Phyto therapeutic Potential of Indigenous Plant Extracts Against Salbutamol-Induced Myocardial Injury and Lipid Dysregulation

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Abstract

Background

Salbutamol, a widely used bronchodilator, is known to induce cardiovascular side effects and cardiotoxicity, exacerbating the global burden of cardiovascular diseases (CVDs). Traditional medicinal plants offer a promising, often safer and more affordable, alternative for managing cardiac disorders. This study aimed to evaluate the cardioprotective and antilipidemic efficacy of indigenous plant extracts against salbutamol-induced myocardial injury in rabbits.

Methods

New Zealand White rabbits were randomly divided into six groups, including healthy control, salbutamol-induced control, and a standard curative group treated with propranolol (Inderal). Other groups were treated with aqueous extracts of *Alpinia zerumbet*, *Citrullus colocynthis*, *Cuscuta reflexa*, *Ranunculus bulbosus*, *Solanum xanthocarpum*, and *Viola tricolor* at 50 µL/mL and 100 µL/mL after salbutamol induction. Plant materials were collected from Lahore, identified, dried, and extracted using standard methods. Myocardial injury was induced by oral administration of salbutamol (60 mg/kg b.wt.) for two days. Biochemical assessments included cardiac marker enzymes (LDH, CK-MB, AST, ALT, AP, ALP, CK-CPK), lipid profiles (Cholesterol, Triglyceride, LDL, HDL), and total blood counts (WBC, RBC, Hb) using commercial kits. Statistical analysis was carried out using one-way ANOVA followed by Tukey's comparison test ($P < 0.05$).

Results

Salbutamol induction significantly elevated cardiac marker enzymes (LDH, CK-MB, AST, ALT, AP, ALP, CK-CPK), increased cholesterol and triglyceride levels, and decreased WBC, RBC, and Hb counts compared to the healthy control. Treatment with Inderal effectively reversed these adverse changes. Most plant extracts, particularly *Alpinia zerumbet*, *Ranunculus bulbosus*, *Solanum xanthocarpum*, *Viola tricolor*, and *Citrullus colocynthis* at 100 µL/mL, demonstrated significant cardioprotective effects, reducing cardiac enzyme levels and improving lipid profiles comparably to, or in some cases, better than Inderal. These extracts also effectively restored hematological parameters towards normal ranges. *Cuscuta reflexa* showed relatively less pronounced protective effects.

Conclusion

The indigenous plant extracts investigated possess significant cardioprotective and antilipidemic properties against salbutamol-induced heart injury, offering a scientific basis for their traditional use. These findings suggest their potential as efficacious, safer, and economically viable alternatives to

synthetic drugs for managing cardiovascular disorders and contributing to public health. Further research is warranted to isolate active compounds and elucidate their precise mechanisms of action.

1 Introduction

Salbutamol (albuterol, Ventolin) is a highly selective β2-adrenergic receptor agonist used to alleviate bronchospasm in various respiratory conditions and to treat high blood potassium (Mahoney et al., 2005; Starkey et al., 2014). Available via inhaler, nebulizer, pill, or IV, its common side effects include shakiness, headache, and fast heart rate, with severe effects potentially including worsening bronchospasm and irregular heartbeat (Mahoney et al., 2005, 2014). First synthesized in 1967 and approved in the U.S. in 1982, it is on the WHO's List of Essential Medicines (World Health Organization, 2014). Salbutamol's therapeutic action involves stimulating β2-adrenergic receptors, increasing cyclic AMP (cAMP) levels, which relaxes bronchial smooth muscle and inhibits bronchoconstrictor release (Abdul, 2014). Its chemical structure, 1-(4-hydroxy-3-hydroxymethylphenyl)-2-(t-butylamino)-ethanol sulphate ((C13H21NO3)2H2SO4, MW 576.7), includes a tertiary butyl group enhancing its β2-receptor selectivity, with the (R)-enantiomer being the pharmacologically active form (Mehta, 2010; Salbutamol-GA Product Information, 2014).

Despite its importance, Salbutamol can cause cardiovascular side effects, including palpitation, tachycardia, and ECG changes, and may induce myocardial damage (cardiotoxicity), a critical medical emergency (Mahoney et al., 2005; Marieb & Nicpon, 2003). Studies in pigs have shown Salbutamol's dose-dependent effects on heart rate and blood pressure, with evidence of tachyphylaxis (White et al., 1989). Globally, cardiovascular diseases (CVDs) remain the leading cause of death, with alarming projections for increased mortality by 2030 (Cao et al., 2013; Clarke et al., 2014; Lopez & Murray, 1998; Pakistan Society of Cardiovascular and Thoracic Surgeries, 2008). The limitations and side effects of synthetic drugs necessitate the exploration of novel, safer therapeutic approaches, particularly from natural products.

