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ORIGINAL RESEARCH

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Mechanism and inotropic actions of the water extracts of the leaves and seeds of *Datura metel* (Solanaceae) on isolated frog's heart

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ABSTRACT

Datura metel leaves are used by athletes to enhance their performances. The water extract of the leaves and seeds was tested for their cardiotonic effect. Langherdoff's model for the measurement of the mechanical activity of the isolated amphibian heart in ventricular perfusion was used to evaluate some parameters of cardiac contractility. The effect of the extracts was also compared to sympathetic agonist and antagonist. The leaf and seed extracts induced a significant ($p < 0.05$) positive inotropic effect (68.65% and 70.42%) when used at 1.5 and 2.5 mg/mL, respectively. No significant change in the heart rate was observed**.** In addition, reversible heart arrest for the leaf and seed extracts was noticed at the dose of 6 and 5 mg/ml, respectively. The stimulatory activity of the tested extracts was inhibited by acetylcholine and amlodipine, suggesting that the extracts have cardiotonic properties, likely through muscarinic receptors and calcium channels.

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KEYWORDS

Datura metel; inotropism; anticholinergic; isolated heart

Introduction

According to the World Health Organization, cardiovascular pathologies cause about 31% of global mortality per year [1]. In addition, heart diseases, including heart failure, are the leading causes of disability and premature death [2,3]. Heart failure by definition is the inability of the heart muscle to pump enough blood or to provide the blood flow needed for the body's oxygen or nutriment requirements [4]. Several drugs such as digoxin have therapeutic effects on heart failure, but their side effects and their inaccessibility limit their use [5,6,7]. It is, therefore, essential to search for novel drugs that are more accessible and well-tolerated by the body. In Africa, *Datura metel* is traditionally used as a pesticide; it has ritual uses in many countries, e.g., in initiation rites in Nigeria and Mozambique. Most commonly, the leaves are smoked, or they are boiled and eaten; seeds are similarly used. Roots, seeds, or leaves are added to alcoholic drinks to increase the intoxication effect [8,9]. Medicinal use also includes the treatment of asthma, convulsions, pain, and rheumatism [8, 9]. The previous studies have shown that the ethanolic extract of this plant has hypolipidemic properties, which may be beneficial in the treatment of cardiovascular diseases [10]. *Datura metel* is rich in phenolic compounds, atropine, scopolamine, cardiac glycosides, triterpenes, and flavonoids 11,12] which are all bioactive compounds known for their effect on the cardiovascular system [8]. In addition, an ethnobotanical survey conducted in the northern regions of Cameroon revealed that this plant is used by athletes to improve their physical performance. If these properties are scientifically confirmed, the plant has a potential to treat heart failure and

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related conditions. Hence, this study was designed to focus on the cardiotonic effects of the leaves and seeds of *D. metel* along with possible mechanisms such as the involvement of the cholinergic system and calcium channels.

Materials and Methods

Reagents and equipment

- ***Ringer for amphibians***: NaCl (9 g/l), KCl (0.42 g/l), $CaCl_2$ (0.24 g/l), dextrose (1.0 g/l), and $NaHCO_3$ (0.5 g/l) in 1 l of distilled water.
- ***Drugs***: Digoxin, adrenaline, amlodipine, acetylcholine, and atropine. The drugs were purchased from local pharmacy store, and only acetylcholine was from Sigma. All these drugs were reconstituted in the Ringer's solution.
- ***Equipment***: Kymograph and Starling heart lever (Orchid Scientific, model SRD-01) were used to record the responses.

Plant material

The leaves and seeds of *D. metel* were collected early in the morning from flowery plants in the city of Koza (Mayo-Tsanaga Division, Far North Region, Cameroon) and shade dried at the room temperature until all the water were evaporated after 14 days. The identity was confirmed by plant taxonomist Froumsia Moksia, from the Faculty of Science of the University of Maroua. A voucher specimen was deposited at the Herbarium of the University Campus.

