

Original Research Article

Investigation of the cardiac depressant effect of *Caralluma tuberculata* N.E.Br on isolated rabbit heart

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Abstract

Purpose: To investigate the histopathological and cardiac depressant effect of the aqueous methanol extract of *Caralluma tuberculata* N.E. Br (AMECT) (family: Asclepiadaceae) and to determine if there is a scientific basis for its cardiovascular diseases-related folkloric use.

Methods: The effect of AMECT in different concentrations ranging from 0.00001 to 1.0 mg/mL were evaluated in isolated perfused rabbit heart to assess their effect on the force of contraction and heart rate using Langendorff's apparatus. Atropine and adrenaline were used to identify the underlying mechanism of response produced by AMECT. The extract was studied for its possible mechanism in the absence and presence of atropine and adrenaline. In addition, sub-chronic toxicity and histopathological study of heart tissues in rats were assessed by administering 500 mg/kg of extract.

Results: At all concentrations, AMECT produced significant ($p < 0.001$) negative inotropic and negative chronotropic effects. The most significant effect was observed at 0.001 mg/mL and higher concentrations hence 0.001 mg/mL was selected for further studies. Pre-incubation with atropine did not significantly inhibit the effects of AMECT. However, AMECT significantly ($p < 0.01$) blocked the cardiac stimulant effect of adrenaline. In the histopathological studies, AMECT did not produce any significant cellular changes or signs of toxicity in the sub-chronic toxicity study.

Conclusion: The cardiac-depressant responses of AMECT may involve the β -adrenergic receptors in the myocardium of isolated rabbit heart thus confirming the rationale for its use in ethnomedicine for cardiac diseases.

Keywords: *Caralluma tuberculata* N.E. Br, Cardiac depressant effect, Atropine, Adrenaline, Isolated rabbit heart, Histopathology

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INTRODUCTION

Cardiovascular diseases are circulatory irregularities, including rheumatism, hypertension, inflammatory heart diseases, cerebrovascular and ischemic heart disorders. In 2019, approximately 17.9 million people died from cardiovascular diseases (CVDs), representing 32 % of all global deaths [1]. In Pakistan, the prevalence of CVDs is approximately 26 % [2]. The mortality rate in developing countries is much higher as compared to developed countries [3]. Although, CVDs treatment largely includes synthetically prepared drugs like amlodipine, enalapril and atenolol etc, some common undesirable effects with these drugs are excessive vasodilatation, dry cough, and withdrawal effects etc.

Herbal medicines remain the popular choice of treatment in developing countries, because drugs from natural sources are inexpensive and easily available [1]. According to the World Health Organization (WHO), traditional medicine use is 60 % globally, while in developing countries almost 80 % of the population rely on herbal medicines as their basic health care requirement [4]. It has been reported that 30 – 50 % of all marketed drugs have their origin from medicinal plants [5]. Therefore, the plant kingdom may be described as a potential target for drug development [6].

Caralluma tuberculata N.E.Br, belonging to Asclepiadaceae, is a perennial herb and widely distributed in the mountainous region of Pakistan. It is one of the important members of the family Asclepiadaceae due to its anti-inflammatory and antinociceptive activity [7], antihyperglycaemic and hypolipidaemic effects etc [8]. Traditionally, AMECT has been reported for BP lowering, anti-diabetic, hepatoprotective, and anti-obesity properties [9]. Phytochemical analysis of plant extract revealed the presence of phytoconstituents like saponins, tannins, phenols, terpenoids, alkaloids, flavonoids, steroids and glycosides. The role of glycosides in cardiac diseases like congestive heart failure (CHF) is well established [10]. In addition, medicinal plant extracts rich in flavonoids, alkaloids and phenols have also shown promising cardiac depressant effects and are also well-known for their antioxidant effects [11].

Thus, the current study was carried out with the aim of determining the cardiovascular effects of aqueous methanolic extract of *Caralluma tuberculata* N.E. Br in isolated rabbit heart.

EXPERIMENTAL

Equipment used

Nikon Advanced Research Microscope OPTIPHOT Model X2T-21E, Langendorff's isolated heart apparatus (ADInstruments, Australia), Lab Chart 6.0 software (AD instruments), Herbal Grinder (China) and analytical weighing balance (Shimadzu Corporation, Japan).