Medicinal plants are rich sources of bioactive compounds, making them economical, effective, and safe drug alternatives (Prakash & Gupta, 2005; Rao, 2003). Traditional systems document numerous plants with cardioprotective, antioxidant, antibacterial, antihypertensive, and hypocholesterolemic properties, such as *Terminalia arjuna*, *Rauvolfia serpentina*, *Elettaria cardamomum*, and *Crataegus oxyacantha* (Deshmukh et al., 2012; Nema et al., 2012; Rasmussen, 2011; Verma et al., 2009). Herbal mixtures often exhibit synergistic effects and fewer side effects than single herbs (Baber et al., 2012; Prince et al., 2008; Rajalakshmy et al., 2011; Tende et al., 2015). For instance, ginger possesses antioxidant and antilipidemic properties with minimal side effects (Badreldin et al., 2007). Phytochemicals from plants serve as a foundation for novel cardiovascular drugs (Arora et al., 2003; Breslow, 1997; Prusti et al., 2008; Torssell, 1997). Hyperlipidemia, a major CVD risk factor, is characterized by elevated cholesterol and triglycerides, and reduced HDL (Grundy, 1986; Saravanan et al., 2003; Smith, 1993). While synthetic hypolipidemic drugs have side effects (Brown, 1996; Speight & Avery, 1987), plant extracts like *Sapidus emerginatus* and *Spinacia oleracea* have demonstrated antihyperlipidemic activity, often attributed to compounds like saponins and flavonoids, which can modulate lipid metabolism and enhance HDL levels (Krista & Peter,

2006; Srikant & Devi, 2009; Thamolwan & Somkiat, 2008). Plant-derived compounds, particularly flavonoids, also offer protection against arterial blockages and heart attacks by reducing cholesterol, possessing vasodilatory potential, and inhibiting platelet aggregation (Arai et al., 2000; Chisaka et al., 1988; Chun et al., 2007; Duarte et al., 1993; Green & Harari, 1992; Hertog et al., 1993; Imai & Nakachi, 1995; Kono et al., 1992; Narayana et al., 2001; Stensvold et al., 1992).

In the present study, *Alpinia zerumbet*, *Citrullus colocynthis*, *Cuscuta reflexa*, *Ranunculus bulbosus*, *Solanum xanthocarpum*, and *Viola tricolor* were selected based on their established ethnomedicinal and pharmacological importance. These plants are recognized in traditional medicine systems for their diverse therapeutic properties, which include uses for inflammation, respiratory ailments, gastrointestinal issues, and general well-being, often contributing indirectly to cardiovascular health through systemic effects (Prakash & Gupta, 2005; Rao, 2003; Rajalakshmy et al., 2011). Their selection aligns with the growing global interest in natural products for various health conditions and formed the foundation for evaluating their specific potential as safer, efficacious, and viable alternatives to synthetic drugs for controlling cardiovascular disorders, thereby providing affordable medicine and supporting Pakistan's pharmaceutical industry and socio-economic development.

2 Methods

The present study was designed to evaluate the cardioprotective potential of certain plants indigenous to Punjab, Pakistan, against myocardial injury induced by the drug salbutamol. Salbutamol-administered rabbits were utilized, and the observed elevated levels of cardiac marker enzymes (CKMB, LDH, AST, ALT) and decreased levels of antioxidant enzymes (SOD, CAT) served as indicators of injury and response.

2.1 Selection and Collection of Plants

Plants for this study were selected based on the following criteria: their common use as folk medicine in Pakistan (indicating traditional medicinal properties), easy availability, ease of cultivation, and economic viability.

The selected plant species, *Alpinia zerumbet*, *Solanum xanthocarpum*, *Ranunculus bulbosus*, and *Cuscuta reflexa*, were collected from various locations within the Lahore region. *Citrullus colocynthis* and *Viola tricolor* were procured from local markets in Lahore. All collected plant materials were formally identified by Prof. Dr. Zaheer ud Din Khan (Government College University, Lahore), and their respective voucher specimens were deposited in the Prem Madan Herbarium (Lahore College for Women University, Lahore). Following collection, all plants were dried under shade and subsequently ground into a fine powder for further processing.

Table 1
Selection of Plants

Sr.No	Botanical Name	Common Name	Family	Part Used	Voucher No
1.	*A. zerumbet*	Shell Ginger	Zingiberaceae	Leaves	0688
2.	*C. colocynthis*	Dhooma	Cucurbitaceae	Fruit	0234
3.	*C. reflexa*	Dodder	Convolvulaceae	Stem	0119
4.	*R. bulbosus*	Butter cup	Ranunculaceae	Leaves	0188
5.	*V. tricolor.*	Banafsha	Violaceae	Aerial part of Plant	0203
6	*S. xanthocarpum*	Kandiarri	Solanaceae	Leaves and Stem	0197

2.2 Preparation of Plant Extract

Upon arrival at the laboratory, all collected plant materials were thoroughly washed with cold water to remove dirt and debris. The fresh weight of all plants was recorded. *Viola tricolor* and *Citrullus colocynthis* were directly ground after washing. The plant material was dried thoroughly in the shade, then cut into small pieces, and subsequently ground using an electrical grinder to obtain a fine powder form. These powdered plant materials were then stored in separate labeled bottles for subsequent use. The crude aqueous extract of the powdered plant material was prepared following standard methods (Onyeyili et al., 2001). Briefly, twenty grams (20 g) of the powdered plant material were mixed with 100 mL of distilled water in a 1L beaker and boiled for 1.5 hours. After boiling, the mixture was allowed to cool to room temperature and then filtered using Whatman filter paper. The resulting extract was stored in a freeze drier at -40°C until use.

