



Cardioprotective Activity Of Ashwagandharishta On Isoproterenol Induced Myocardial Infarction

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Abstract

The present study was designed to evaluate the cardio protective activity of Ashwagandharishta-T, Ashwagandharishta-M prepared by traditional and modern methods respectively and its marketed preparation on isoproterenol (ISO) induced myocardial infarction (MI) in albino rats. Wistar albino rats of either sex were randomly divided into 06 groups comprising 06 animals in each group as normal control, ISO control, pretreatment with Inderal*10 (10 mg/kg) per os, pretreatment with Ashwagandharishta-T, M and its marketed preparation at the dose of 2 ml/kg per os per day for 30 days. MI was induced in all the groups except normal control, by administering ISO (85 mg/kg) intraperitoneally, on 29th and 30th day. On 31st day, level of serum marker enzymes was determined and serum lipid profile was also measured. Then, animals were subsequently sacrificed, hearts were removed, weighed and immediately processed for biochemical studies. Pretreatment with Inderal*10 and all the test preparations of Ashwagandharishta significantly prevented the ISO-induced adverse changes in the levels of serum marker enzymes as creatine kinase (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and also improved serum lipid profile. All the test formulations pretreated groups showed significant increase in glutathione (GSH) content and significantly reduced malonyldialdehyde (MDA). Thus, experimental finding suggests that the cardio protective activity of Ashwagandharishta-T, M and its marketed preparation may be due to an augmentation of endogenous antioxidants as GSH and inhibition of lipid peroxidation of cardiac membrane.

Key words: Myocardial infarction, Isoproterenol, Ashwagandharishta

Introduction

Myocardial infarction (MI) is the most lethal manifestation of cardiovascular diseases and has been the object of intense investigation by clinicians and basic medical Scientists. It is the necrotic condition that occurs due to imbalance between coronary blood supply and demand¹. Currently, there is increasing realization that herbs can influence the course of heart diseases and its treatment by providing an integrated structure of nutritional substances which aid in restoring and maintaining balanced body systems²⁻³. Use of herbs for the treatment of cardiovascular diseases in Ayurveda, Chinese and Unani systems of medicine has given a new lead to understand the pathophysiology of these diseases. Therefore, it is rational to use the formulations which have been prepared by using natural resources for identifying and selecting inexpensive and safer approaches for the management of cardiovascular diseases along with the current therapy.

Ashwagandharishta is a polyherbal hydro alcoholic preparation and is used as rasayana. Rasayanas are used to promote health and longevity by increasing defense against disease, arresting the ageing process and revitalizing the body in debilitated conditions⁴. The chief ingredient of Ashwagandharishta is roots of Ashwagandha, *Withania somnifera*, commonly known for its usefulness in the treatment of hypercholesterolemia, arthritis in combination with other drugs, is also credited to be hypoglycemic and diuretic⁵. The pharmacological effect of the roots of *Withania somnifera* is attributed to withanolides, a group of steroidal lactones⁶.

Besides *Withania* roots, the other ingredients of Ashwagandharishta as arjuna (bark of *Terminalia arjuna*), liquorice (roots of *Glycyrrhiza glabra*), majith (roots of *Rubia cordifolia*), rasna (roots of *Alpinia chinensis*), taj (inner bark of *Cinnamomum zeylanicum*), nagarmotha (rhizomes of *Cyperus rotundus*), haritaki (fruits of *Terminalia chebula*), turmeric (rhizomes of *Curcuma longa*), nagakesara (stamens of *Mesua ferrea*) etc. contain a rich quantity of

polyphenolic compounds and flavonoids and possess significant antioxidant activity⁷⁻⁸. Therefore, we undertook the present investigation to evaluate the cardio protective effect of Ashwagandharishta-T and Ashwagandharishta-M prepared by traditional and modern methods respectively on isoproterenol (ISO) induced myocardial infarction (MI) in albino rats.

Material and methods

Preparation of Ashwagandharishta-T

This was prepared by the method as given in the Ayurvedic Formulary of India⁴. The ingredients of Ashwagandharishta were procured from local market, Jamnagar. Identification of all the individual plant material was done as per Ayurvedic Pharmacopoeia of India. Authentication of all these ingredients was done by Dr. G D Bagchi, Scientist, Department of Taxonomy and Pharmacognosy, Central Institute of Medicinal and Aromatic Plants, Lucknow. Prepared herbarium has been deposited in the CIMAP for future reference.