Chemicals and drugs

Adrenaline and atropine sulphate were used as standard drugs, while the chemicals used to prepare Krebs-Henseleit solution were also of analytical grade. All chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals

Rabbits (1,000 – 1,500 g) and Sprague Dawley (SD) rats (220 - 280 g) of either sex were used. The animals below or above the standard weight range were not selected. The rabbit's hearts were used for the cardiac study while the SD rats were used for the toxicity study.

All the experimental animals were obtained from the Animal House, University of Sargodha, Pakistan. Rabbits were selected due to the ease of their availability and use. Rats were used for the histopathological and other toxicity studies due to their cost effectiveness and minimum per kg dose requirement. The experimental rats/rabbits were housed at standard condition in animal house of University of Sargodha, Pakistan. Approval for the use of animals for this work was obtained from the College of Pharmacy, University of Sargodha, Pakistan (approval no. 33-B12 IEC UOS) and the animals were handled according to standard protocols [12].

Plant material collection and identification

Almost 5 kg of fresh plant shoots or aerial parts from the hilly area of district Malakand, Pakistan were collected. The plant material was validated by a taxonomist. A voucher (Cv-AP-09/12) was also submitted in the herbarium, Department of Botany, UOS.

The authentication of the plant was alternatively confirmed through <http://www.theplantlist.org>. No special permissions were necessary to collect plant samples.

Extraction procedure

The plant shoots were used for the study because in local society, shoots were used in cardiac problems. The shoots were shed dried at room temperature and then crushed into powder form using appropriate milling machines. Aqueous methanol (30:70) solvent was used for the extraction because it is relatively inexpensive and lots of compounds dissolve in it due to its maximum polarity. The plant powder (2 kg) was soaked in aqueous methanol (3 L) and kept for almost three days. Cold maceration was followed for extract preparation. At the end of this procedure, a dark brown color extract was obtained. The percentage yield of AMECT was 13.2 % [13].

Procedures

Effects of AMECT on various cardiac parameters by Langendorff's method

Almost half hour before the dissection, the heparin (1000 IU) was injected into the marginal vein of rabbit ear. After few minutes, the rabbit was made unconscious by cervical dislocation. Then the rabbit heart was isolated and into Petri-dish filled with cold Krebs-Henseleit solution. The experiment on the isolated rabbit heart was further carried out following the procedure adopted by Al-Hashem *et al* [14]. The signals recorded were saved for later analysis. After stabilization, different concentrations (0.00001, 0.0001, 0.001, 0.01, 0.1 and 1 mg/mL) of the extract were applied in ascending order to assess various cardiac parameters like force of contraction and heart rate. Before the addition of different concentrations, the tracing was considered as control. A stock solution (10 mg/mL) of the plant extract was prepared by dissolving the extract in distilled water. Then from this stock solution, more dilutions were prepared in a tenfold dosing protocol (1 to 0.00001 mg/mL). Each dilution was first filtered with microfilter. Then in a fixed volume of (5 mL), different concentration(s) were injected with 5 mL syringe through a three-way port, to observe the possible effect of the crude extract on isolated beating heart of rabbit ($n = 4 - 6$). To elucidate the possible underlying mechanism, isolated rabbit heart ($n = 4 - 6$) was used to determine the effect of the selected most effective concentration (0.001 mg/mL) of AMECT. This was investigated both in the absence and presence of atropine (10^{-5} M); a muscarinic receptor antagonist and β -adrenergic receptors blocking agent; adrenaline (10^{-5} M), respectively.

Sub-chronic toxicity and histopathological examination

The rats were randomly divided into two groups ($n = 4$). The control group received normal saline while a dose of 500 mg/kg (PO) once daily of the aqueous methanolic extract was administered to the treated group. Both groups were treated for 28 days. The extract was administered for 28 days because it is a suitable time period for an extract to show its effect. On the 29th day, the SD rats were anaesthetized with a 10 % chloroform-soaked cotton swab. The lowest possible concentration of chloroform for a short time period is not considered to change the histology of the organs examined in the present study. The heart of normal control and treated SD rats were isolated and observed for any pathological changes. Subsequently, the procedure of Daher *et al* [15], was followed for H (hematoxylin) and E (eosin) staining and analysis of histo-slides through microscope.

Statistics

The GraphPad Prism was used for statistical analysis and plotting of the graphs, followed by Tukey's test. Results are presented as mean \pm standard error of the mean (SEM) and $p \leq 0.05$ was reflected as statistically significant.