2.3 *In vivo* studies

2.3.1 Animals Model

Rabbits (*Oryctolagus cuniculus*) were preferred for evaluating cardiac and antilipidemic activity. Rabbits of both sexes, aged 5–6 weeks and weighing between 700–1000 gm, were used as experimental animals. Rabbits were selected based on their adaptability to a wide variety of conditions, ease of handling, and their amenable behavior as domestic animals. Rabbits were purchased from Tollenton Market, Lahore, and housed in the designated "Animal House" facility at LCWU, Lahore. Earthen water pots and salt pieces were provided within the animal house. The facility, measuring twenty-two feet in length and twelve feet in width, was maintained under standard conditions concerning temperature, relative humidity, and day/night cycles. The rabbits were fed at regular intervals with green leafy vegetables, carrots, and cucumbers. Tap water was provided ad libitum in earthen pots. Wooden dust was spread across the entire floor of the Animal House to absorb animal feces and moisture. The wooden dust was changed every 24 hours to maintain hygiene. The floor of the house was cemented to prevent digging. The rabbits were housed in the animal facility for three days prior to experimentation. This acclimatization period ensured the animals were stable and free of stress within the environment of the animal house before the

commencement of the study. Detailed information regarding each rabbit group, including age, gender, and initial body weight (in grams), was meticulously recorded at the beginning of the experiment. These records were utilized for monitoring the outcome of the treatments on the rabbits and for the final interpretation of the results.

2.4 Preparation of Doses

Salbutamol (60 mg/kg) was utilized as the drug to induce myocardial injury in rabbits. Salbutamol administration was known to elevate levels of cardiac marker enzymes such as LDH, indicating myocardial damage (White et al., 1989). Inderoll (propranolol hydrochloride) is a beta-blocker that affects heart function and circulation. It was used as a standard synthetic drug to treat tremors, angina (chest pain), hypertension (high blood pressure), heart rhythm disorders, and to prevent heart attack. In this study, the recommended daily dosage for myocardial infarction prevention, typically ranging from 180 mg to 240 mg per day in divided doses (e.g., 40 mg t.i.d. titrated up), was considered for the standard curative group. A stock solution was prepared by dissolving 0.1 g of the plant extract in distilled water. Serial dilutions of 50 mg/mL and 100 mg/mL were prepared from the stock solution to achieve the desired concentrations for administration.

2.5 Experimental Protocol

All rabbits were randomly divided into six distinct experimental groups, with each group comprising three rabbits. **Group I (Normal Controls)**: Rabbits in this group received only a standard diet throughout the study. **Group II (Salbutamol Control Group)**: Salbutamol was orally administered to the rabbits at a dose of 60 mg/kg body weight for two consecutive days to induce oxidative stress and myocardial cell necrosis. **Group III (Baseline Group)**: Plant extract (100 mg/kg body weight) was orally administered to rabbits in this group once daily for three weeks. This group aimed to assess the baseline effects of the plant extracts without prior cardiotoxicity induction. **Group IV (Preventive Group)**: Rabbits in this group were pretreated with plant extracts at 100 mg/kg body weight, orally administered once daily for three weeks. Following the pretreatment, these rabbits were then treated with two consecutive doses of Salbutamol (60 mg/kg) to evaluate the preventive potential of the plant extracts. **Group V (Standard Curative Group - Synthetic Drug)**: Rabbits were initially treated orally with Salbutamol (60 mg/kg) for two days to induce cardiotoxicity. Subsequently, these cardiotoxic rabbits were treated with a standard drug, Propranolol hydrochloride (Inderal), once daily for five days. Blood samples were collected daily from this group to monitor the therapeutic effect. **Group VI (Curative Groups)**: Rabbits were treated with Salbutamol (60 mg/kg) for two days to induce cardiotoxicity. Following this, these cardiotoxic rabbits were treated with 200 mg/kg body weight of the respective plant samples twice daily for five days. Blood samples were collected daily from these groups to check the post-treatment effect of the plant extracts. The Curative Groups (Group VI) were further subdivided based on the specific plant samples and their concentrations: **Group A**: A-1: Salbutamol-induced rabbits treated with 25 µL/mL aqueous extract of *C. colocynthis*. A-2: Salbutamol-induced rabbits treated with 100 µL/mL aqueous extract of *C. colocynthis*. **Group B**: B-1: Salbutamol-induced rabbits treated with 25 µL/mL aqueous extract of *A. zerumbet*. B-2: Salbutamol-induced rabbits treated with 100 µL/mL aqueous extract of *A. zerumbet*. **Group C**: C-1: Salbutamol-induced rabbits treated with 25 µL/mL aqueous extract of *V. tricolor*. C-2: Salbutamol-induced rabbits

treated with 100 µL/mL aqueous extract of *V. tricolor*. **Group D**: D-1: Salbutamol-induced rabbits treated with 25 µL/mL aqueous extract of *C. reflexa*. D-2: Salbutamol-induced rabbits treated with 100 µL/mL aqueous extract of *C. reflexa*. **Group E**: E-1: Salbutamol-induced rabbits treated with 25 µL/mL aqueous extract of *S. xanthocarpum*. E-2: Salbutamol-induced rabbits treated with 100 µL/mL aqueous extract of *S. xanthocarpum*. **Group F**: F-1: Salbutamol-induced rabbits treated with 25 µL/mL aqueous extract of *R. bulbosus*. F-2: Salbutamol-induced rabbits treated with 100 µL/mL aqueous extract of *R. bulbosus*.