According to this method, coarsely powdered ashwagandha roots (*Withania somnifera*) with prescribed ingredients were placed in polished vessel of brass along with prescribed quantity of water (24.576 l), and allowed to steep. After 12 h of steeping, this material was warmed at medium flame until the water for decoction reduced to one eighths of the prescribed quantity (3.072 l), then the heating was stopped and it was filtered in cleaned vessel and after that honey was added. Then, dhataki flowers (*Woodfordia floribunda*) and prakshepa dravyas as sonth, marich, pippali, tvak, tejpatra, priyangu and nagakesara were added and this sweet filtered material was placed for fermentation in incubator for fifteen days at 33°C±1°C. After 15 days, completion of fermentation was confirmed by standard tests⁹. The fermented preparation was filtered with cotton cloth and kept in cleaned covered vessel for further next seven days. Then, the preparation was poured in amber colored glass bottles, packed and properly labeled.

Preparation of Ashwagandharishta-M

Method of preparation was same as followed with Ashwagandharishta-T only dhataki flowers were replaced with yeast for inducing fermentation¹⁰.

Animals

Adult wistar albino rats, weighing between 200-220g of either sex were acclimatized to normal environmental conditions in the animal house for one week. The animals were housed in standard polypropylene cages and maintained under controlled room temperature ($22^{\circ}\text{C}\pm 2^{\circ}\text{C}$) and humidity ($55\pm 5\%$) with 12:12 hour light and dark cycle. All the animals were given a standard chow diet (Hindustan Lever Limited), and water *ad libitum*. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India were followed and prior permission was granted from the Institutional Animal Ethics Committee (CPCSEA No. 07/09).

Experimental procedure

The cardio protective effect of Ashwagandharishta-T, Ashwagandharishta-M and marketed Ashwagandharishta was determined on ISO-induced MI in albino rats¹¹. All the animals were randomly divided into six groups comprising six animals in each group. Animals of normal control and positive control group received normal saline as vehicle and positive control animals received ISO (85 mg/kg) intraperitoneally (i.p.). Remaining groups were pretreated with Inderal*10 (Piramal Healthcare Limited, Baddi, India) which contains propranolol hydrochloride 10 mg at the dose of 10 mg/kg per os per day¹² and with Ashwagandharishta-T, Ashwagandharishta-M and marketed Ashwagandharishta at the dose of 2 ml/kg per os per day for thirty days to all the ISO treated animals. MI was induced in all the groups except normal control by administering ISO (85 mg/kg)

intraperitoneally on 29th and 30th day, at an interval of 24 h. At the end of the experimental period, i.e. 24 h after the last injection of ISO, on 31st day, the blood samples were withdrawn by retro orbital bleeding under mild ether anaesthesia and were centrifuged at 2000 rpm for 10 minutes for the separation of serum. The animals were subsequently sacrificed with an over dose of ether anaesthesia, hearts were removed, weighed and immediately processed for biochemical studies. The ratio of heart weight to body weight (mg/g) was also measured.

Biochemical analysis of serum

The separated serum was analysed for various serum marker enzymes as lactate dehydrogenase¹³, creatine kinase¹⁴, alanine aminotransferase and aspartate aminotransferase¹⁵. Serum was also assessed for lipid profile as serum cholesterol¹⁶, serum HDL and LDL¹⁷ and triglycerides¹⁸. Span and Erba diagnostic kits were used for the measurement of all these serum marker enzymes.

Biochemical analysis of myocardial tissue

A 10% homogenate of myocardial tissue was prepared in 50 mM phosphate buffer of pH 7.4. This homogenate was centrifuged at 2000 rpm for 10 min and an aliquot of the supernatant was used for the estimation of malonyldialdehyde¹⁹ and glutathione²⁰.

Statistical analysis

The results are expressed as mean \pm SEM. Statistical analysis of data among the various groups was performed by using one way analysis of variance (ANOVA) followed by Tukey's test using Graph Pad Prism software of statistics.

