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Antihypertrophic effects of the seed ethanolic extract of *Aframomum pruinosum* Gagnep. (Zingiberaceae) against isoproterenol-induced cardiac hypertrophy in male Wistar rat

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ABSTRACT

The seeds of *Aframomum pruinosum* are popularly used in the management of cardiovascular conditions. This study was undertaken to evaluate the capacity of the seed ethanolic extract of *A. pruinosum* (EE) to prevent the development of cardiac hypertrophy in rats. Isoproterenol (0.3 mg/kg/day, *sc*) was injected to male rats alone or concomitantly with EE (37.5, 75, or 150 mg/kg, *per os*) or propranolol (20 mg/kg/day, *per os*) for 7 consecutive days and systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate measurements were performed. Cardiac homogenates were used to assay myeloperoxidase (MPO), superoxide dismutase (SOD), catalase, nitric oxide (NO) and reduced glutathione (GSH). Also, sections of heart tissue were stained with Hematoxylin-Eosin, Masson trichrome, or for immunohistological labelling of atrial natriuretic peptide (ANP). Isoproterenol administration caused a decline in SBP and DBP ($p < 0.001$). Heart rate, cardiac mass, cardiomyocyte surface, and MPO levels were significantly ($p < 0.001$) increased. All these alterations were significantly prevented ($p < 0.01$ and $p < 0.001$) by EE. EE inhibited immune cell infiltration and cardiac fibrosis elicited by isoproterenol injection. The overexpression of ANP in the atrium and ventricle induced by the isoproterenol was significantly ($p < 0.001$) prevented by EE. EE possesses antihypertrophic effect against isoproterenol-induced cardiac hypertrophy that may result from its antifibrotic, anti-inflammatory properties, as well as its capacity to down regulate the expression of ANP.

1. Introduction

Cardiac hypertrophy (CH) is the enlargement of the heart that results from an individual increase in cardiomyocyte size [1]. CH is considered as a compensatory response of the cardiac muscle to overcome the increased workload brought on by mechanical and neurohormonal stress [2,3]. This compensatory response becomes overwhelmed and maladaptive if the stressful conditions last for a long time, paving the way to heart failure, cardiac arrhythmias, and sudden death [2,4].

CH, especially left ventricular hypertrophy, is found in 15–20 % of the general population [5]. Obese people, the elderly, Black people and patients suffering from hypertension are more likely to develop CH [5,6]. Indeed, the prevalence of CH was shown to range from 19 % to 48 % in untreated hypertensives and from 58 % to 77 % in high-risk

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hypertension patients, according to an analysis of 37700 people's echocardiographic data [5].

CH is accompanied by various structural, biochemical, and molecular alterations within cardiomyocytes including sarcomeric reorganization, fibrosis, increase production of reactive oxygen species, calcium handling abnormalities, and reactivation of some cardiac fetal genes such as atrial natriuretic peptide (ANP), brain natriuretic peptide, β-myosin heavy chain, and skeletal α-actin [2,4,7]. CH is a common feature of several cardiovascular conditions including hypertension, myocardial infarction, dilated cardiomyopathy, hypertrophic cardiomyopathy, valvular diseases [2,7] and sudden death. Thus, prevention and attenuation of CH is a good therapeutic strategy to prevent sudden death. Despite the huge progress in medicine, cardiovascular diseases, including CH, remain the leading cause of death worldwide. Therefore, there is an emergency to find new therapeutic alternative that can effectively prevent or reverse CH.

Aframomum pruinosum Gagnep. (Zingiberaceae) is a plant widely used in Cameroon for medicinal, ethno-dietary, cultural and spiritual purposes [8]. The seeds are used as a tranquilizer and to treat cardiac palpitations, difficult respiration [9,10]. *A. pruinosum* is also used to cure schizophrenia, female infertility, and to bring peace and strength in family with twins [11]. Mabeku et al. [12] demonstrated the anti-ulcerogenic effect of *A. pruinosum* seeds. Recently, the cardioprotective effect of seed extracts from *A. pruinosum* against isoproterenol (ISO)--induced myocardial infarction was evidenced by Nguelefack-Mbuyo et al. [13]. ISO also causes a convenient, reproducible, and rapid model of cardiac hypertrophy that closely share common disease transcriptomes with human cardiac hypertrophy [14]. Based on these informations, we hypothesized that the ethanolic extract from the seeds of *A. pruinosum* may prevent the change of normal cardiac phenotype to a hypertrophic phenotype through the improvement of hemodynamic parameters, thus reducing cardiac workload. Hence, the present study was undertaken to assess the inhibitory effect of *A. pruinosum* seed ethanolic extract against ISO-induced cardiac hypertrophy in rat. A preprint of this paper has previously been published.

2. Materials and methods

2.1. Reagents

ISO (Isoprenaline Hydrochloride) and hexadecyltrimethylammonium were purchased from Sigma Aldrich (Germany). Propranolol tablets were from Teva laboratory, France. DMSO and EDTA were bought from Carl-Roth (Kalshur, Germany). NaCl, and orthophosphoric acid were bought from BDH (chemicals Ltd Poole England). Antibodies used in this study were rabbit antibodies, anti-rat and anti-human ANP (EMD Millipore Corporation, USA; no cross-reactivity with BNP) diluted 1:1000 with Tris-HCl. The block protein, post-block protein, HRP polymer and DAB were from ZYTOCHEM-Plus HRP polymer Kit (Berlin, Germany).

2.2. Extract preparation and characterization

The fresh fruits of *A. pruinosum* were purchased from a local producer at Banguè in the municipality of Yokadouma, East region of Cameroon, in September 2019. A specimen was authenticated at the national herbarium of Cameroon in comparison with an existing voucher specimen 45393/HNC. After shade-drying, the sheath that covers the seeds was removed and the seeds were reduced into a powder and used for the extract preparation. Three hundred grams of *A. pruinosum* powder were extracted in 2.25 L of ethanol for 24 hours at room temperature with occasional shaking. After filtration, the residue was extracted again following the same protocol for another 24 h and filtered. The two filtrates were concentrated at 60 °C using a rotary evaporator under reduced pressure. The extraction procedure yielded 19.21 g of ethanolic extract.

The prepared ethanolic extract of *Aframomum pruinosum* was chemically characterized by gas chromatography coupled to mass spectrometry (GC-MS) analytical technique. The obtained results are in the supplementary file (Supplementary file S1).

