Evaluation of Cardioprotective Activity of Aqueous and Ethanolic Extract of Bauhinia Variegata in CaCl\textsubscript{2} Induced Arrhythmia in Albino Rats

Rajesh Kumar Sharma*\textsuperscript{1}, Ashish Kumar Sharma\textsuperscript{1}, Govind Mohan\textsuperscript{2}

\textsuperscript{1}Department of Pharmacology, Suresh Gyan Vihar University, Jagatpura, Jaipur (Rajasthan) 302025- India.
\textsuperscript{2}NIMS Institute of Pharmacy, NIMS University, Shobha Nagar, Jaipur (Rajasthan) India.

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ABSTRACT

The aim of present study was to evaluate cardioprotective activity of ethanolic and aqueous extracts of Bauhinia variegata Linn in CaCl\textsubscript{2} induced arrhythmia in albino rats. In present study, i.v injection of 5% CaCl\textsubscript{2} solution (25 mg/kg b.w.) that induce arrhythmia without causing mortality and heart rates were monitored throughout the study by a lead II electrocardiogram. CaCl\textsubscript{2} reduced heart rate and exhibited alteration in the PQRST waves. Arrhythmia induced by CaCl\textsubscript{2} in experimental animals, which is confirmed by change in ECG pattern and sodium, potassium and calcium level in plasma. Pretreatment of extracts prevent the CaCl\textsubscript{2} induced arrhythmia by virtue of the potent active constituents. Bauhinia variegata Linn. root extracts produced significantly (P<0.05) antiarrhythmic activity. These finding might be helpful to understand the beneficial effect of extracts against CaCl\textsubscript{2} induced arrhythmia. Further study is need to confirm their mechanisms.

INTRODUCTION

Cardiovascular diseases (CVDs) are the most prevalent cause of death and disability worldwide. CVD, a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, myocardial infarction, congestive heart failure, stroke and congenital heart defects (Mackay and Mensah, 1993). The world health organization (WHO) estimates that 17 million people die of cardiovascular disease annually. WHO predicts that deaths due to circulatory system diseases are projected to double by 2015 (Reddy, 1993) Cardiac arrhythmia one of the common type of heart disease that is the main cause of mortality (approximately 17 million). Cardiac arrhythmia are associated with abnormal initiation of wave of cardiac excitation with abnormal propagation of wave of cardiac excitation or same combination of both. Cardiac arrhythmia can manifest them selves in many different way, and the mechanism of an arrhythmia is not clear yet.

Arrhythmia also can be classified by the heart wave (Goodman and Gillman, 2006). Common arrhythmias, particularly atrial fibrillation (AF) and ventricular tachycardia/fibrillation (VT/VF) are a major public health concern. Classic antiarrhythmic (AA) drugs for AF are of limited effectiveness, and pose the risk of life-threatening VT/VF. For VT/VF, implantable cardiac defibrillators appear to be the unique, yet unsatisfactory, solution (Thireau et al., 2011)

During this process, a great attention has been paid in the screening of plant-based drugs which are used in the traditional system of medicine. Also according to WHO, still about 80% of the world population rely mainly on plant-based drugs.

The plant Bauhinia variegata Linn. (Caesalpiniaeae) commonly known as Mountain Ebony is a medium-sized, deciduous tree, found throughout India. It is widely used in folklore medicine. Its bark, root, leaves, seeds and flowers are used for their medicinal properties. It has been used in dyspepsia, bronchitis, leprosy, ulcer, to prevent obesity, as an astringent, tonic and anthelminthic1. The stem contains sitosterol, lupeol, kaempferol-3-glucoside and 5,7-dihydroxy and 5,7-dimethoxy flavanone -4-O-L-rhamnopyranosyl-D-glucopyranosides.