2.6 Biochemical Assessment

2.6.1 Estimation of Cardiac Biomarkers

Blood samples were systematically collected from the jugular vein of all rabbits. Serum was then separated and used for the analysis of various cardiac biomarkers, including lactate dehydrogenase (LDH), creatine kinase-MB fraction (CKMB), aspartate transaminase (AST), and alanine transaminase (ALT). In addition, lipid parameters such as total cholesterol, triglyceride, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were also estimated. All these analyses were performed using commercially available diagnostic kits and a chemistry analyzer (Semar S 1000-elite).

2.6.2 Estimation of Antioxidant Enzymes in Heart Tissues

After the experimental period, the animals were humanely sacrificed, and heart tissues were carefully separated and washed with isotonic saline. The heart tissues were then homogenized in 10% ice-cold phosphate buffer (pH = 7). The resulting mixture was centrifuged, and the supernatant was collected for the analysis of antioxidant enzymes, specifically Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx), following the established method by Hameed

2.7 Statistical Analysis

The results for all experimental assessments were expressed as the mean ± standard error of the mean (SEM) for the three rabbits in each group. The statistical analysis was performed using Minitab 16.0 software. Analysis of variance (ANOVA) was conducted using a one-way ANOVA model, followed by Tukey's post-hoc comparison test to identify specific differences between groups. A P value of < 0.05 was considered statistically significant.

3 Results

Table 2
Fresh Weight, Dry Weight, Percentage of Moisture Content and Extract/kg of Selected Medicinal Plants

Sr.No	Name of Plant	Fresh. wt. (kg)	Dry. wt. (gm)	%Moisture Content	Extract (g)
1.	*C. colocynthis*	01	1000	40.7	189.4
2.	*A. zerumbet*	01	873.3	67.3	398
3.	*V. tricolor.*	01	1000	57.4	397.2
4.	*C. reflexa*	01	670.1	42.9	513
5.	*S. xanthocarpum*	01	820.2	77.9	176.1
6.	*R. bulbosus*	01	752.2	64.7	328.7

This section presents the findings from the experimental evaluation of the cardioprotective and antilipidemic effects of various plant extracts against salbutamol-induced myocardial injury in rabbits. The results are systematically categorized into cardiac biomarker levels, lipid profiles, and total blood count parameters. All data are presented as mean ± Standard Error of the Mean (SEM), and statistical significance was set at $P < 0.05$.

Table 3
Effects of Salbutamol Induction, Healthy Control, and Inderal Treatment on Biochemical Parameters

Parameter	Control	Salbutamol	Inderal	Units
Cardiac Biomarkers				
LDH	250.2	283.0	197.7	U/L
CK-MB	359.4	383.0	201.8	U/L
AST	117.0	148.0	57.9	U/L
ALT	43.0	49.0	33.3	U/L
AP	2.5	3.8	1.6	U/L
ALP	19.4	24.3	11.0	U/L
CK-CPK	371.1	400.8	312.8	U/L
Lipids				
Cholesterol	16.4	100.0	47.7	mg/dL
Triglyceride	1.63	1.80	1.59	mmol/L
Total Blood Count				
WBC	11.7	3.3	8.9	g/dL
RBC	6.9	2.4	5.2	g/dL
Hb	13.3	4.4	13.2	g/dL

Table 3 presents the levels of cardiac biomarkers, lipid profile, and total blood count parameters in healthy control rabbits, salbutamol-induced cardiotoxic rabbits, and salbutamol-induced rabbits treated with the standard synthetic drug, Inderal. Salbutamol administration significantly elevated cardiac marker enzymes and adversely affected lipid and hematological parameters, indicative of successful myocardial injury induction. Inderal treatment effectively mitigated these changes, restoring parameters towards normal ranges.