2.3. Animal handling

Male Wistar rats, aged 10–12 weeks and weighing 180 g to 200 g were used. They were raised in the animal facilities of the Laboratory of Animal Physiology and Phytopharmacology of the University of Dschang. They were maintained at a room temperature of 23 ± 2°C under natural light/dark cycle and provided with tap water and ordinary laboratory chow *ad libitum*. All animals' procedures were carried out following the ethical guidelines for animal use and care as outlined by the law 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and approved by the institutional ethical committee (022/23/401FSa).

2.4. Evaluation of the antihypertrophic effect of the seed ethanolic extract of Aframomum pruinosum

2.4.1. Animal grouping and dosing

This study was carried out following the protocol previously described by Silva et al. [15]. Before the beginning of the experiment, rats were accustomed with the indirect blood pressure and heart rate recording using the tail-cuff plethysmography method (IITC Life Science, Woodland Hills, CA, USA). Sixty rats were assigned into 6 groups as follows:

i) the naive group: 0. 9 % NaCl subcutanously + distilled water (*per os*)

ii) the disease control group (placebo): ISO (0. 3 mg/kg/day) + 5 % DMSO

iii) the propranolol (reference) group received ISO (0.3 mg/kg/day) + propranolol (20 mg/kg /day),

iv) EE treated groups (4, 5, and 6) received ISO (0. 3 mg/kg/day) + ethanolic extract of *A. pruinosum* seeds at doses of 37. 5, 75, or 150 mg/kg by gavage. The doses of the ethanolic extract of *A. pruinosum* used in this study were selected from a previous study [13]. ISO (0.3 mg/kg/day) was administered to rats for 7 days by subcutaneous injection. The plant extract or propranolol (used as a reference drug) was orally administered by gavage (1 mL /100 g) immediately after ISO injection for the same duration as ISO. Identical volume of isotonic saline was injected to naive control rats.

Body weight, blood pressure, and heart rate were recorded before the beginning of the experiment and at the end of the treatment. Twenty-four hours after the administration of the last dose, the rats were anesthetized with diazepam (10 mg/kg) and ketamine (50 mg/kg). Blood was collected by catheterization of the abdominal artery. The heart was harvested, weighed and separated into 2 sets. The first set of hearts (3 rats per group) was fixed in 10 % formaldehyde solution for histological and immunohistochemistry analysis. The second set of heart (from 07 rats per group) was homogenized with TRIS buffer pH 7.4 and then centrifuged at 4 °C at 10,000 rpm during 10 min. The supernatant was removed and used for the assessment of nitric oxide (NO), myeloperoxidase (MPO), and oxidative stress parameters: malondialdehyde (MDA), superoxide dismutase (SOD), Catalase, reduced glutathione (GSH).

2.4.2. Biochemical analysis

MPO was assessed in left ventricle homogenate prepared as previously described [13]. The activity of MPO in the homogenate was determined according to the method of [16]. The optical density was read at 450 nm using an ELISA plate reader (Multiskan FC, 51119000).

The NO content in cardiac homogenate was measured spectrophotometrically using the Griess method [13].

SOD was quantified according to Misra and Fridovich [17]. The

adenochrome absorbance was read at 480 nm with the plate reader. The percentage inhibition was calculated as follows: % Inhibition (I) = 100 - (OD sample / OD standard) x 100.

50 % inhibition corresponding to one unit, the activity of SOD (A) was expressed in units per quantity of proteins according to the formula: A = I / (50 x Amount of protein)

Catalase activity was assessed as described by Sinha [18]. Optical densities were read at 570 nm and catalase activity was expressed as the amount of hydrogen peroxide decomposed/min/milligram of protein using a calibration curve for hydrogen peroxide.

GSH was quantified according to a method described by Şehirli et al. [19]. The spectrophotometer wave length was set at 412 nm and 1.36×10^4 M^{-1} Cm^{-1} was used as the molar extinction coefficient for GSH.

The quantification of MDA was done according to a protocol described by Chen et al. [20]. 1.56×10^5 M^{-1} Cm^{-1} was used as the molar extinction coefficient for MDA. The anti-peroxidation activity or ability to inhibit peroxidation of cell membranes was inversely proportional to the concentration of MDA.

2.4.3. Histopathological examination

Atrial and ventricular tissues were fixed in 10 % formaldehyde prepared in an isotonic NaCl solution. These tissue samples were dehydrated in graded alcohol concentrations (70–90–100°) and xylene, then embedded into paraffin. Tissue Sections (4–5 μm) were stained with hematoxylin and eosin or with Masson trichrome after dewaxing. Sections were then examined and photographed under a DN-107T light microscope for histo-architectural changes. Total heart surface, cardiomyocyte surface, and heart thickness were measured using Image J software 2.0.

2.4.4. Immunohistochemistry staining procedure

For immunohistochemistry staining procedure, atrial and ventricular Sections (4–5 μm) prepared as described in the histopathological examination section, were mounted on poly-L-lysine coated slides, deparaffinized with xylene, and rehydrated. After dewaxing for 30 min in Tris-EDTA buffer pH 8, the sections were treated with 3 % hydrogen peroxide for 5 min to inhibit intrinsic peroxidase activity and washed in Tris-HCl buffer pH 7.4. Immunostaining for atrial natriuretic peptide (ANP) detection was performed according to the manufacturer's instructions ZYTOCHEM - Plus HRP polymer Kit (Berlin Germany). The expression of ANP in heart slices was measured using image J software 2.0.

2.5. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM), and the analysis were performed using GraphPad Prism 8.4.2 software (GraphPad, USA). The differences of continuous variables among various groups were tested using one-way analysis of variance (ANOVA), followed by post hoc Tukey's multiple comparison test (heart rate, body weight, cardiac mass, cardiac morphometries, stress parameters, myeloperoxidase and nitric oxide assessment). Two-way ANOVA with repeated measures followed by Bonferroni post hoc test were used to analyze longitudinal data (blood pressure). Statistical significance was adjudged at p-value less than 0.05.