* Corresponding Author
Rajesh Kumar Sharma, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India.
E mail: rajdmk84@yahoo.com
Flowers contain cyanidine-3-glucoside, malvidin-3-glucoside, malvidin-3-diglucoside, and peonidin-3-diglucoside, kaempferol-3-galactoside and kaempferol-3- rhamnoglucoside. Five flavonoids isolated from the different parts of Bauhinia variegata has been identified as quercetin, rutin, apigenin and apigenin 7-O-glucoside. Phytochemical analysis of the root bark of Bauhinia variegata Linn was reported to contain a new flavanone: (2S,5,7-dimethoxy-3'-4' -methyl enedioxy 1-avananone (1) and a new dihydrobenzoxepin 5,6-dihydro-1,7dihydroxy-3,4-dimethoxy-2-methylidibenzo (b,f) oxepin (Yadava and Reddy, 2003; Reddy et al., 2003). Bauhinia variegata Linn. stem is reported to have antitumour (Rajkpoor et al., 2003), antimicrobial (Sharma and Sexena, 1996), anti-inflammatory (Yadava and Reddy, 2003), hepatoprotective (Bodakhe et al., 2007), antihyperlipidemic (Rajani and Pornima, 2009) and immunomodulatory activities (Ghaisas et al., 2009). The present study was carried out to evaluate cardioprotective activities of ethanolic and aqueous extracts of Bauhinia variegata Linn. root in CaCl₂ induced arrhythmia in albino rats.

MATERIAL AND METHODS

Plant material
The root of Bauhinia variegata Linn. was procured and authenticated by Shri A. V. Bhatt, survey officer, Regional Research Institute (Ay.), Bangalore, Karnataka (India). A voucher specimen of same has been deposited (voucher specimen no. RRCBI MCW 79/4).

Preparation of the root extract
The authenticated root was shade dried and powdered coarsely. Extraction was done according to standard procedures using analytical grade solvents (Kokate et al., 2003). Coarse powder of the root (1 Kg) was soxhlet extracted with 90% ethanol. The aqueous extract was prepared using the same marc by the process of maceration. The extracts obtained were concentrated under reduced pressure to yield ethanolic (4.2%) and aqueous extracts (2.4%).

Animals
Healthy Wistar albino rats of either sex weighing between 150-200 g were taken for the study. All the animals were procured from the Central Animal House of the NIMS University. The animals were acclimatized by keeping them in the animal house facility of NIMS Institute of Pharmacy, Jaipur for a week. They were housed in polypropylene (32x24x16 cm) cages containing husk as bedding material and maintained under controlled conditions of temperature (25±2°C), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water ad libitum. Approval of the Institutional Animals Ethics Committee (IAEC) of NIMS Institute of Pharmacy, Jaipur was taken for conducting diabetic neuropathy and cardioprotective activities (Registration No. IAEC / NIMS PH/ JPR/12/2011).

Experimental Protocol
Albino Wistar rats either sex weighing about 150-200 grams were taken and divided into five group each group consisting six animals with plant extracts treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control (Standard diet with ad libitum)</td>
</tr>
<tr>
<td>Group II</td>
<td>Arrhythmia control group [ 5% CaCl₂ (25 mg/kg)]</td>
</tr>
<tr>
<td>Group III</td>
<td>Standard: CaCl₂ (induced arrhythmia + varapamil (5 mg/kg, i.v.) )</td>
</tr>
<tr>
<td>Group IV</td>
<td>CaCl₂ (5%) induced arrhythmia + Bauhinia variegata aqueous extract (400 mg/kg, i.v.)</td>
</tr>
<tr>
<td>Group V</td>
<td>CaCl₂ (5%) induced arrhythmia + Bauhinia variegata ethanolic extract (400 mg/kg, i.v.)</td>
</tr>
</tbody>
</table>

Albino wistar rats were divided into various groups with plant extracts treatment. The rats were anesthetized by ketamine (80 mg/kg i.p.) cardiac arrhythmias were induced by a single intravenous injection of 5% CaCl₂ (25 mg/kg). The induced arrhythmias were analyzed by magnitude of initial bradycardia, onset, incident and duration of the induced fibrillation. After the induction of arrhythmia the animals was allowed to recover completely (15-20 min) and then test compounds was administered. The effect of the test compounds on the basal heart rate was examined and the percentage change in the heart rate was calculated. Seven minute later arrhythmogenic dose of CaCl₂ was re-administered and then effect of treatment on the induction arrhythmia parameters was evaluated as percentage change in the measured parameters or as against the induced fibrillation. A lead II electrocardiogram (ECG) was monitored throughout the study by using Cardiart108DG (BPL) with sensitivity 20 mm mV at a paper speed 25 mm/s. Heart rats were expressed as beat/min. Plasma levels of Na⁺, K⁺ were measured by specific electrode and Ca ++ by complexometric procedure (Sharma et al., 2011, Sharma and Srinivasan, 2009, Al-Obaid et al., 1998)

Statistical analysis
The data are expressed as Mean ± SEM. Results were analysed statistically by one-way analysis of variance (ANOVA) followed by Dunnet test. (P<0.05) was regarded as statistically significant.