Table 4
Baseline Effects of Plant Extracts (Without Salbutamol Induction) on Biochemical Parameters

Parameter	*C. colocynthis*	*A. zerumbet*	*C. reflexa*	*R. bulbosus*	*V. tricolor.*	*S. xanthocarpum*	Units
Cardiac Biomarkers							
LDH	220.0	203.0	240.0	196.2	197.0	195.1	U/L
CK-MB	230.0	227.7	352.0	250.0	249.2	247.0	U/L
AST	69.0	69.8	113.0	54.2	56.3	58.0	U/L
ALT	27.0	24.0	35.8	17.8	23.0	27.5	U/L
AP	1.8	1.53	2.3	1.6	1.9	1.54	U/L
ALP	15.2	12.7	15.0	7.8	11.0	12.0	U/L
CK-CPK	193.7	225.0	342.3	253.0	256.0	253.3	U/L
Lipids							
Cholesterol	54.3	56.0	60.0	50.1	59.8	58.2	mg/dL
Triglyceride	1.63	1.52	1.61	1.59	1.59	1.30	mmol/L
Total Blood Count							
WBC	9.4	8.0	9.0	9.4	8.7	7.1	g/dL
RBC	5.5	5.8	6.7	5.6	5.5	5.4	g/dL
Hb	12.7	13.9	15.2	13.3	14.8	13.6	g/dL

Table 4 summarizes the biochemical parameters in rabbits treated solely with plant extracts (Baseline Groups), providing insight into the inherent effects of these plants without prior salbutamol induction. Generally, these plant extracts maintained the parameters within or close to the normal physiological ranges.

Table 5
Curative Effects of Plant Extracts (50 µL/mL) on Biochemical Parameters in Salbutamol-Induced Rabbits

Parameter	*C. colocynthis*	*A. zerumbet*	*C. reflexa*	*R. bulbosus*	*V. tricolor.*	*S. xanthocarpum*	Units
Cardiac Biomarkers							
LDH	204.0	198.7	250.8	194.0	194.0	193.7	U/L
CK-MB	228.2	231.0	359.0	248.0	245.8	248.5	U/L
AST	70.1	65.5	117.3	53.7	57.0	55.2	U/L
ALT	24.4	22.7	43.7	15.6	22.8	25.4	U/L
AP	1.6	1.49	2.5	1.9	1.74	1.32	U/L
ALP	13.8	13.0	19.1	13.2	12.1	11.9	U/L
CK-CPK	196.6	229.9	370.5	251.0	252.8	265.0	U/L
Lipids							
Cholesterol	56.0	53.3	77.3	59.8	55.7	55.3	mg/dL
Triglyceride	1.64	1.58	1.69	1.55	1.40	1.50	mmol/L
Total Blood Count							
WBC	8.8	8.5	12.7	9.0	7.8	6.9	g/dL
RBC	5.1	6.1	7.3	5.6	5.6	5.4	g/dL
Hb	13.2	14.0	16.8	12.9	14.1	13.9	g/dL

Table 5 details the curative effects of different plant extracts at a concentration of 50 µL/mL on salbutamol-induced biochemical alterations. Most extracts at this concentration demonstrated a significant amelioration of the adverse effects caused by salbutamol, bringing the levels of cardiac biomarkers, lipids, and blood cell counts closer to the normal ranges.

Table 6
Curative Effects of Plant Extracts (100 µL/mL) on Biochemical Parameters in Salbutamol-Induced Rabbits

Parameter	*C. colocynthis*	*A. zerumbet*	*C. reflexa*	*R. bulbosus*	*V. tricolor.*	*S. xanthocarpum*	Units
Cardiac Biomarkers							
LDH	201.0	196.0	251.0	195.7	197.0	195.0	U/L
CK-MB	225.5	228.9	359.1	247.7	243.3	250.0	U/L
AST	74.3	63.3	118.5	54.0	55.2	57.8	U/L
ALT	23.4	25.0	43.0	22.4	25.9	27.9	U/L
AP	1.4	1.57	2.5	2.0	1.76	1.45	U/L
ALP	11.9	12.8	18.7	12.7	11.9	13.7	U/L
CK-CPK	216.0	244.0	370.9	249.0	258.0	264.4	U/L
Lipids							
Cholesterol	54.4	54.0	79.0	61.1	58.2	59.4	mg/dL
Triglyceride	1.51	1.61	1.72	1.58	1.30	1.60	mmol/L
Total Blood Count							
WBC	7.9	9.0	11.3	8.7	7.9	6.7	g/dL
RBC	5.3	5.5	6.7	5.1	5.5	5.5	g/dL
Hb	14.1	13.9	15.2	14.0	12.5	13.5	g/dL

Table 6 presents the curative effects of different plant extracts at a higher concentration of 100 µL/mL on salbutamol-induced biochemical alterations. At this concentration, several plant extracts demonstrated enhanced efficacy in normalizing the measured parameters, often showing results comparable to or better than the standard drug, Inderal, and significantly ameliorating the cardiotoxic and lipid-disturbing effects of salbutamol.

4 Discussion

The escalating global burden of cardiovascular diseases (CVDs) underscores the urgent need for novel and safer therapeutic strategies, particularly in developing countries like Pakistan where access to expensive synthetic drugs can be limited (Cao et al., 2013; Pakistan Society of Cardiovascular and Thoracic Surgeries, 2008). This study investigated the cardioprotective and antilipidemic efficacy of various indigenous plant extracts against salbutamol-induced myocardial injury in rabbits, comparing their

effects with those of the standard synthetic drug, Inderal (propranolol hydrochloride). Our findings provide compelling evidence for the therapeutic potential of these herbal remedies.

