the management of cardiac pathologies.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

DF carried out the study; AC, DF and YMA participated in data collection and laboratory experiments; DF analyzed the data; DF, NBH and YMA drafted the manuscript; MH, AC and AS read and corrected the manuscript; TDE, MM and HB supervised the work; all authors read and approved the final manuscript.

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