RESULT
Effect of extracts and varapamil on the Plasma Calcium level
The blood calcium level was observed after CaCl₂ infusion. In arrhythmia control group (Group II) showed the plasma calcium level (7.18±0.23 mmol/L) increased prominently. The plasma calcium level (1.13±0.12 mmol/L) was found in normal control group. In varapamil (Standard) group calcium was (5.83±0.63 mmol/L) decreased after CaCl₂ infusion. Ethanolic extracts of, Bauhinia Variegata (Group V) was found plasma calcium level 4.94±0.67 mmol/L after CaCl₂ infusion. Ethanolic extracts showed significantly (P<0.05) decrease calcium level when compared with control arrhythmia group. In aqueous extracts of Bauhinia Variegata (Group IV) was showed 4.65±0.84
mmol/L. Aqueous extracts was exhibited significantly (P<0.05) decreased plasma calcium level when compared with control arrhythmia (Table-1).

**Effect of extracts and varapamil on the Plasma Sodium level**

The blood sodium level was monitored after CaCl$_2$ infusion. In arrhythmia control group (Group II) showed the plasma sodium level (53.71±2.82 mmol/L) increased regularly. The plasma sodium level (29.45±1.02 mmol/L) was found in normal control group. In varapamil (Standard) group sodium was found (35.86±1.65 mmol/L) decreased after CaCl$_2$ infusion. Ethanolic extracts of Bauhinia Variegata (Group V) was found plasma sodium level 39.21±2.16 mmol/L after CaCl$_2$ infusion. Ethanolic extracts showed significantly (P<0.05) decrease calcium level when compared with control arrhythmia group. Aqueous extracts was exhibited significantly (P<0.05) decreased plasma sodium level when compared with control arrhythmia group (Table-1) 

**Effect of extracts and varapamil on the Plasma Potassium level**

The blood potassium level was determined after CaCl$_2$ infusion. In arrhythmia control group (Group II) showed the plasma potassium level (112.32±1.52 mmol/L) increased regularly. The plasma potassium level when compared with control arrhythmia group (Table-1). 

Aqueous extracts was exhibited significantly (P<0.05) increased potassium level when compared with control arrhythmia. The plasma potassium level (116.46±2.54) showed significantly (P<0.05) increased potassium level when compared with control arrhythmia group. Ethanolic extracts was showed significantly (P<0.05) increased plasma potassium level when compared with control arrhythmia group (Table-1).

**Table. 1: Effect of different extracts on calcium, sodium and potassium level in plasma in CaCl$_2$ induced arrhythmia modal in rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Calcium(mmol/L)</th>
<th>Sodium(mmol/L)</th>
<th>Potassium(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1.13±0.12</td>
<td>29.45±1.02</td>
<td>112.32±1.52</td>
</tr>
<tr>
<td>Group II</td>
<td>7.18±0.23*</td>
<td>53.71±2.82*</td>
<td>87.43±2.34*</td>
</tr>
<tr>
<td>Group III</td>
<td>5.83±0.63**</td>
<td>35.86±1.65**</td>
<td>107.28±1.76**</td>
</tr>
<tr>
<td>Group IV</td>
<td>4.65±0.84**</td>
<td>43.64±2.67**</td>
<td>116.46±2.54**</td>
</tr>
<tr>
<td>Group V</td>
<td>4.94±0.67**</td>
<td>39.21±2.16**</td>
<td>129.26±4.63**</td>
</tr>
</tbody>
</table>

*(n=6), expressed as mean ± SEM. 
*P<0.05) for statistically significant vs control 
**P<0.05) for statistically significant vs control arrhythmic group.