4.1 Cardioprotective Effects on Cardiac Biomarkers

The administration of salbutamol at 60 mg/kg body weight for two days successfully induced myocardial injury in rabbits, as evidenced by the significant elevation of cardiac marker enzymes including Lactate Dehydrogenase (LDH), Creatine Kinase MB Fraction (CK-MB), Aspartate Transaminase (AST), Alanine Transaminase (ALT), Acid Phosphatase (AP), Alkaline Phosphatase (ALP), and Creatine Kinase (CK-CPK) in the salbutamol-induced control group compared to the healthy control group. These enzymes are typically released into the bloodstream upon cellular damage, serving as reliable indicators of myocardial necrosis and integrity disruption (Marieb & Nicpon, 2003). This observation aligns with previous research on salbutamol's cardiovascular effects (White et al., 1989).

Treatment with Inderal, a well-known β-blocker, significantly ameliorated these elevated cardiac biomarker levels, restoring them close to the normal physiological ranges. This is consistent with its established cardioprotective mechanisms, primarily through reducing cardiac workload and oxygen demand, and stabilizing myocardial cell membranes (Mahoney et al., 2005).

Crucially, most of the investigated plant extracts demonstrated remarkable cardioprotective effects, significantly reducing the elevated levels of cardiac enzymes in salbutamol-induced rabbits. Specifically, the higher concentration (100 µL/mL) of extracts from *Alpinia zerumbet*, *Ranunculus bulbosus*, *Solanum xanthocarpum*, and *Viola tricolor* showed efficacy comparable to, and in some instances, even superior to that of Inderal in reducing LDH, CK-MB, AST, and ALT levels. For example, LDH levels in the *Alpinia zerumbet* (100 µL/mL) group (196.0 U/L) were notably lower than the salbutamol-induced group (283.0 U/L) and comparable to the Inderal group (197.7 U/L), falling well within the normal range (132-252 U/L). This suggests that these plant extracts possess compounds capable of protecting myocardial cells from salbutamol-induced damage, likely by stabilizing cell membranes or reducing oxidative stress. The beneficial effects observed could be attributed to the rich presence of various phytochemicals such as flavonoids, phenolic compounds, and glycosides, which are known for their antioxidant, anti-inflammatory, and cardioprotective properties (Nema et al., 2012; Rasmussen, 2011; Tende et al., 2015; Verma et al., 2009). Antioxidants are critical in counteracting the reactive oxygen species generated during myocardial injury, thus preventing further cellular damage. In contrast, *Cuscuta reflexa* exhibited less pronounced protective effects on cardiac biomarkers, suggesting a comparatively weaker cardioprotective potential under the experimental conditions.

4.2 Antilipidemic Effects on Lipid Profile

Salbutamol induction also resulted in elevated levels of total cholesterol (100.0 mg/dL) and triglycerides (1.80 mmol/L), indicating the development of hyperlipidemia alongside myocardial injury. Hyperlipidemia is a well-established risk factor for cardiovascular diseases and can exacerbate cardiac damage (Grundy, 1986; Smith, 1993; Saravanan et al., 2003).

Inderal treatment successfully lowered cholesterol levels to 47.7 mg/dL and maintained triglycerides within the normal range (1.59 mmol/L), showcasing its ability to ameliorate lipid disturbances.

Consistent with their cardioprotective actions, the plant extracts also demonstrated significant hypolipidemic activity. The 100 µL/mL concentrations of most plant extracts, particularly *Citrullus colocynthis* (Cholesterol: 54.4 mg/dL; Triglyceride: 1.51 mmol/L), *Alpinia zerumbet* (Cholesterol: 54.0 mg/dL; Triglyceride: 1.61 mmol/L), and *Viola tricolor* (Triglyceride: 1.30 mmol/L), effectively reduced cholesterol and normalized triglyceride levels. These findings are supported by previous studies highlighting the antilipidemic potential of herbal medicines due to compounds like saponins and flavonoids (Srikant & Devi, 2009; Thamolwan & Somkiat, 2008). These phytochemicals may exert their effects by inhibiting cholesterol absorption, interfering with lipid synthesis pathways in the liver, or enhancing cholesterol excretion, as observed in studies on green tea and other plant extracts (Green & Harari, 1992; Imai & Nakachi, 1995). The consistent reduction in lipid parameters suggests that these plants could offer a multi-faceted approach to managing cardiovascular risk factors.

4.3 Effects on Total Blood Count

The salbutamol-induced group exhibited a reduction in White Blood Cell (WBC), Red Blood Cell (RBC), and Hemoglobin (Hb) levels, suggesting a detrimental impact on hematopoietic parameters. While the direct mechanism of salbutamol on these blood parameters is less commonly emphasized in cardiotoxicity studies, severe physiological stress and organ damage can indirectly affect blood cell production and survival.