Animal

Frogs (Bufonidae) weighing between 40 and 65 g were kept in an artificial pond located at the Maroua Protestant College. The frogs were given free access to food throughout the acclimatization period. Their diet consisted of insects such as termites and small crickets; a vitamin complement was added to the insects before giving them to frogs. Animal procedures were conducted with strict adherence to the NIH Guide for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985).

Preparation of the aqueous extract of the leaves and seeds of D. metel

A dried material was powdered and extracted (200 g each in 1.750 l dH_2O) for 1 hour at 70°C. After cooling and filtration, the filtrate obtained was evaporated in a ventilated oven at a temperature of 55°C to yield 9.75% and 11.50% extract residues of the leaves and seeds, respectively.

Experimental design

Isolated frogs' heart preparation

The isolation of the frogs' hearts was done following the protocol described by Neerati et al. [13]. The frogs were decerebrated, and the medulla was removed by pithing and then placed on a dissecting board. An incision was made at the midline of the belly and the sternum and thoracic musculature split. A triangular cut was made at the level of the thorax so as to clear the heart entirely. The heart was then gently removed from the pericardium. The aorta was sectioned and thread passed underneath to fix it on the cannula. A small incision was made in the aorta to introduce the cannula filled with the physiologic solution (Ringer), which was inserted into the heart [14].

A thin pin hook was passed through the tip of the ventricle and with the help of a fine thread attached to the hook; it was tied to the free limb of the Sterling heart lever which was fixed to a stand. A proper tension was adjusted by altering the height of the lever. The perfusion fluid in the cannula was completely displaced by Ringer containing the substances to be investigated.

A study of the cardiotonic activity

Cardiotonic potential on the normal isolated heart: The cardiotonic potential was evaluated using the aqueous extracts of the leaves and seeds of *D. metel* 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 the force of contraction (FC), and cardiac output (CO) was calculated. The frog hearts were rinsed for about 2 minutes with Ringer's solution after every administration of the reference drug or tested extract until they returned to resting levels. Digoxin was tested at the concentration of 0.5 mg/mL; the water extract of the leaves of *D. metel* (WELDM) was prepared at 0.3, 1.5, and 3 mg/ml; and the aqueous extract of the seeds of *D. metel* (WESDM) was prepared at 1.5, 2.5, and 3.5 mg/ml. A new heart was prepared each time; the previous one no longer had normal rhythmic contractions. The basal cardiac contraction was recorded after the administration of the Ringer's solution.

Determination of the possible mechanism of action

Comparison with calcium channel blocker

Once the basal cardiac contraction was recorded, amlodipine was injected into the cannula at 10^{-6}

mg/ml. After approximately 4 minutes of amlodipine injection, each heart received a 10^{-2} mg/ml of adrenaline injection and 1.5 and 2.5 mg/ml of the aqueous extract of the leaves or seeds of *D. metel*.

Comparison with muscarinic receptor blocker

The basal cardiac contraction was again recorded, and each heart was injected with acetylcholine 10^{-6} M followed by atropine 0.1 mg/ml, to test the sensitivity of the tissue. In another set of experiments, propranolol (10^{-4} mg/ml), a β-adrenergic blocker, was used instead of acetylcholine. After rinsing, the experiment was repeated, using the aqueous extract of the leaves or seeds of *D. metel* at the concentrations of 1.5 and 2.5 mg/ml, respectively, instead of atropine.

Statistical analysis

The statistical analysis of the results was performed using the GraphPad Prism 5.00 software, and the results were presented as mean ± standard error on average (ESM), for a number of $n = 6$ cores per group. After analyses of variances using one-way analysis of variance test, multiple comparisons of averages were achieved using nonparametric Tukey test. The significant differences were considered at the level of $p < 0.05$.

Results

Cardiotonic potential on normal isolated heart

Administration of the aqueous extract of the leaves and seeds of *D. metel* caused a significant increase in the FC and CO ($p < 0.05$). This inotropic effect was more than 60%, over that of digoxin (52%) (Figs. 1 and 2). No significant variation was observed in the HR. The testing extract induced a reversible cardiac arrest at 5 and 6 mg/ml for the leaf and seed extracts, respectively (Fig. 3).