3. Results

3.1. Effect of Aframomum pruinosum seeds on blood pressure and heart rate

The effect of the ethanolic seed extract of *A. pruinosum* or propranolol on blood pressure following ISO administration is depicted in Fig. 1. ISO when administered alone induced a 10.67 % drop in systolic blood pressure $F_{(5, 108)} = 63.64$, $p < 0.001$) and a 15.30 % decrease in diastolic

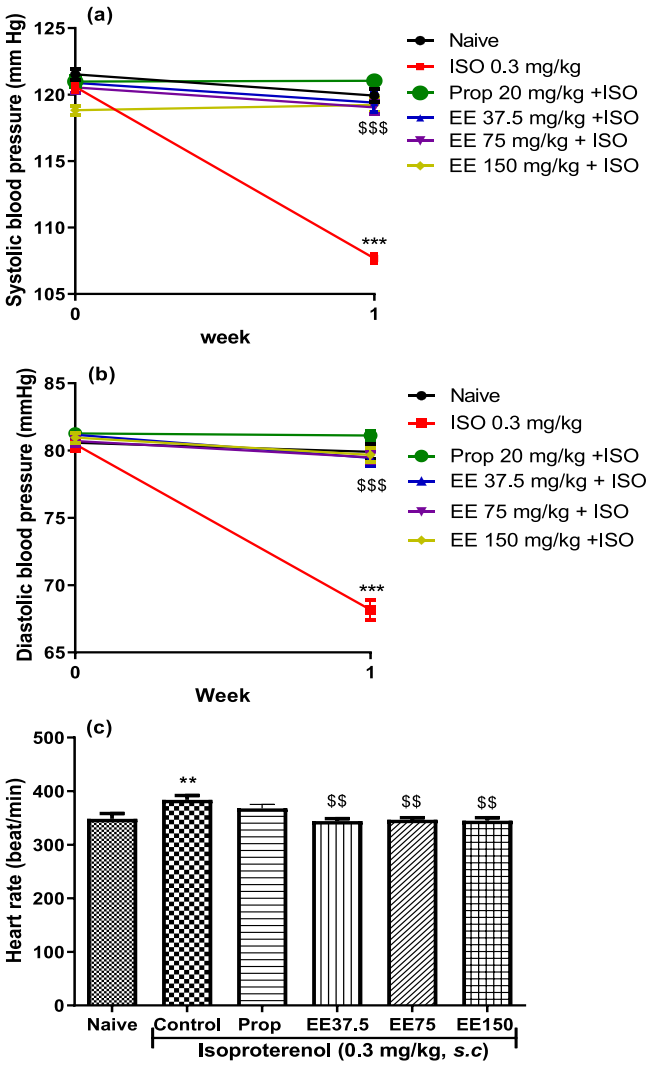

Fig. 1. Oral administration of the seed ethanolic extract of *Aframomum pruinosum* prevents the decrease in systolic (A) and diastolic blood pressure (B) as well as the surge in heart rate (C) elicited by isoproterenol. Data are expressed as mean ± SEM (n = 9).***$p < 0.001$ statistically different compared to the naive group. $^{\$\$\$}p < 0.001$ statistically different compared to the control group. The numbers in the legend refer to the dose in mg/kg. Prop: propranolol; EE: Ethanolic Extract of *A.pruinosum*, ISO: isoproterenol.

blood pressure ($F_{(5, 106)} = 58.45$, $p < 0.001$) compared to the baseline value. Treatment of rats with the plant extract at all doses significantly ($p < 0.001$) prevented the fall in blood pressure elicited by ISO (Fig. 1a and b). Heart rate was significantly increased after ISO administration and *A. pruinosum* extract at all doses used significantly inhibited ($F_{(5, 54)} = 6.77$, $p = 0.0003$) the effect of ISO (Fig. 1c).

3.2. Effect of Aframomum pruinosum seeds on body weight and cardiac mass

As observed in Fig. 2, no significant change in body weight was noticed among the different experimental groups ($F_{(5, 54)} = 1.532$, $p = 0.1954$). The entire cardiac mass and the left ventricular mass were significantly high in rats treated with isoproterenol alone ($F_{(5, 36)} = 10.7$, $p < 0.001$). In rats that received both isoproterenol and *A. pruinosum*, the cardiac mass and the left ventricular mass were reduced especially at the dose of 37.5 mg/kg ($F_{(5, 36)} = 10.7$, $p = 0.0002$ and $F_{(5, 36)} = 5.191$, $p = 0.0299$).

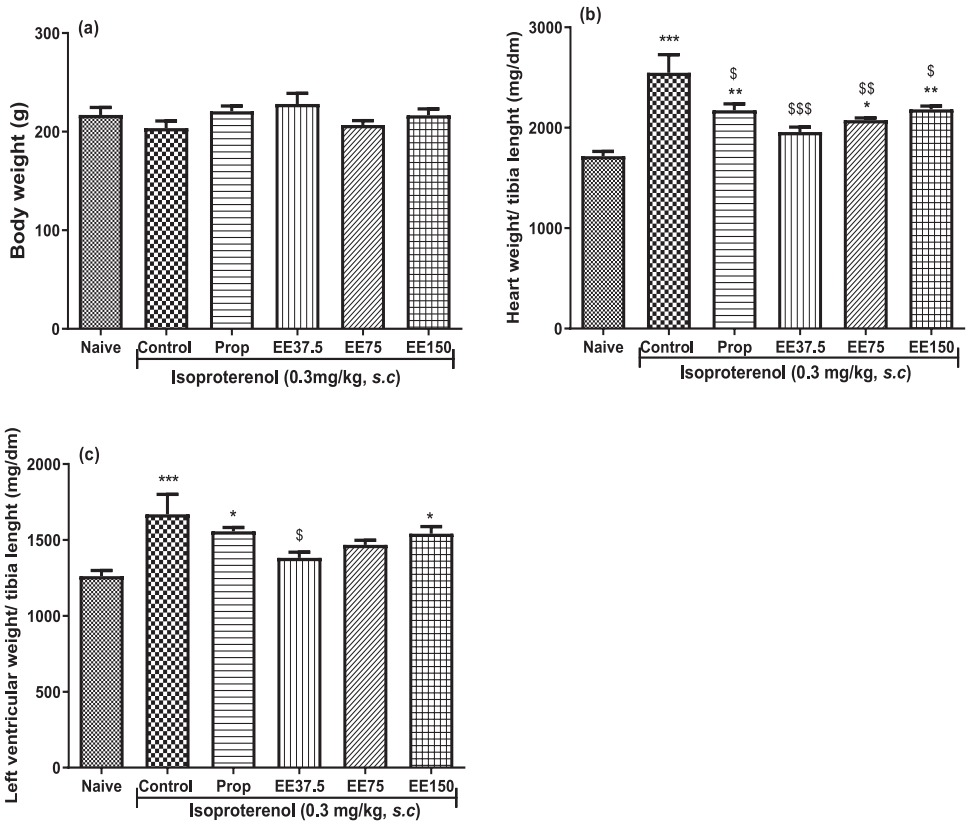

Fig. 2. The seed ethanolic extract of *Aframomum pruinosum* showed no effect on body weight (A) but mitigates isoproterenol-induced cardiac (B) and left ventricular hypertrophy (C). Each bar represents the mean ± SEM. ($7 \leq n \geq 9$) *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ statistically different compared to naive. $^{\$}p < 0.05$; $^{\$}p < 0.01$; $^{\$\$\$}p < 0.001$ statistically different compared to control group. Numbers in the legend refer to the dose in mg/kg. Prop: propranolol; EE: Ethanolic Extract of *A.pruinosum*.