Both Inderal and the plant extracts demonstrated a remarkable ability to restore these hematological parameters towards their normal physiological ranges. For instance, treatment with *Citrullus colocynthis* (100 µL/mL) increased WBC to 7.9 x g/dL, RBC to 5.3 x g/dL , and Hb to 14.1 g/dL, closely approaching the control values. This restorative effect on blood counts indicates a broader systemic protective or ameliorative action of these extracts, possibly through reducing overall systemic inflammation or supporting bone marrow function compromised during the myocardial injury. This aspect warrants further investigation to elucidate the exact mechanisms.

4.4 Overall Significance and Future Directions

This study provides strong preliminary evidence for the cardioprotective and antilipidemic efficacy of several indigenous plants against salbutamol-induced heart injury in rabbits. The observed amelioration of cardiac enzyme elevations, lipid profile disturbances, and hematological abnormalities highlights their potential as natural alternatives to synthetic drugs. Their easy availability, economic feasibility, and historical use in folk medicine further underscore their importance in providing safer and cheaper medicine, particularly relevant for public health initiatives in Pakistan (Prakash & Gupta, 2005). Supporting research into these natural products can foster the pharmaceutical industry in Pakistan and contribute to socio-economic development.

Despite these promising results, certain limitations of the study must be acknowledged. The sample size of three rabbits per group, while chosen with ethical considerations to minimize animal use, may limit the generalizability and statistical power of the findings. Future studies should aim for larger sample sizes to enhance the robustness of the statistical analysis. Furthermore, while this study demonstrates the efficacy of crude aqueous extracts, the specific phytochemicals responsible for these effects were not isolated and identified.

Future research should focus on:

Isolation and Characterization: Identifying and isolating the active compounds within the most promising plant extracts (e.g., *A. zerumbet*, *R. bulbosus*, *S. xanthocarpum*, *V. tricolor*, *C. colocynthis*) responsible for the observed cardioprotective and antilipidemic activities.

Mechanistic Studies: Conducting in-depth in-vitro and molecular studies to elucidate the precise mechanisms of action of these active compounds at the cellular and subcellular levels.

Dose-Response and Long-Term Studies: Performing comprehensive dose-response studies to establish optimal therapeutic concentrations and conducting longer-term studies to assess chronic effects and potential toxicity.

Clinical Trials: Eventually, translating these promising preclinical findings into human clinical trials to validate their efficacy and safety in a clinical setting.

Comparative Analysis: Further investigations could compare the antioxidant enzyme levels (SOD, CAT, GPx) in heart tissues across all groups, as mentioned in the methodology, to provide a more complete picture of the antioxidant defense.

5 Conclusion

This study successfully demonstrated the salbutamol-induced myocardial injury and dyslipidemia in rabbits, providing a robust experimental model for investigating cardioprotective and antilipidemic agents. Crucially, the research revealed the significant therapeutic potential of several indigenous plant extracts, including *Alpinia zerumbet*, *Ranunculus bulbosus*, *Solanum xanthocarpum*, *Viola tricolor*, and *Citrullus colocynthis*. These extracts effectively ameliorated the elevated cardiac biomarker levels, normalized lipid profiles, and restored hematological parameters towards physiological ranges, exhibiting effects comparable to, and in some cases, even surpassing those of the standard synthetic drug, Inderal.The findings underscore the promising role of these medicinal plants as natural, accessible, and potentially safer alternatives for the management and prevention of cardiovascular diseases. This research provides a scientific foundation for their traditional use and highlights their value in the development of novel, affordable phytotherapeutic interventions, particularly beneficial for healthcare systems in regions like Pakistan. Further investigations into the active compounds and their precise mechanisms of action are warranted to translate these preclinical successes into clinical applications.

Declarations

Authors' Contributions

Ayesha Saqib: Main Researcher

Tahira Mughal: Supervisor

Abdul Rehman Riaz: Co supervisor

Conflict of Interest

The authors 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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Ethics Approval

The animal study protocol was approved by the Institutional Animal Care and Use Committee of LCW Protocol Code FIH-653, Date of Approval 4-April-2024. All procedures involving animals adhered to the relevant guidelines)

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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Figures

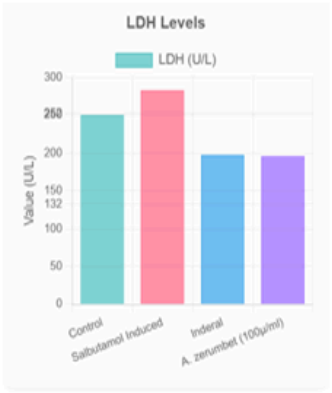

Figure 1a: Effect on Lactate Dehydrogenase (LDH) Levels.

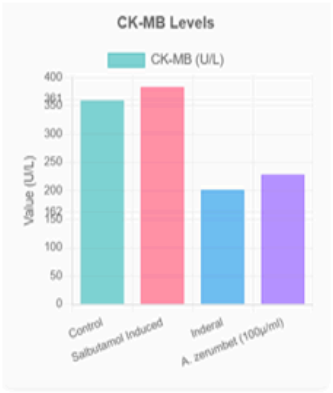

Figure 1b: Effect on Creatine Kinase MB Fraction (CK-MB) Levels.