Each value represents the mean ± ESM, $n = 6$. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$: statistically significant when compared to normal. $^{a}p < 0.05$ and $^{c}p < 0.001$: statistically significant when compared to the positive control (digoxin). WELDM: water extract of the leaves of *D. metel.*

Each value represents the mean ± ESM, $n = 6$. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$: statistically significant when compared to normal. $^{a}p < 0.05$ and $^{c}p < 0.001$: statistically significant when compared to the positive control (digoxin). WESDM: water extract of the seeds of *D. metel.*

Determination of the possible mechanism of action

Comparison with calcium channel blocker

Adrenaline as well as the aqueous extract of the leaves and seeds of *D. metel* induced a significant increase ($p < 0.05$) in the force of contraction (54.10%, 55.88%, and 43.17%, respectively). The cardiac output was also increased about 62.21%, 60.95%, and 57.87%, respectively, for adrenaline, the aqueous extract of the leaves of *D. metel*, and the aqueous extract of the seeds of *D. metel* (Figs. 4–6). Amlodipine significantly reduced FC ($p < 0.05$) in *D. metel*-treated hearts treated with adrenaline and extracts of the leaves and seeds by 71.2%, 70.3%, and 71.65%, respectively. This was accompanied by a similar reduction in CO of 81.37%, 81.01%, and 79.86%, respectively, for the same substances.

Each value represents the average ± ESM, $n = 6$. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$: statistically significant when compared to normal. $^{a}p < 0.05$ and $^{c}p < 0.001$: statistically significant when compared

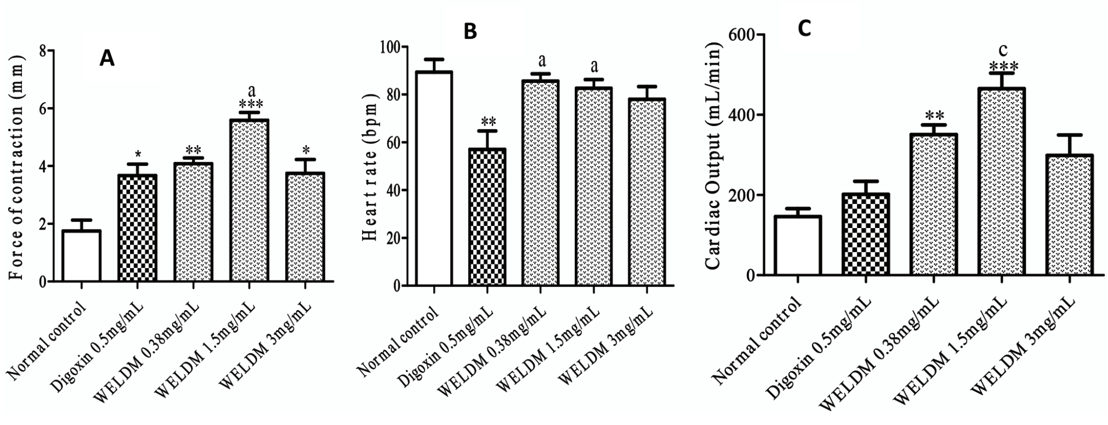

Figure 1. Effects of the aqueous extract of *D. metel* leaves on contraction force (A), cardiac frequency (B), and cardiac output(C) of the isolated frog hearts.