RESULTS

Cardiac parameters

The AMECT at concentrations (0.0001 to 1 mg/mL) exhibited a significant ($p < 0.05 - 0.01$) fall in the FC and HR. In comparison, maximum response in both parameters was observed at 0.001 mg/mL concentration and above. Hence, 0.001 mg/mL concentration was chosen for further study. This can be seen in Table 1 and Figure 1.

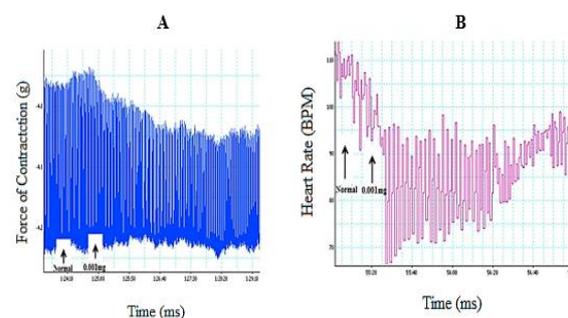


Figure 1: The original tracings show the response of AMECT (0.001 mg) on (A) FC (force of contraction) and (B) HR (heart rate) of isolated rabbit heart

Table 1: Effect of AMECT on various cardiac parameters of isolated rabbit heart

AMECT Conc. (mg/mL)	Force of contraction (FC)	Heart rate (HR)
Change from control (%)		
0.00001	-0.09 ± 0.06	-0.14 ± 0.07
0.0001	-14 ± 1.02*	-12 ± 2.02*
0.001	-30 ± 2.05***	-27 ± 3.12***
0.01	-32 ± 3.23***	-28 ± 1.05***
0.1	-33 ± 2.03***	-27 ± 2.08***
1	-33 ± 1.03***	-27 ± 3.02***

Data are shown as means ± SEM (n = 10). ***p < 0.001 and *p < 0.05 vs. control

Anti-muscarinic activity

When the isolated hearts were pretreated with atropine (10⁻⁵ M), the AMECT (0.001 mg) revealed a significant (p < 0.001) decrease in the FC and HR. Atropine (10⁻⁵ M) was unable to significantly block the negative inotropic and chronotropic effects of AMECT, as shown in Figure 2.

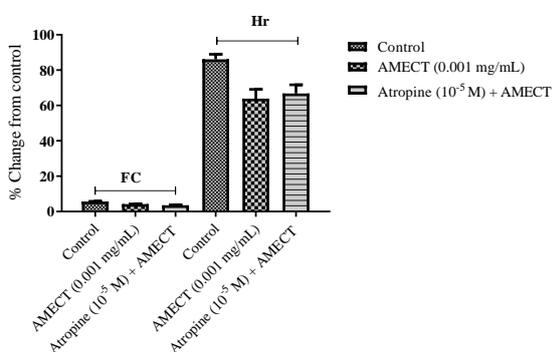


Figure 2: Effect of AMECT on FC (force of contraction) and HR (heart rate) on rabbit hearts in the absence and presence of atropine as compared to normal control (n = 4 - 6)

Activity on β-adrenergic receptors

The findings revealed that adrenaline showed a significant (p < 0.001) rise in FC and HR of isolated heart in the absence of AMECT (0.001 mg/mL). However, adrenaline (10⁻⁵ M) response was changed significantly in the presence of AMECT (0.001 mg/mL), as seen in Figure 3.

Sub-chronic toxicity and histopathological examination

In the histopathological study, zero mortality rate was reported. The physical appearance of SD rats such as the skin, fur and eyes remained normal up till the end of the study. No symptoms of diarrhea, vomiting and anorexia were observed. Histopathological slides of SD rat heart

samples treated with AMECT were also observed and no significant changes in the histopathology of the treated group were observed in comparison with the normal control group (Figure 4).

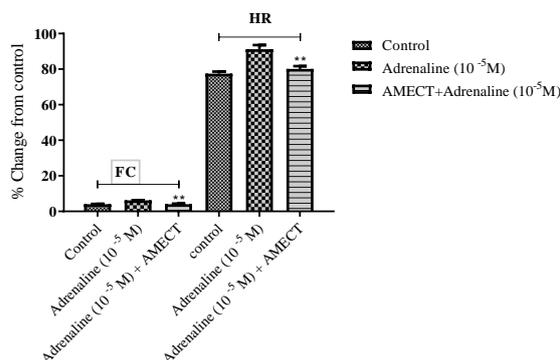


Figure 3: The response of adrenaline (10⁻⁵ M) with and without extract of *Caralluma tuberculata* (0.001 mg) on HR (heart rate) on spontaneous beating rabbit heart. **p < 0.01 compared to control (n = 4 - 6)