3.3. Effect of Aframomum pruinosum on oxidative stress markers, myeloperoxydase and nitric oxide

ISO administration did not alter any oxidative stress parameter ($p > 0.05$) as depicted in Fig. 3; but the seed ethanolic extract of *A. pruinosum* at doses of 75 and 150 mg/kg significantly increased the activity of catalase in the cardiac muscle ($F_{(5, 30)} = 3.517$, $p = 0.0318$ and $p = 0.0134$) (Fig. 3c).

From the results depicted in Fig. 3e, it can be noticed that ISO induced a 44.21 % rise in MPO levels that was however not statistically significant ($F_{(5, 29)} = 9.592$, $p = 0.1293$). Treatment with propranolol or with the plant extract at all doses significantly decreased cardiac MPO levels ($F_{(5, 29)} = 9.592$, $p < 0.001$ and $p < 0.01$). ISO injection had no effect on the cardiac NO content but propranolol and EE increased this parameter. The effect was marked with EE which induced a significant increase ($F_{(5, 29)} = 6.771$, $p = 0.0005$) as compared to both naive and control groups (Fig. 3f).

3.4. Effect of Aframomum pruinosum seeds on cardiac morphometry

As shown in Fig. 4a and summarized in Fig. 4b, the whole heart surface of control animals greatly increased as a result of ISO administration ($F_{(5, 12)} = 12.54$, $p = 0.0007$). This effect was significantly inhibited by the plant extract whatever the doses used ($F_{(5, 12)} = 12.54$, $p = 0.0115$ and $p = 0.0097$). The left ventricle surface did not significantly change among experimental groups (Fig. 4c) while a slight enlargement of the right ventricle was observed in rats of the control group. Propranolol significantly reduced the right ventricle surface ($F_{(5, 12)} = 4.879$, $p = 0.0062$) while, no significant effect was observed in plant extract-treated groups when compared to both naive and control groups (Fig. 4d). The thickness of both ventricular chambers augmented following ISO administration alone as compared to the naive group ($F_{(5, 12)} = 3.825$, $p = 0.0247$ for the left ventricle and $F_{(5, 12)} = 6.25$, $p = 0.0053$ for the right ventricle). Propranolol and the seed ethanolic extract of *A. pruinosum* inhibited this effect with the doses of 37.5 mg/kg and 75 mg/kg being the most effective on left ventricular thickness and right ventricular thickness, respectively ($F_{(5, 12)} = 3.825$, $p = 0.0426$ and $F_{(5, 12)} = 6.25$, $p = 0.0068$; Figs. 4e and 4f).

3.5. Effect of Aframomum pruinosum seeds on cardiac histology

When compared to the naive group, it was observed that short-term administration of ISO alone resulted in an increased synthesis of collagen as revealed by the Masson's trichrome staining of the cardiac tissue in the control group (Fig. 5a). The cardiac histology of animals treated with propranolol or the plant extract at the dose of 37.5 mg/kg were similar to that of the naive group. Doses of 75 and 150 mg/kg of the ethanolic seed extract of *A. pruinosum* mitigated ISO-induced fibrosis as evidenced by the low intensity of the blue staining indicating collagen production.

The transverse section of the heart apex using hematoxylin and eosin staining (Fig. 5b) shows that animals from the naive group (normal control rats) had well-structured cardiac tissue. The muscle fibers had a normal architecture without immune cell infiltration and cell damages. In animals treated with ISO alone, cardiac fibers are enlarged, and large areas of leukocyte infiltration are observed, as well as cell damages. Animals treated with either propranolol or *A. pruinosum* extract concomitantly with ISO injection had a nearly normal cardiac histology.

As seen in Fig. 5c, ISO administration has significantly ($F_{(5, 12)} = 49.65$; $p < 0.001$) increased the cardiomyocyte surface compared to the naive group. Treatment with *A. pruinosum* at all doses used or propranolol drastically reduced isoproterenol-induced cardiomyocyte