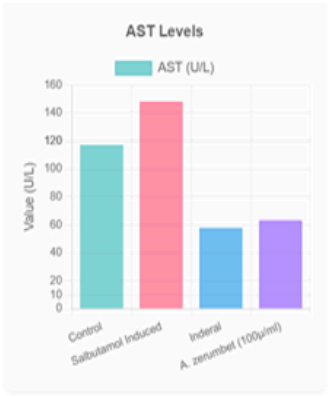

Figure 1c: Effect on Aspartate Transaminase (AST) Levels.

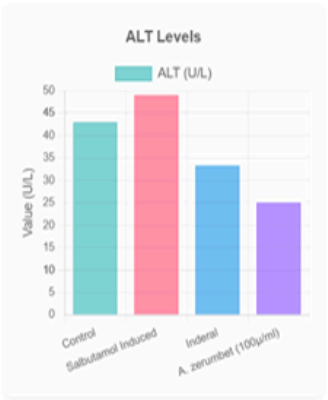

Figure 1d: Effect on Alanine Transaminase (ALT) Levels.

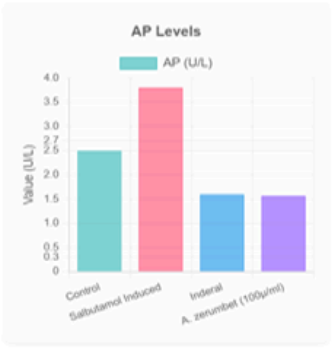

Figure 1e: Effect on Acid Phosphatase (AP) Levels.

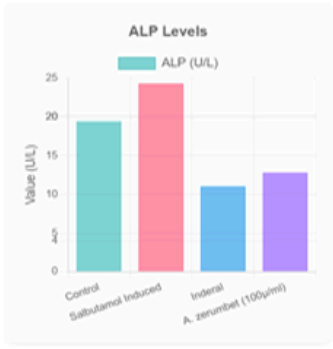

Figure 1f: Effect on Alkaline Phosphatase (ALP) Levels.

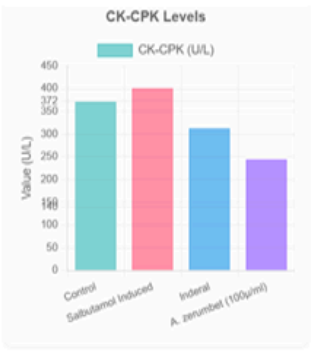

Figure 1g: Effect on Creatine Kinase (CK-CPK) Levels.

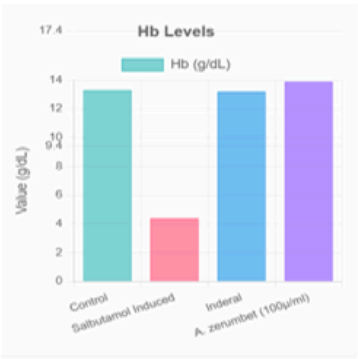

Figure 3c: Effect on Hemoglobin (Hb) Levels.

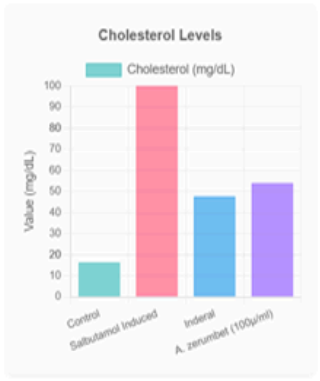

Figure 2a: Effect on Cholesterol Levels.

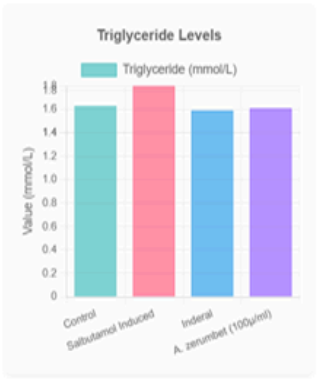

Figure 2b: Effect on Triglyceride Levels.

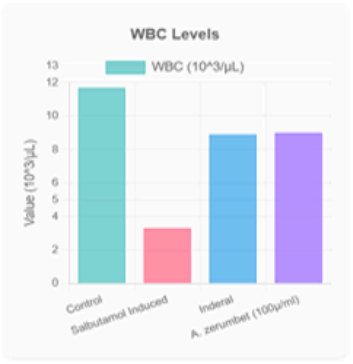

Figure 3a: Effect on White Blood Cell (WBC) Count.

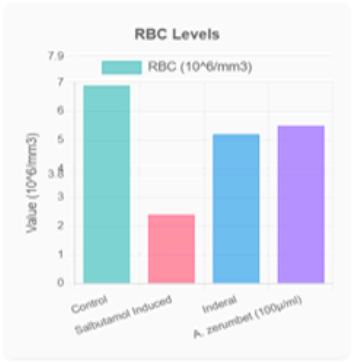

Figure 3b: Effect on Red Blood Cell (RBC) Count.

Figure 1

Teal/Blue-Green: Control Group, Red: Salbutamol Induced Group, Blue: Inderal (Standard Curative) Group, Purple: Plant Extract Group

Supplementary Files

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