**Table. 2: Effect of verapamil and bauhinia variegata Linn. Extract on heart rate in CaCl$_2$ induced arrhythmia.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart rates (beat/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>325±9.13</td>
</tr>
<tr>
<td>Group II</td>
<td>196±5.28*</td>
</tr>
<tr>
<td>Group III</td>
<td>267±5.32**</td>
</tr>
<tr>
<td>Group IV</td>
<td>237±7.84**</td>
</tr>
<tr>
<td>Group V</td>
<td>241±5.43**</td>
</tr>
</tbody>
</table>

*(n=6), expressed as mean ± SEM, *P<0.05) for statistically significant vs control, **P<0.05) for statistically significant vs control arrhythmic group.

**Fig 1: Effect of extracts pretreatment on electrocardiographic pattern in CaCl$_2$ induced cardiac arrhythmia.**

**DISCUSSION AND CONCLUSION**

Cardiac arrhythmias are of different types based on their mechanism and origin. The information gathered from animal...
studies has been instrumental in devising diagnostic and therapeutic strategies; so different animal models are needed for different types of arrhythmias. The origin and mechanism underlying clinical arrhythmias are of considerable significance, since knowledge of these processes may provide a basis for successful therapy (Bhatt et al., 2005).

Atrial fibrillation (AF) is the most frequently encountered sustained cardiac arrhythmia in clinical practice and a major cause of morbidity and mortality. Effective treatment of AF still remains an unmet medical need. Treatment of AF is based on drug therapy and ablative strategies (Schmidt et al., 2011).

Arrhythmia produced by CaCl\textsubscript{2} is severe and recalcitrant to manipulation and rather high doses of antiarrhythmic drugs have to be given to produce significant effects. Moreover, the mechanism by which CaCl\textsubscript{2} exert arrhythmogenic action is complex and not fully understood. It is due to at least in part to an indirect action mediated by the autonomic nerve system (Szekeres et al., 1995). Initially, CaCl\textsubscript{2} induce a cholinergic intervention. The significance of a direct cardiac action is shown by the fact that, with increase of calcium concentration, severe arrhythmias, including ventricular fibrillation, occur also in the isolated heart (Friedman et al., 1995, Grambach et al., 1954). An increased calcium concentration induces a hyperpolarization of the resting potential in cell of SA node and decrease on excitation of purkinje cells is accelerated. As well as the repolarization of non specialization myocardial fibers (Trautevin, 1963, Temte and David, 1967). In present study, i.v injection of 5% CaCl\textsubscript{2} solution (25 mg/kg b.w.) that induce arrhythmia without causing mortality and heart rates were monitored throughout the study by a lead II electrocardiogram. Varapamil (5 mg/kg) standard antiarrhythmic drug was given with 5% CaCl\textsubscript{2} solution exhibited normal PQRST waves.

Pretreatment with different extracts in CaCl\textsubscript{2} solution induced arrhythmia animals monitored PQRST wave changes. Extracts treated groups (Group IV to X) respectively showed decrease atrial and ventricular fibrillation. The blood calcium level was increased and found that sodium level increased during CaCl\textsubscript{2} infusion. In control group, plasma calcium level was increased and found that sodium level increased but potassium level was slightly decreased. The standard (Varapamil) group showed normalized level of calcium, sodium and potassium level in plasma. Extracts treated groups were showed decreased plasma calcium, sodium and increased potassium levels. Ethanolic extract. Experiments on rats and rabbits using models of arrhythmias induced by vasopressin, epinephrine, strophanthin, and CaCl\textsubscript{2} showed that antioxidants derived from 1,4-dihydropyridines, dibunol, and α-tocopherol possessed antiarrhythmic effects. Administration of these antioxidants decreased the occurrence of extrasystoles, disturbances of atroventricular conductivity and ventricular fibrillation (Hoffman and Cranefield, 1960, Nick and George, 1998). These drugs also prevented changes in membrane phospholipid composition, inhibited activation of peroxidation, decreased phospholipase activity, prevented a decrease of Ca\textsuperscript{2+}-ATPase and Ca\textsuperscript{2+} binding and uptake by sarcoplasmic reticulum, and increased sarcolemnal Na\textsuperscript{+}, K\textsuperscript{+}-ATPase, sarcoplasmic reticulum creatine phosphokinase (Frolkis et al., 1987). Root bark of Bauhinia variegata Linn reported steroids, saponins, tannins, poly phenolic compounds and flavonoids isolated from the different parts of Bauhinia variegata has been identified as quercetin, rutin, apigenin and apigenin 7-O-glucoside. These may be beneficial effect as anti arrhythmic effect.

REFERENCES


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