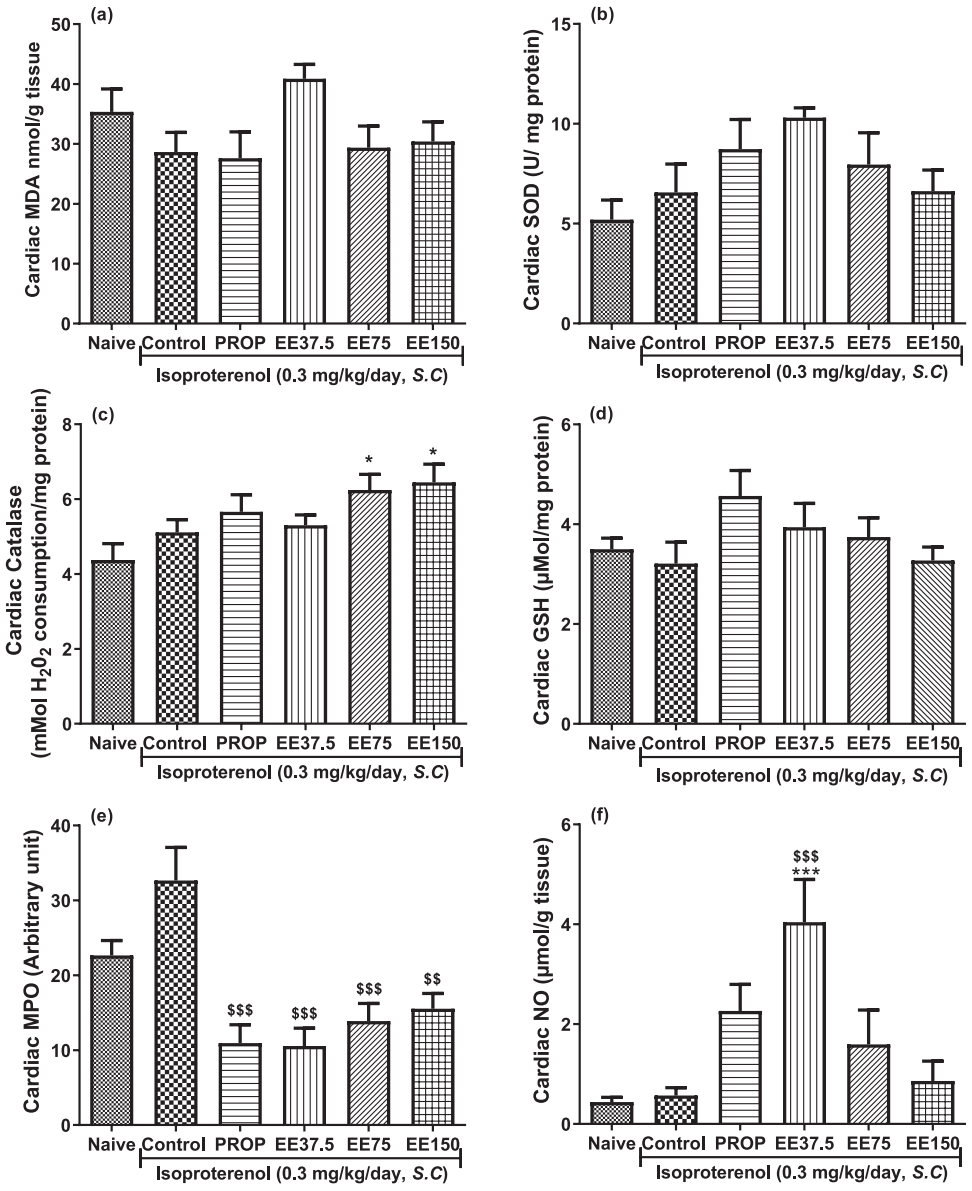

Fig. 3. The seed ethanolic extract of *Aframomum pruinosum* increased catalase activity (c) and nitric oxide content (f), reduced myeloperoxidase (e) and had no effect on MDA, SOD, and glutathione (GSH) levels (panels a, b and d) in the heart of isoproterenol-treated rats. Each bar represents the mean ± SEM. (n = 6) *$p < 0.05$, ***$p < 0.001$; statistically different compared to the naive group. $^{\$\$}p < 0.01$; $^{\$\$\$}p < 0.001$ statistically different compared to isoproterenol group. The numbers in the legend refer to the dose in mg/kg. Prop: propranolol; EE: Ethanolic Extract of *A.pruinosum*, NO: nitric oxide MPO: myeloperoxidase.

surface enlargement ($F_{(5, 12)} = 49.65$, $p < 0.001$) with the dose of 75 mg/kg being the most effective.

3.6. Effect of Aframomum pruinosum seeds on atrial and ventricular expression of Atrial Natriuretic Peptide (ANP)

Following isoproterenol injection in rat, ANP expression was markly increased in the atrium and ventricle by 640.86 % and 486.56 %, respectively ($F_{(5, 24)} = 214.1$, $p < 0.001$) when compared to the naïve group. As seen in Fig. 6, this effect was significantly blunted, especially in the ventricle of rats treated with either propranolol or with the plant extract whatever the dose ($F_{(5, 54)} = 6.77$, $p < 0.001$).

4. Discussion

Cardiac hypertrophy, which first develops as an adaptive response to an increased workload, is recognized as an independent risk factor for adverse clinical outcomes and cardiovascular diseases including heart failure, arrhythmias, and sudden death [2,21,22]. The present study evaluates the protective effect of the ethanolic extract from the seeds of *Aframomum pruinosum* on an animal model of chemically-induced cardiac hypertrophy. ISO-induced cardiac hypertrophy in rats is extensively used to evaluate the cardioprotective effect of natural products [1,2,23]. The model is simple and exhibits biochemical, histological, and electrocardiographic changes that closely mimic those seen in humans.

The results obtained show that ISO administration decreased both systolic and diastolic blood pressure. ISO is a non-selective β-adrenergic agonist and stimulation of β_2-adrenergic receptors is known to lower blood pressure [24]. Thus, the fall in blood pressure observed in ISO-treated rats may result from peripheral activation of β_2-adrenergic receptors with a subsequent decrease in peripheral resistance. Also, ISO induced an increase in heart rate as a consequence of its well-known positive inotropic and chronotropic effects [23,25]. The administration of the ethanolic extract from *A. pruinosum* seeds successfully prevented these hemodynamic alterations. A similar result was observed in a previous study conducted on a model of myocardial infarction using