Results

The effects of pretreatment of

Ashwagandharishta-T, Ashwagandharishta-M and its marketed preparation on serum lactate dehydrogenase (LDH), creatine kinase (CK-MB), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in ISO-induced MI in albino rats have been shown in **Table 1**. Results showed that in ISO-control group significant ($P < 0.001$) increase was observed in the level of serum marker enzymes as serum LDH, CK-MB, AST and ALT as compared to normal control group. Pretreatment with Ashwagandharishta-T, M at the dose of 2 ml/kg orally for thirty days significantly ($P < 0.001$) reduced serum LDH, CK-MB, AST and ALT in ISO-induced MI in albino rats as compared to ISO-control group. Pre-treatment with marketed Ashwagandharishta also showed similar effects on serum LDH, CK-MB, AST and ALT nearby same as produced by Ashwagandharishta-T and M in ISO-induced MI in albino rats.

Pretreatment with all the test preparations of Ashwagandharishta significantly improved serum lipid profile in ISO-induced MI in albino rats as compared to ISO-control group as shown in **Table 2**.

Pretreatment with Ashwagandharishta-T, M and its marketed preparation significantly ($P < 0.001$) reduced serum cholesterol, triglycerides (TG), serum low density lipoproteins (LDL) while showed significant ($P < 0.001$) increase in serum HDL as compared to ISO-control group.

Ashwagandharishta-T, M and its marketed preparation pretreated groups significantly ($P < 0.001$) reduced the increased heart weight and heart to body weight ratio as compared to ISO-control group as shown in **Table 3**.

It was observed that ISO-control group showed significant ($P < 0.001$) rise in the basal level of myocardial lipid per-oxidation marker malonyldialdehyde (MDA) in myocardial tissue and caused significant ($P < 0.001$) decrease in glutathione (GSH) content in cardiac tissue. Pretreatment with Ashwagandharishta-T, M and its marketed preparation significantly ($P < 0.001$) reduced MDA content and showed significant ($P < 0.001$) rise in GSH content in cardiac tissue as compared to ISO-control group as shown in **Table 4**.

| Group | Dose (ml/kg/day p.o.) | LDH (U/L) | CK-MB (U/L) | ALT (IU/L) | AST (IU/L) |
|----------------|-----------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| Normal control | 2ml normal saline | 192.51± 2.48 | 107.35± 1.96 | 64.21± 4.72 | 118.54± 4.61 |
| ISO control | 2 ml normal saline | 506.12± 6.25 ^a | 278.50± 3.24 ^a | 176.15± 6.48 ^a | 304.48± 3.82 ^a |
| Inderal*10+ISO | 10 mg | 212.42± 2.92 ^b | 123.56± 4.28 ^b | 85.42± 3.17 ^b | 167.24± 4.26 ^b |
| Ashw-T+ISO | 2ml | 246.14± 2.64 ^b | 138.47± 3.26 ^b | 97.44± 1.48 ^b | 181.56± 4.28 ^b |
| Ashw-M+ISO | 2ml | 249.42± 3.17 ^b | 142.15± 2.73 ^b | 100.25± 3.92 ^b | 184.82± 2.48 ^b |
| Marketed Ashw | 2ml | 248.21± 4.62 ^b | 140.54± 1.97 ^b | 98.64± 4.18 ^b | 183.15± 3.78 ^b |

Table 1. Effect of Ashwagandharishta-T, M and marketed Ashwagandharishta on serum LDH, CK-MB, ALT and AST in ISO-induced MI in albino rats

All values are expressed as mean ± standard error mean (n = 6).