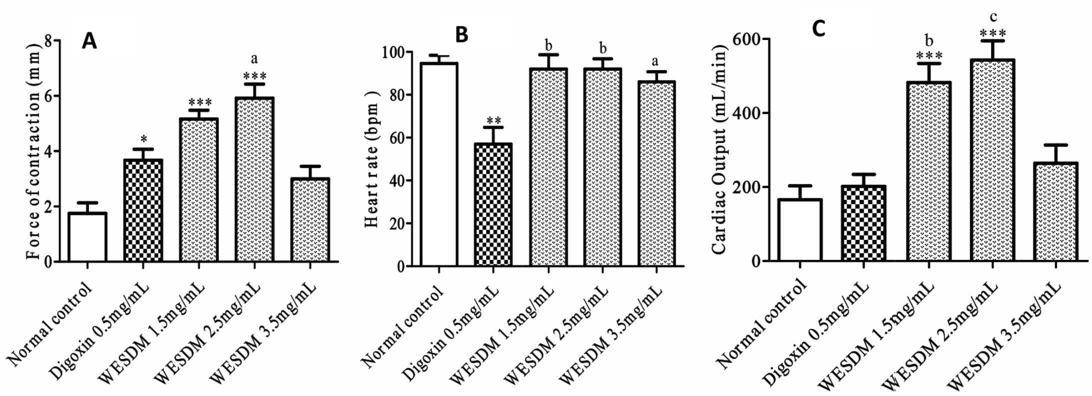

Figure 2. Effects of the aqueous extract of *D. metel* seeds on contraction force (A), cardiac frequency (B), and cardiac output(C) of the isolated frog hearts.

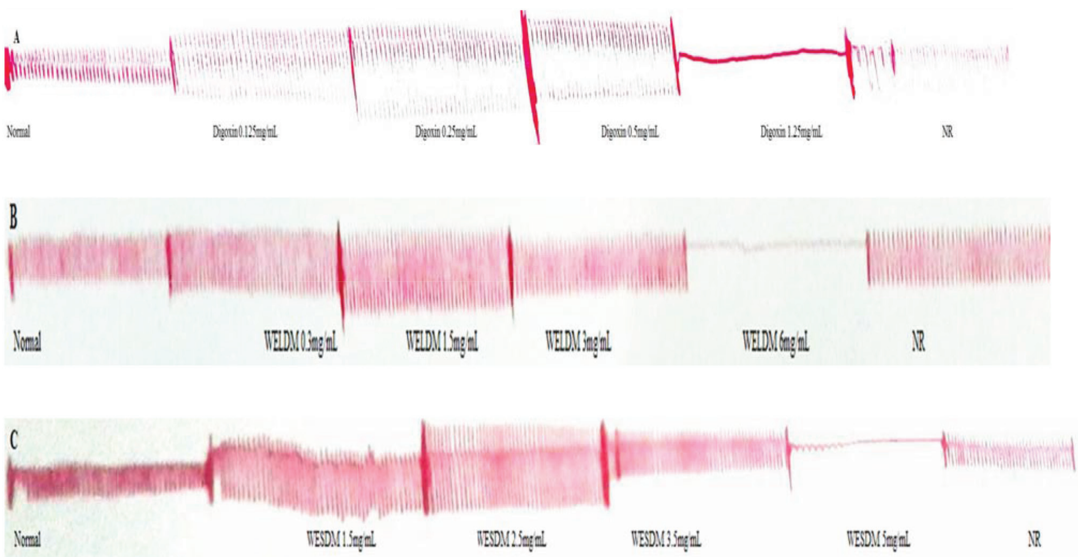

Figure 3. Recordings of the hearts perfused with digoxin (A) aqueous leaf extract (B) and seed extract (C) of *D. metel*. WELDM: water extract of the leaves of *D. metel*; WESDM: water extract of the seeds of *D. metel.*

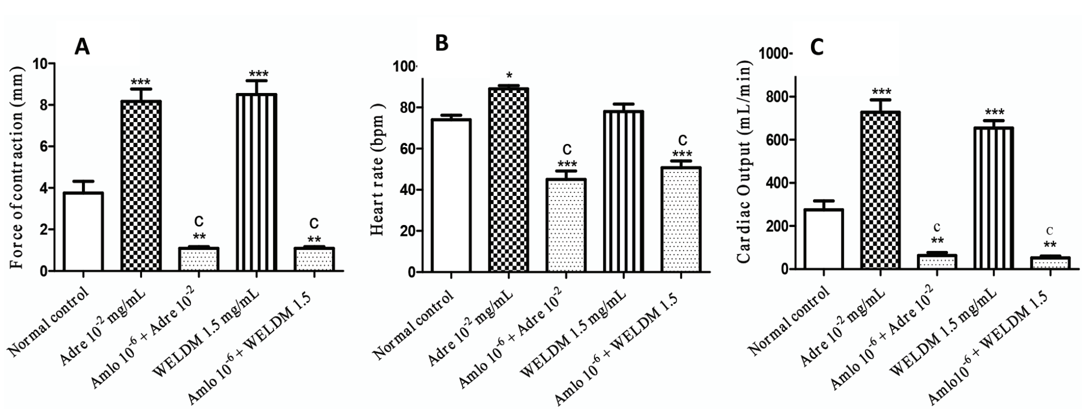

Figure 4. Interaction between amlodipine-adrenaline and amlodipine-aqueous extract of *D. metel* leaves on the contraction force (A), cardiac frequency (B), and cardiac output (C).