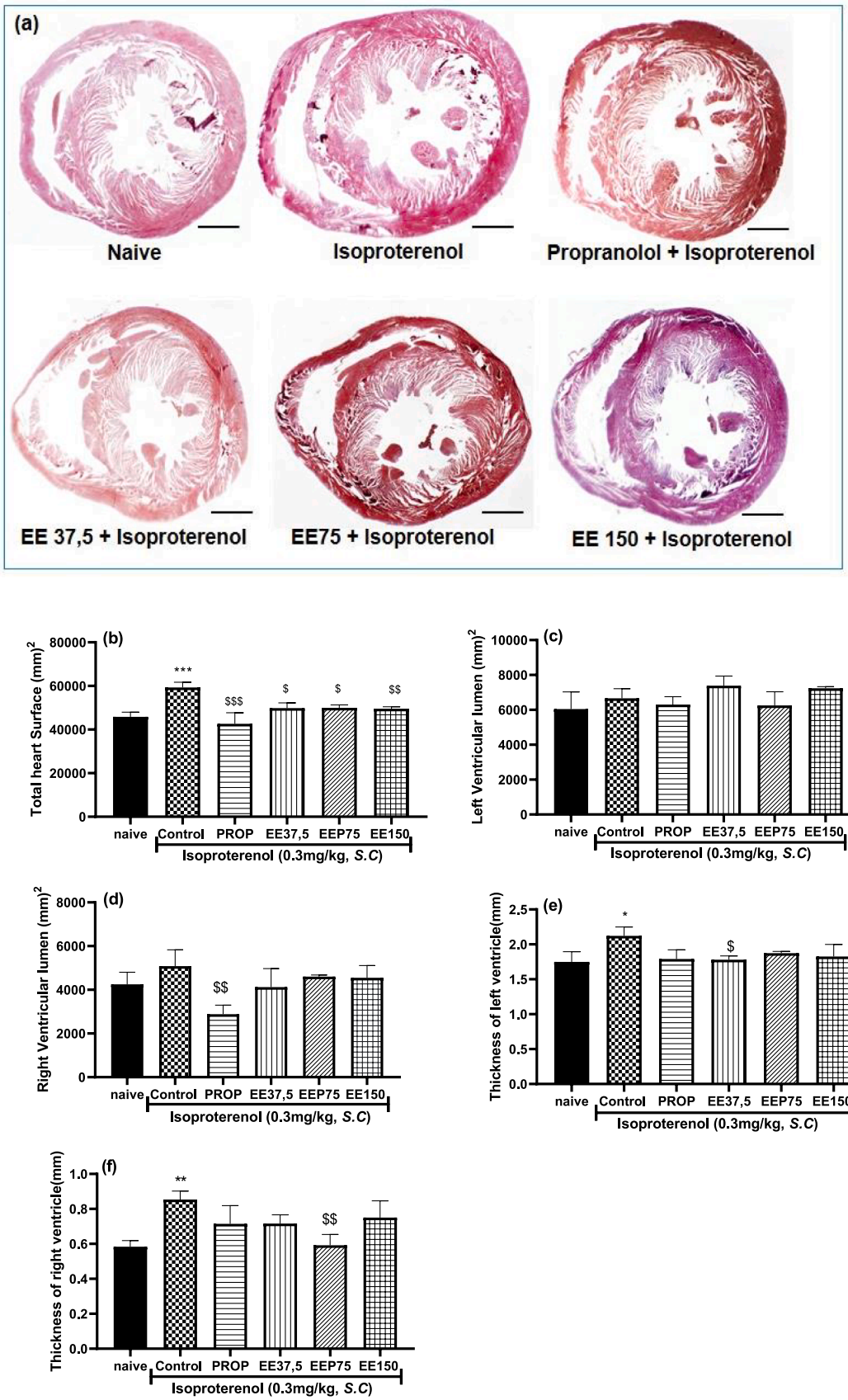

Fig. 4. The seed ethanolic extract of *Aframomum pruinosum* improved total heart surface (b), and ventricular thickness (e and f) but had no effect on ventricular lumens (c and d). Panel (a) represents cardiac sections stained with hematoxylin and eosin magnification (X15). Each bar represents the mean ± SEM. (n = 3). $^{**}p < 0.01$; $^{***}p < 0.001$ statistically different compared to the naive group. $^{\$}p < 0.05$; $^{\$\$}p < 0.01$; $^{\$\$\$}p < 0.001$; statistically different compared to isoproterenol group. The numbers in the legend refer to the dose in mg/kg. Prop: propranolol; EE: Ethanolic Extract of *A. pruinosum*.