a $P < 0.001$ significant as compared to normal control

b $P < 0.001$ significant as compared to ISO control

ISO, isoproterenol; MI, myocardial infarction; Ashw, Ashwagandharishta

| Group | Dose (ml/kg p.o./day) | Serum cholesterol (mg/dl) | Serum HDL (mg/dl) | Serum LDL (mg/dl) | Serum triglycerides (mg/dl) |
|-----------------|-----------------------|---------------------------|-------------------------|--------------------------|-----------------------------|
| Normal control | 2 ml normal saline | 148.52±5.81 | 53.24±3.72 | 76.92±6.46 | 89.74±4.67 |
| ISO control | 2 ml normal saline | 320.29±6.24 ^a | 29.30±1.72 ^a | 252.41±9.26 ^a | 206.15±5.92 ^a |
| Inderal*10 +ISO | 10 mg | 161.48±12.21 ^b | 50.12±4.26 ^b | 90.25±1.48 ^b | 102.41±2.73 ^b |
| Ashw-T+ISO | 2 ml | 170.52±2.47 ^b | 48.46±4.12 ^b | 99.63±1.84 ^b | 112.15±3.65 ^b |
| Ashw-M+ISO | 2 ml | 173.14±3.76 ^b | 47.98±2.53 ^b | 100.48±3.71 ^b | 123.40±4.14 ^b |
| marketed Ashw | 2 ml | 171.64±1.98 ^b | 48.17±3.81 ^b | 100.54±4.19 ^b | 114.65±2.57 ^b |

Table 2. Effect of Ashwagandharishta-T, M and marketed Ashwagandharishta on serum lipid profile in ISO-induced MI in albino rats

All values are expressed as mean ± standard error mean (n = 6).

a P<0.001 significant as compared to normal control

b P<0.001 significant as compared to ISO control

ISO, isoproterenol; MI, myocardial infarction; Ashw, Ashwagandharishta

| Group | Dose (ml/kg p.o./day) | Heart weight (mg) | Body Weight (g) | | Heart to body weight ratio (mg/g) |
|-------------------|-----------------------|----------------------|------------------------|---------------|-----------------------------------|
| | | | On 1 st Day | After 14 days | |
| Normal control | 2 ml normal saline | 972±46 | 208.6±3.8 | 209.2±2.7 | 4.646±0.42 |
| ISO control | 2 ml normal saline | 1215±37 ^a | 208.2±4.6 | 208.4±2.2 | 5.830±0.38 ^a |
| ISO+Inderal*10 | 10 mg | 994±42 ^b | 208.1±2.4 | 207.9±4.1 | 4.781±0.51 ^b |
| ISO+Ashw-T | 2 ml | 1023±44 ^b | 210.7±1.8 | 210.5±3.7 | 4.859±0.26 ^b |
| ISO+Ashw-M | 2 ml | 1025±53 ^b | 210.4±5.2 | 210.2±4.6 | 4.876±0.34 ^{b*} |
| ISO+marketed Ashw | 2 ml | 1026±48 ^b | 210.6±3.8 | 210.5±2.8 | 4.874±0.38 ^{b*} |

Table 3. Effect of Ashwagandharishta-T, M and marketed Ashwagandharishta on heart weight and heart to body weight ratio in ISO-induced MI in albino rats

All values are expressed as mean ± standard error mean (n = 6).

a P<0.001 significant as compared to normal control

b P<0.001; b*P<0.01 significant as compared to ISO control

ISO, isoproterenol; MI, myocardial infarction; Ashw, Ashwagandharishta

| Group | Dose (ml/kg p.o./Day) | MDA (nmol/g tissue) | GSH (μ mol/g tissue) |
|----------------|-----------------------|--------------------------------|-------------------------------|
| Normal control | 2 ml normal saline | 110.12 \pm 4.28 | 1.48 \pm 0.081 |
| ISO control | 2 ml normal saline | 246.23 \pm 7.43 ^a | 0.89 \pm 0.043 ^a |
| Inderal*10+ISO | 10 mg | 125.27 \pm 3.72 ^b | 1.21 \pm 0.036 ^b |
| Ashw-T+ISO | 2 ml | 140.49 \pm 1.98 ^b | 1.16 \pm 0.039 ^b |
| Ashw-M+ISO | 2 ml | 142.18 \pm 2.47 ^b | 1.15 \pm 0.048 ^b |
| marketed Ashw | 2 ml | 141.54 \pm 4.37 ^b | 1.16 \pm 0.054 ^b |

Table 4. Effect of Ashwagandharishta-T, M and marketed Ashwagandharishta on heart MDA and GSH concentration in ISO-induced MI in albino rats

All values are expressed as mean \pm standard error mean (n = 6).

a P<0.001 significant as compared to normal control

b P<0.001 significant as compared to ISO control

ISO, isoproterenol; MI, myocardial infarction; Ashw, Ashwagandharishta

Discussion

Isoproterenol (ISO), a synthetic catecholamine in higher dose produces cardiotoxic effects on the myocardium. Amongst the various mechanisms proposed to explain ISO-induced cardiac damage, generation of highly cytotoxic free radicals through the auto-oxidation of catecholamines has been implicated as one of the important causative factor²¹. This free radical mediated lipid peroxidation of membrane phospholipids and consequent changes in membrane permeability is the primary target responsible for cardio toxicity induced by ISO.