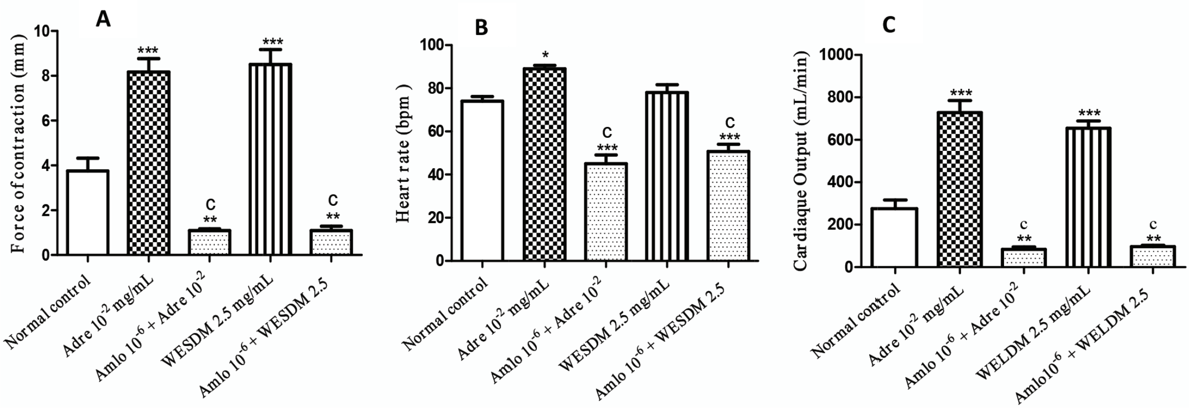

Figure 5. Interaction between amlodipine-adrenaline and amlodipine-aqueous extract of *D. metel* seeds on the contraction force (A), cardiac frequency (B), and cardiac output (C). Each value represents the average ± ESM, $n = 6$. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$: statistically significant when compared to normal. $^{a}p < 0.05$ and $^{c}p < 0.001$: statistically significant when compared to the positive control (adrenaline). adre: adrenaline; Amlo: amlodipine; WELDM: water extract of the leaves of *D. metel;* WESDM: water extract of the seeds of *D. metel.*