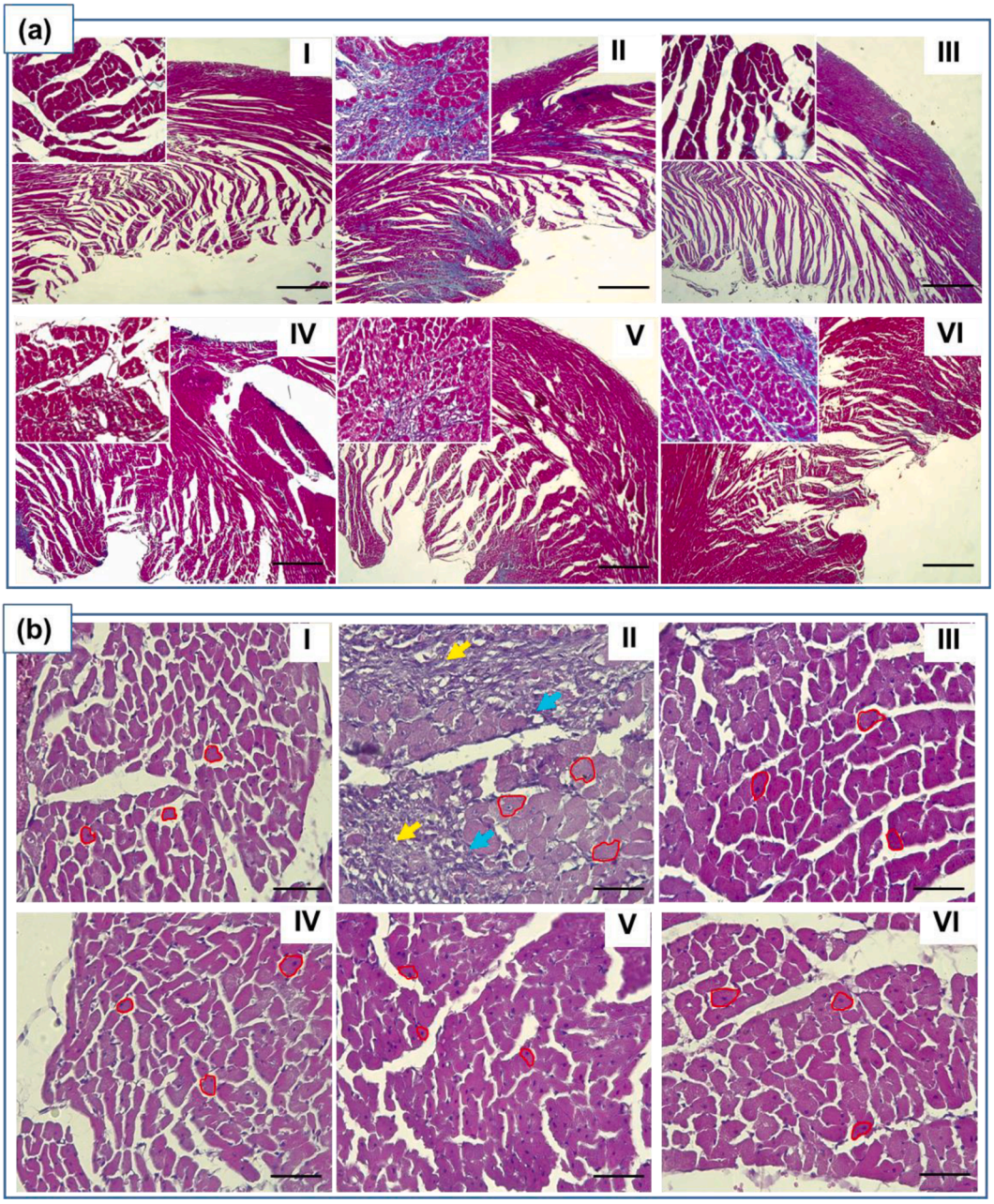

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Fig. 5. Transverse sections of the heart stained with Masson's trichrome (a) and transverse section of the heart apex stained with Hematoxylin and eosin staining (b). Panel (a) shows the inhibition of fibrosis formation (blue staining) by the seed ethanolic extract of *Aframomum pruinosum*. Panel (b) shows the inhibitory effect of the seed ethanolic extract of *Aframomum pruinosum* on leukocyte infiltration, and cardiomyocyte surface increase. The light blue arrow indicates inflammatory cell infiltration and the yellow arrow designates cell damage. The red surrounding indicates the cell surface. In panel (a) the scale bar correspond to 36 μm while panel (b), it correspond to 14 μm. I: naïve group, II: isoproterenol treated group, III: Isoproterenol + Propranolol, IV: Isoproterenol + EE 37.5 mg/kg, V: Isoproterenol + EE 75 mg/kg, VI: isoproterenol + EE 150 mg/kg. Panel (c) numbers in the legend refer to the dose in mg/kg. (n = 3) $^{**}p < 0.01$; $^{***}p < 0.001$ statistically different compared to the naive group. $^{\$\$\$}p < 0001$ statistically different compared to control group. Isoproterenol was injected subcutaneously at the dose of 0.3 mg/kg/day for 7 days. Prop: propranolol; EE: Ethanolic Extract of *A. pruinosum*.

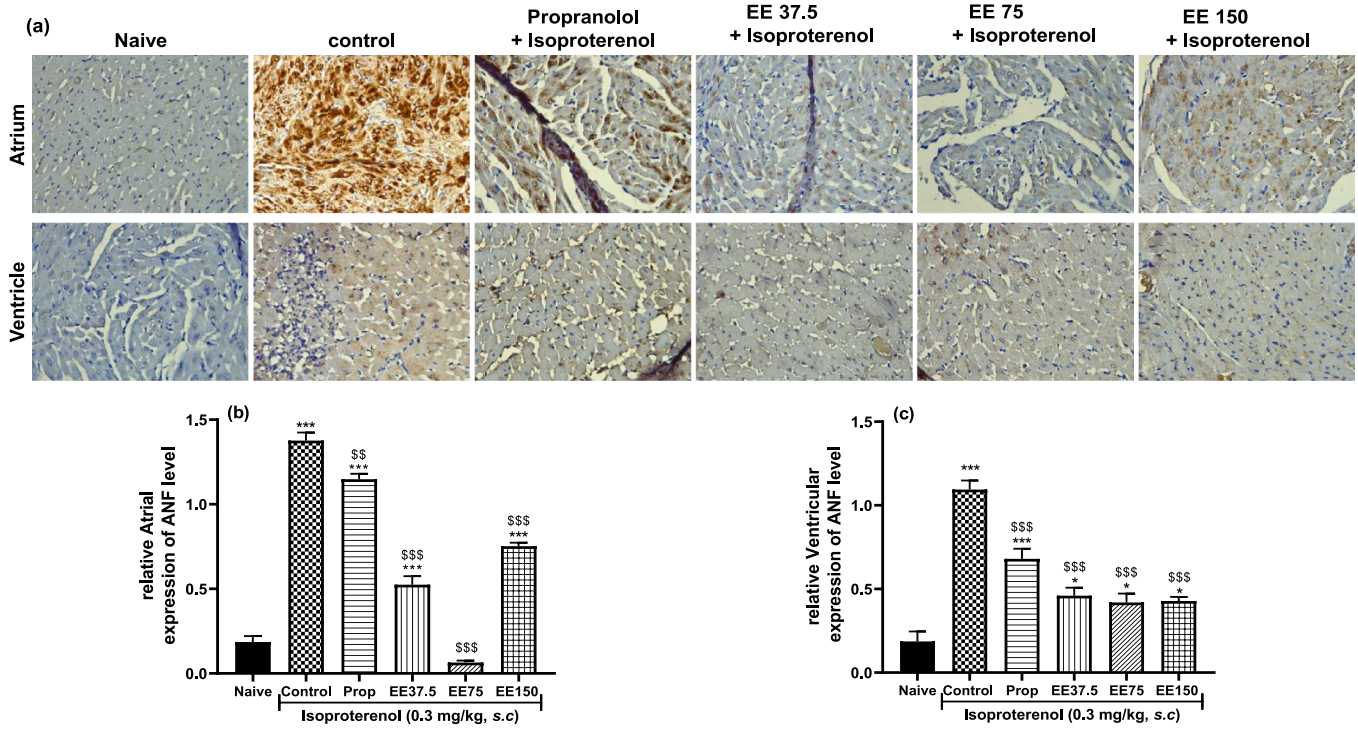

Fig. 6. Immunohistochemistry staining of the atrium (A and B) and ventricle (A and C) showing the inhibitory effect of the seed ethanolic extract of *Aframomum pruinosum* against isoproterenol-induced Atrial Natriuretic Factor upregulation. Each bar represents the mean ± SEM. (n = 3) $^{*}p < 0.05$; $^{***}p < 0.001$ statistically different compared to naive group. $^{\$\$}p < 0.01$; $^{\$\$\$}p < 0.001$; statistically different compared to control group. Numbers in the legend refer to the dose in mg/kg. Prop: Propranolol; EE: Ethanolic Extract, ISO: Isoproterenol.

high dose ISO [13]. In a recent study, we demonstrated that *A. pruinosum* extracts have antihypertensive and vasorelaxant effects and that, this vasorelaxant activity was partially mediated by the inhibition of calcium influx [26]. These effects may contribute to reducing ISO-induced increased cardiac workload and thus prevent hemodynamic abnormalities. This result strongly suggests that the ethanolic extract of *A. pruinosum* interacts with β-adrenergic signaling. Moreover, nerolidol, the major compound identified in this plant extract, has been shown to prevent ISO-induced QTc prolongation on the ECG, a risk factor for the occurrence of ventricular arrhythmias [27]. This suggests that the effect of *A. pruinosum* on heart rate could be at least partially attributed to the presence of nerolidol.

The heart weight/tibia length and left ventricular weight/tibia length ratios were significantly increased in rats that received only ISO, as well as the total heart surface and the left and right ventricle thickness. However, the ventricular luminal surface was not affected. These results show that ISO caused a concentric hypertrophy of all cardiac chambers. Right ventricular remodeling is generally considered an adaptive mechanism to increase pulmonary pressure [28]. Knowing that ISO is used in the treatment of pulmonary hypertension, this observation seems confusing. However, the increase in right ventricular thickness seen in this study may result from inotropic stimuli caused by exposure to ISO. These changes were highly attenuated or even completely prevented in some cases by the treatment with the ethanolic extract from *A. pruinosum* seeds, especially at lower doses. These results clearly show that the plant extract exhibits an antihypertrophic effect.