Studies have shown that oxidative stress results in the reduction of the efficacy of the β -adrenoceptor agonists probably due to reduction in cAMP formation. The reduction in of maximal β -adrenoceptor mediated response might be the result of cytotoxic aldehydes that are produced during the oxidative stress. This β -adrenoceptor hyper stimulation leads to cardio toxicity²². Oxidative stress may also depress the sarcolemmal Ca²⁺ transport and result in the development of intracellular Ca²⁺ overload and ventricular dysfunction²³.

Hence, therapeutic intervention with therapeutic activity may be useful in preventing these deleterious changes.

Changes in serum LDH and CK-MB activities have been considered some of the important biomarkers of MI. A significant increase in serum LDH, CK-MB, AST and ALT was observed in ISO control group. Pre-treatment with Ashwagandharishta-T, Ashwagandharishta-M and marketed Ashwagandharishta in ISO-induced MI in albino rats significantly restored serum LDH, CK-MB, AST and ALT activity as compared to the ISO control group was suggestive of their cardio-protective effect.

In ISO control group significant rise in serum lipid profile was also observed. Pre-treatment with Ashwagandharishta-T, Ashwagandharishta-M and marketed Ashwagandharishta for thirty days significantly reduced serum cholesterol, LDL and TG level while showed significant rise in serum HDL level in ISO-induced MI in albino rats. A rise in LDL may cause deposition of cholesterol in the arteries and aorta and hence it is a direct risk factor for coronary heart disease. LDL carries cholesterol from liver to the peripheral cells and smooth muscles and

cells of the arteries²⁴. HDL promotes the removal of cholesterol from peripheral cells and facilitates its delivery back to the liver. Therefore, increased levels of HDL are desirable²⁵.

In the ISO control group, a significant increase in heart weight and heart weight to body weight ratio was observed which was reversed by Ashwagandharishta-T, Ashwagandharishta-M and marketed Ashwagandharishta treatment in ISO-induced MI in albino rats. It suggests the cardio-protective property of all these test formulations.

In the current investigation, ISO-induced MI produced oxidative stress as indicated by increased heart lipid peroxides as MDA and decreased heart GSH content. Pre-treatment with Ashwagandharishta-T, Ashwagandharishta-M and marketed Ashwagandharishta significantly reduced heart lipid peroxides level as MDA and showed significant rise in GSH content in ISO-induced MI in albino rats. Thus, all the test formulations as Ashwagandharishta-T, M and marketed Ashwagandharishta maintained membrane integrity as evidenced by decline in cardiac MDA levels.

In summary, the present study strongly suggests that multiple mechanisms may be responsible for the cardio-protective effect of Ashwagandharishta-T, Ashwagandharishta-M and marketed Ashwagandharishta. All these test formulations as Ashwagandharishta-T, Ashwagandharishta-M and marketed Ashwagandharishta produced myocardial adaptive changes (augmentation of endogenous antioxidants as GSH) on chronic administration. In addition, they restored the integrity of the myocardium, subsequent to ISO-induced oxidative stress. Ashwagandharishta mainly contains withanolides and the rich concentration of polyphenolic compounds which possess good antioxidant activity. Thus, the obtained result suggests that presence of self generated alcohol could be beneficial in the faster absorption of polyphenolic compounds present in Ashwagandharishta which might be responsible for showing scavenging of ISO-induced free radicals.

The present study provides scientific basis for the

cardio protective potential of Ashwagandharishta validating their usage in Ayurveda. Considering its safety, efficacy and traditional acceptability, clinical trials should be conducted to support its therapeutic use in ischemic heart diseases.

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