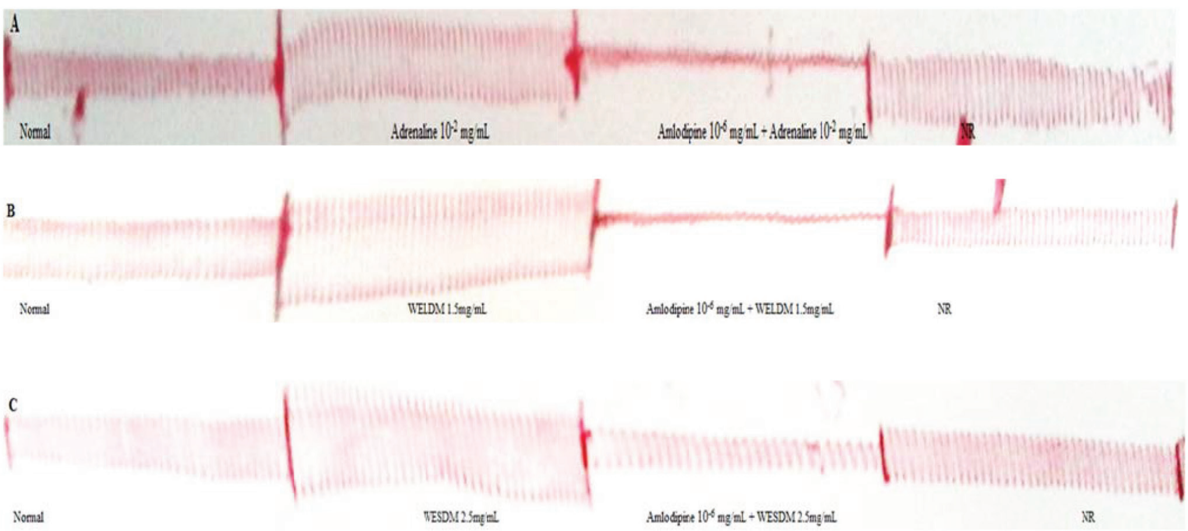

Figure 6. Recordings of the hearts perfused with amlodipine (A), aqueous leaf extract (B), and seed extract (C) of *D. metel* only in the presence of amlodipine. WELDM: water extract of the leaves of *D. metel;* WESDM: water extract of the seeds of *D. metel.*

to the positive control (adrenaline). adre: adrenaline; Amlo: amlodipine; WELDM: water extract of the leaves of *D. metel.*

Comparison with muscarinic receptor blocker

Acetylcholine significantly depressed FC ($p < 0.05$) as well as HR and CO (Figs. 7–9). These effects were inhibited by atropine and aqueous extract of the leaves and seeds of *D. metel* at the doses used (Fig. 9). The increase in FC was, hence, 88.23%; 88.67%; 87.66% and 87.99% for adrenaline, and the aqueous extract of the leaves and seeds of *D. metel,* respectively, whereas CO increased was 93.29%; 92.76%; 92.64% and 91.89%, respectively.

Each value represents the average ± ESM, $n = 6$. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$: statistically significant when compared to normal. $^{a}p < 0.05$ and $^{c}p < 0.001$: statistically significant when compared to the positive control (acetylcholine). ach: acetylcholine; WELDM: water extract of the leaves of *D. metel;* WESDM: water extract of the seeds of *D. metel.*

Discussion

The different parts of *D. metel* were extracted and studied for their cardiotonic potential. The cardiotonic action was also studied by comparing the effects of the extracts to that of a sympathetic agonist and an antagonist. Administration of the

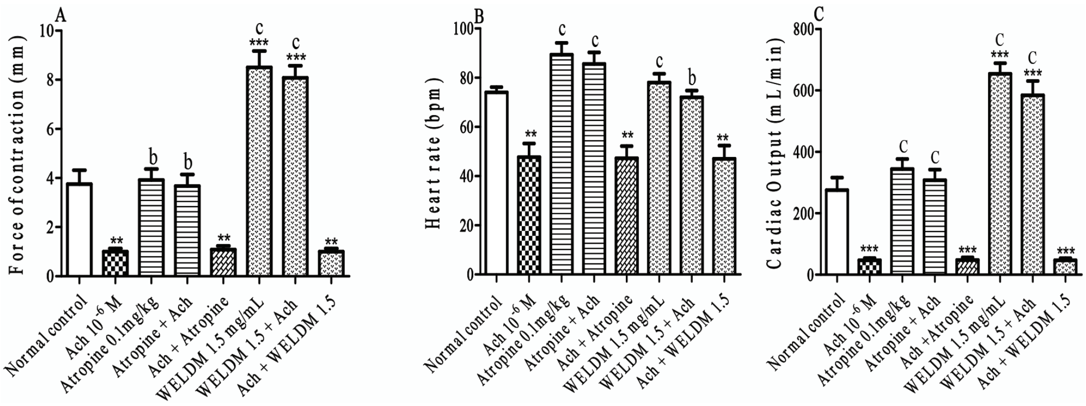

Figure 7. Interaction between atropine-acetylcholine and aqueous extract of the leaves of *D. metel*-acetylcholine on the contraction force (A), cardiac frequency (B), and cardiac output (C).