In the state of cardiac hypertrophy, there is a gradual enlargement of cardiomyocyte cells accompanied by a series of changes, including fibrosis and the reactivation of fetal genes like atrial natriuretic peptide (ANP) [3,4,29]. The reprogramming of these fetal genes has been reported to trigger pathological cardiac remodeling [30]. Thus, inhibiting the expression of these genes could be an effective therapeutic target for the management of cardiac hypertrophy. In this study, low doses of *A. pruinosum* (37.5 and 75 mg/kg) completely prevented ISO-induced cardiomyocyte surface increase, significantly decreased atrial and ventricular ANP expression, and mitigated fibrosis. Though mechanisms by which the plant extract impedes ANP expression is still unknown, this result shows that both the inhibition of ANP expression and the reduction of collagen synthesis contribute to the antihypertrophic effect of the plant extract, although the mechanisms behind this antihypertrophic effect are still to be investigated.

Oxidative stress, which results from an imbalance between reactive oxygen species (ROS) production and the antioxidant defense mechanism of the organism, has been associated with cardiac hypertrophy. Indeed, oxidative stress activates ROS-sensitive pro-hypertrophic and remodeling cascades [31]. In the present study, although an increase in catalase activity was observed in rats treated with the plant extract, no evidence of oxidative stress was noticed. Nalban et al. [32] reported a

state of oxidative stress after injecting 5 mg/kg of ISO into male mice for 10 consecutive days. In this work, no alteration in the redox status of ISO-treated rats was observed. The difference in the dose of ISO used in the two studies could explain this discrepancy. In this study, cardiac hypertrophy was induced using 0.3 mg/kg ISO for seven consecutive days versus 5 mg/kg for 10 days in the study of Nalban et al. [32] that suggests that in the ISO-induced cardiac hypertrophy model, oxidative stress may occur as a consequence of cardiac enlargement.

NO is well known for its cardioprotective effect. In this study, NO levels were increased in animals that received the ethanolic extract of *A. pruinosum* but this increase was only significant at the lowest dose showing the contribution of NO to the antihypertrophic effect of this extract. This result is in accordance with a previous finding which showed that the vasorelaxant effect of the ethanolic extract of *A. pruinosum* was partially mediated by NO [13]. Plant extracts contains a variety of active principles that may act synergistically at lowest doses and antagonistically at higher doses. This may explain the fact that greater doses of the extract fairly boosted NO production. Inflammation plays a key role in the pathogenesis of cardiac hypertrophy [32,33]. Increased production of pro-inflammatory mediators has been reported in ISO-induced cardiac hypertrophy [32]. In the present study, histological sections of the heart tissue from the ISO group showed a marked immune cell infiltration. Additionally, ISO caused a surge in cardiac MPO, an inflammation marker released during neutrophil degranulation which was prevented by the administration of the plant extract. The inhibition of MPO release by *A. pruinosum* was demonstrated in a previous study by Nguelefack-Mbuyo et al. [13] and could be associated with the ability of the plant extract to prevent immune cell infiltration. Indeed, Kim et al. [34] and Lockhart and Sumagin [35] reported that MPO activates neutrophil recruitment and promotes cytokine and chemokine release. Moreover, MPO release in cardiac tissue has been associated with cardiac hypertrophy [36]. Thus, the inhibition of MPO appears as a mechanism contributing to the antihypertrophic effect of *A. pruinosum* seeds. Nerolidol, the major compound identified in the plant extract has been reported to exhibit anti-inflammatory activity [37,38]. The inhibition of immune cell invasion coupled with the inhibition of MPO release observed in this work could result from the presence of nerolidol (Supplementary file S1, Table S1).

5. Conclusion

In conclusion, this study provides evidence for the antihypertrophic effect of *A. pruinosum* seed ethanolic extract against ISO-induced cardiac hypertrophy. This antihypertrophic effect of the plant was similar to that of propranolol and no clear dose-response effect was observed. In some parameters, the lowest dose seems to be more effective while, the contrary was observed in other parameters. The antihypertrophic effect of *A. pruinosum* results at least partly from mechanisms including negative chronotropism, inhibition of fibrosis, and anti-inflammatory activity. Negative chronotropism contributes to reducing cardiac workload, which is one of the stimuli causing cardiac hypertrophy. Inhibition of fibrosis by the plant extract is mediated by the reduction in the expression of ANP, whose reactivation is known to trigger cardiac remodeling, while the anti-inflammatory response of *A. pruinosum* occurs through the inhibition of MPO release. Yet, investigating the interaction of this extract with β-adrenergic receptors, transforming growth factor beta signaling, and pro-inflammatory cytokines will shed more light into the mechanism of action of *A. pruinosum*.

Nerolidol was found to be the major component of this plant extract and might be responsible for the cardioprotective effect of the plant extract. However, additional studies are needed to ascertain this. The findings of this study suggest that *A. pruinosum* could be a good therapeutic option for the management of cardiac hypertrophy. However, the assessment of the safety of *A. pruinosum* and its efficacy on human subjects need to be investigated prior to its clinical use.

Author statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

CRediT authorship contribution statement

Ariane Falone Goumtsa: Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Télesphore Benoît Nguelefack:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Florence Nokam:** Investigation. **Elvine Pami Nguelefack-Mbuyo:** Writing – original draft, Validation, Project administration, Methodology, Data curation, Conceptualization. **Cherif Mouhamed Moustapha Dial:** Validation, Resources. **Cédric Wamba Koho:** Investigation.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Grammarly free writing AI assistance to improve language style for readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ariane Falone Goumtsa reports financial support was provided by Deutscher Akademischer Austauschdienst. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' Contributions

Ariane Falone Goumtsa, Elvine Pami Nguelefack-Mbuyo, and Télesphore Benoît Nguelefack conceived and designed the study, analyzed the data. Ariane Falone Goumtsa collected the data and drafted the manuscript. Florence Nokam and Koho Wamba Cedric collected the data. Cherif Mouhamed Moustapha Dial analyzed data of the histological analysis. Elvine Pami Nguelefack-Mbuyo and Télesphore Benoît Nguelefack critically revised the manuscript. All authors read, edited and approved the final manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2024.101855.

Data availability

Data will be made available on request.

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