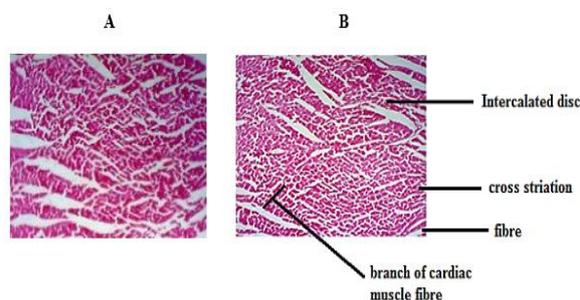


Figure 4: H&E-stained section of heart high magnification (40x) view showing ventricle slide tissue specimen. The ventricle slides of A (control group) and B (treated group) of the SD rat, treated with 500 mg/kg body weight, comparatively no significant change was identified

DISCUSSION

The natural products and discovery of medicines are connected by folkloric claims and the use of medicinal plants from time immemorial. Some important drugs have been derived from medicinal plants such as reserpine, aspirin and digitalis in the history of human pharmacotherapy. Several natural products derived from medicinal plants are launched in the market, examples are galantamine, nitisinone, and tiotropium or in the final phases of clinical trials, like quercetin and resveratrol etc [16]. Despite competition with other sources and approaches of drug discovery, medicinal plants are still searched globally by scientist, to find a remedy for different diseases [17]. Regarding this pharmacological aspect, many medicinal plant extracts are reported for their significant effects against cardiovascular diseases [5].

Plants in the apocynaceae family have been reported to possess different pharmacological activities. The extracts of these plants mainly consist of some important phytochemicals such as alkaloids and glycosides [18]. In this study, AMECT was used to detect its possible response on heart related parameters, including FC and HR.

The results showed that AMECT exhibited a significant negative inotropic and negative chronotropic response on isolated rabbit heart. These findings are in agreement with previously conducted studies [19]. Such cardiac depressant responses can be considered for such drugs which are reported as anti-muscarinic, inhibit β_1 -receptor or calcium channels antagonist [20]. So, with pretreatment of atropine (10^{-5} M), the negative inotropic and negative chronotropic effects of the extract was not blocked. These findings suggest that methanolic extract have not mediated its effects through muscarinic receptors.

To investigate the possible involvement of the β -adrenergic receptors in the cardiac depressant effect of AMECT, the extract was studied in the presence of adrenaline. The data revealed that AMECT significantly inhibited the cardiac stimulant response of adrenaline (10^{-5} M) on isolated rabbit heart, which confirmed that AMECT response is mainly mediated through β_1 -adrenergic receptors. According to literature, adrenaline is a potent cardiac stimulant drug, acting on the β -1 receptors present in heart myocardium [21]. In aggregate, the inhibition of the effect of AMECT by adrenaline suggests the presence of β -blocker phytochemicals in the extract. Moreover, β -blockers are reported to decrease cardiac output and produced vasodilation which in response leads to a decrease in blood pressure. β -adrenergic receptor blocking agents also decrease blood pressure through anti-renin activity and inhibition of catecholamine release [22]. So the plant extract could be exerting its effects via any of the aforementioned mechanism(s).

The histopathological study revealed no mortality and the physical appearance of the animals was normal. Also the histopathological slides exhibited no observable changes in heart cellular structure of the treated group in comparison with the control group. So, the histopathological studies of AMECT have revealed that its use might be safe in cardiovascular problems, as no apparent changes in cellular structure were observed in the treated rats.

CONCLUSION

It is conceivable from this study that AMECT possesses cardiac-depressant effect and the various bioactive compounds present in the extract might exert their effect through β -adrenergic receptors in the myocardium of isolated rabbit heart. This study, therefore, requires further fractionation of this extract to identify the responsible active principle(s) and to reveal its mechanism(s) in detail against high blood pressure and other related cardiovascular disorders.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Taseer Ahmad, Muhammad Naveed Mushtaq and Muhammad Nasir Hayat Malik carried out the experimental work, data collection and evaluation, literature search and manuscript preparation. Alamgeer, and Mater H. Mahnashi, designed the experiments and also supervised the research work, Malik A. Altayar, Mohammed M Jalal and Abdullah Albloshi refined and reviewed the manuscript for publication. Adil

Javed and Maira Ahmad are involved in project administration, proof reading and software. All authors read and approved the final manuscript for publication.

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