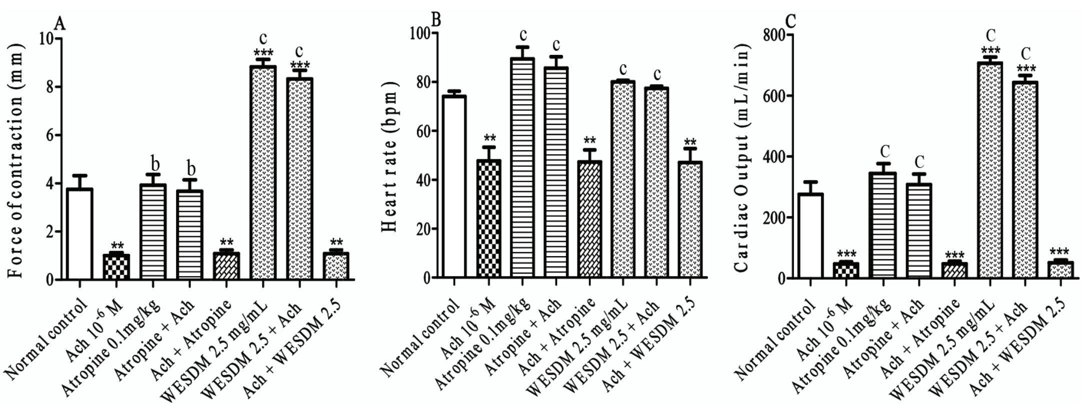

Figure 8. EInteraction between atropine-acetylcholine and aqueous extract of the seeds of *D. metel* -acetylcholine on the contraction force (A), cardiac frequency (B), and cardiac output (C). Each value represents the average ± ESM, $n = 6$. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$: statistically significant when compared to normal. $^{a}p < 0.05$ and $^{c}p < 0.001$: statistically significant when compared to the positive control (acetylcholine). ach: acetylcholine; WELDM: water extract of the leaves of *D. metel*; WESDM: water extract of the seeds of *D. metel.*

aqueous extracts of the leaves and seeds produced a positive inotropic effect (increase in force of contraction as evidenced by increased height of tracing), without any change in the rate of contraction. This effect was different from that of digoxin which produces positive inotropic and negative chronotropic effects. In addition, the inotropic effect of the tested extracts was abolished in the presence of amlodipine, a calcium channel blocker [14,15], and acetylcholine. *D. metel* aqueous-extract-induced positive inotropic effects were not altered by propranolol, a β-adrenergic blocker (data were not shown). A positive inotropism observed in this study might, therefore, come from a competition toward muscarinic receptor activation. Some cardiotonic agents are known to open the slow-type calcium channels [14], and the positive inotropic action of the leaves and seeds of *D. metel* might be closely related to the entry of calcium ions in myocardial cells. Currently, antimuscarinic drugs are important in anesthesia [16], and they are also an important component of the treatment of several pathophysiological conditions including heart failure and acetylcholine overdose [14,17]. In this study, a differentiation was noticed between positive inotropic and chronotropic action of the extracts used, and this extracts can stand as a better option for known antimuscarinic agents, given that these drugs are believed to be used with more discrimination [18]. Furthermore, the dose at which digoxin showed cardiac arrest was 1.25 mg/ml, and the test extracts showed a therapeutic effect in the range of 0.3–6.0 and 1.5–5.0 mg/ml for the leaf and seed extract, respectively. The calculated therapeutic index was, therefore, 10 for digoxin and 10 and 2.33 for the tested extracts, indicating a wide safety margin for the seed extract when compared to Digitalis. The previous studies demonstrated

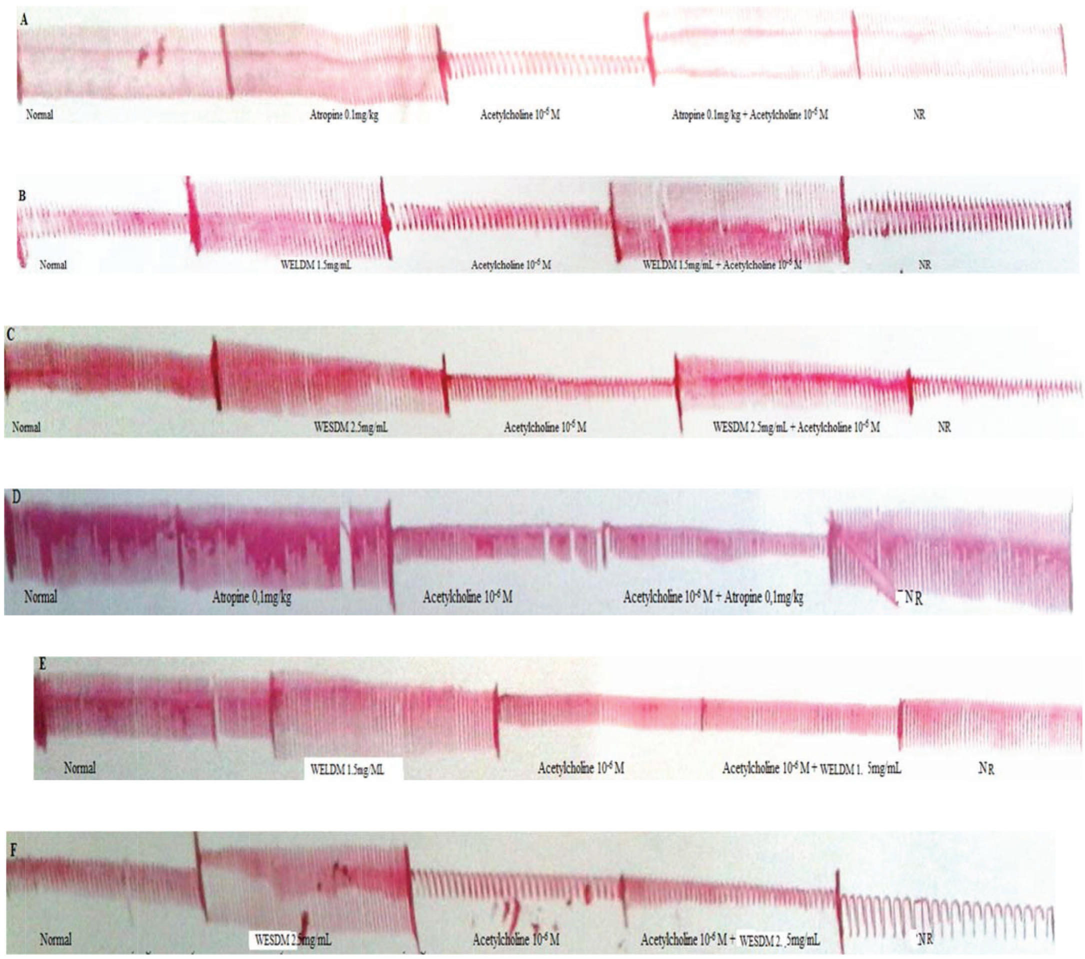

Figure 9. Recordings of the hearts perfused with atropine and acetylcholine (A); WELDM, acetylcholine, and WELDM + acetylcholine (B); WESDM, acetylcholine, and WESDM + acetylcholine (C); atropine, acetylcholine, and acetylcholine + atropine (D); WELDM and acetylcholine + WELDM (E); and WESDM, acetylcholine, and acetylcholine + WEGDM **(**F**)**. WELDM: water extract of the leaves of *D. metel;* WESDM: water extract of the seeds of *D. metel.*

that antimuscarinics come from a group of plants within the family Solanaceae [19]. All the above indicates an improvement of ventricular function by the leaves and seeds of *D. metel*, without increasing in HR. These effects, therefore, have promise for the use of *D. metel* for the therapy of cardiac diseases. However, further studies are necessary to ensure that the concentrations used do not promote side effects that were attributed to the use of *D. metel* when taken at substantial quantities. These side effects include dry mouth, dizziness, cardiac arrhythmia, flushing, and faintness [8].

Conclusion

In summary, the study concludes that the water extracts of the leaves and seeds of *D. metel* showed a positive inotropic action on isolated preparation of frog's heart by acting on cholinergic receptor, and the therapeutic index was the same or better than that of digoxin. Further studies on the identification of the active components are thus well-merited.

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