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ORIGINAL ARTICLE

Novel Approach for Manufacturing and Biological Evaluation of Ayurvedic Fermented Cardioprotective Formulation Arjunarishta

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ABSTRACT
The ayurvedic liquid formulation arishta is prepared by fermenting decoction of key herbal drugs. The traditional methods of preparation of this formulation do not have evidence-based set procedures and thus hoist quality and efficacy issues. In this study, an attempt has been made to manufacture ayurvedic fermented cardioprotective formulation arjunarishta by novel approach and explore its biological activity. This manufacturing approach involves the addition of isolated yeast and fermentation with standardized process parameters. The formulation evaluated for in vitro antioxidant capacity and cardioprotective effect on isoproterenol (ISO) induced myocardial ischaemia in rat model. At the end of fermentation, the novel arjunarishta demonstrated production of 7.68% v/v alcohol. The amounts of reducing and non-reducing sugar were found to be 5.92 and 0.19% w/v respectively. The amount of gallic acid increased from 0.143 to 0.296 mg/ml during fermentation. It had exhibited an EC_{50} value of 9.397 µg/ml by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay; 2.17 ± 0.05 mg/ml (12.329 mM) ascorbic acid equivalent by ferric reducing antioxidant power (FRAP) assay. The 3 ml formulation dose could demonstrate significant cardioprotective effect and can counteract oxidative cardiac injury in an animal model. The reduction in the serum biomarker enzyme creatine kinase isoform MB (CK-MB) and lactate dehydrogenase (LDH) have been observed. The novel arjunarishta also controls elevated total cholesterol and low-density lipoprotein (LDL). This method of manufacturing ensures the quality, reproducibility and efficacy of arishta product. Based on defined preparation and proving the efficacy we feel it can be used as the basis of standardized preparation methodology.
KEYWORDS: *Arishta, Arjunarishta, fermentation, cardioprotective, myocardial infarction, traditional medicine.*

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INTRODUCTION

One of the leading causes of death from cardiovascular diseases is myocardial infarction (MI) [1]. Oxidative stress is a well-established etiopathogenic factor of ischaemic heart disease and its consequences. Prolonged ischemia will lead to poor force generation, contracture, arrhythmia, calcium overload, a decrease in tissue pH and release of cytosolic enzymes from cells and cellular necrosis [2]. Cardiac cell protection and the prevention of myocardial necrosis have been therapeutic targets for a long time. Research on the prevention and treatment of cardiovascular diseases is progressive. Other than conventional medical and interventional treatment, several forms of alternative therapies are also suitable for managing cardiovascular diseases [3]. Herbal medicines are highly popular and extensively used substitutes for synthetic drugs due to easy availability, economic value and belief of no side effects. The stem bark of *Terminalia arjuna* (Family: Combretaceae) has been comprehensively used for centuries in ayurvedic medicine as a cardiotonic for treating cardiac disorders [4]. It has excellent hypolipidemic

[5] and cardioprotective activity [6,7]. Saponin glycosides present in *T. arjuna* account for its primary activity in improving cardiac muscle function and pumping effect of the heart, whereas the flavonoids afford antioxidant action and vascular strengthening [8]. Arishta are alcoholic medicaments prepared by the fermentation of herbal decoction. They exhibit better keeping quality, enhanced therapeutic properties, improvement in the efficiency of extraction of drug molecules from the herbs and improvement in drug delivery to human body sites [9]. Over the counter oral liquid Arjunarishta (Synonym: Parthadyarishta) is one of the popular traditional fermented cardioprotective formulations consisting *T. arjuna* stem bark. Several ethnopharmacological products have quality issues, which affect their effectiveness. In our opinion, different methodology used currently without standardised yeast strains or mixing other microbial contaminants need to be regularised. This will give the needed effect of medicine. Wine industries are well aware of the importance of fermentation in manufacturing; and they are consistently adopting newer technologies and principles for the quality and safety of products. With due consideration of all these parameters hereby, an attempt has been made to make arishta with easiness by virtue of the modern scientific approach without affecting the ayurvedic potentials of formulation. Indeed, such advancements in traditional fermented medicines will explore new areas for research and definitely bring reliability to the product. The arjunarishta manufactured by a systematic methodology was verified for its *in vitro* antioxidant capacity and cardioprotective effect on isoproterenol (ISO)-induced myocardial ischaemia in rat model. The synthetic -adrenergic agonist ISO is a catecholamine and generates significant stress in the myocardium, causing heart muscle necrosis [10] and, in high dosages, myocardial infarction [11]. Additionally, it produces free radicals and promotes lipid peroxidation, which may be the causal factors of irreversible cardiac membrane damage [12]. Consequently, there was a significant increase in the activity of cardiac injury marker enzymes and the concentration of serum lipids [10]. An approach for detection of cardiac injury involves the measurement of cardiac marker enzymes such as creatine kinase (CK), creatine kinase MB isoform (CK-MB), aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH), and myoglobin [11,13].

MATERIAL AND METHODS

Plant materials such as the bark of *Terminalia arjuna* (Family: Combretaceae), flowers of *Madhuca indica* (Family: Sapotaceae) and *Woodfordia fruticosa* (Family: Lythraceae) were harvested from Western Ghats, India, verified by botanist from BST College, Sangamner, MS, India; fruits of *Vitis vinifera* (Family: Vitaceae) and jaggery procured from local market. Isoproterenol hydrochloride was procured from Sigma-Aldrich, India, and other chemicals were purchased from Loba Chemie, Mumbai, India.

Preparation of Arjunarishta

The formulation composition and raw material standard prescribed in The Ayurvedic Pharmacopoiea of India was adopted for the manufacturing of *Arjunarishta* [14, 15]. Moderately fine powder of dry *T. arjuna* soaked overnight in water along with *Vitis vinifera* dried fruits and *M. indica* flowers. The resultant mixture was concentrated to quarter by boiling and strained through muslin cloth. This decoction was cooled, jaggery dissolved in it and filtered again. Afterwards, the filtrate taken in conical flasks, dried flowers of *W. fruticosa* were added to filtrate and pH adjusted to 4.7. The flasks were closed with a cotton plug and sterilized by moist heat. The suspension of yeast isolated from the flowers of *W. fruticosa* was aseptically transferred in sterile flasks, sealed to make airtight and kept at 330C for a month. Finally, filtered liquid was collected in an amber bottle, clarified and tested [16].

Evaluation of Arjunarishta

The prepared *Arjunarishta* was tested for phenolic content [17,18], total sugar [19], reducing sugar [20,21], amount of alcohol and gallic acid [16].

Cardioprotective Effect of Novel Arjunarishta

Isoproternol (ISO) induced myocardial infarction in rats was used as an animal model to study cardioprotective effect of novel *Arjunarishta*. The experiments on animal models were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by Institutional Animal Ethics Committee (IAEC) Protocol number: 1153/PO/ac/08/007/CPCSEA. The albino Sprague-Dawley rats in a good health weighing between 150 and 170 g were used for the experiment. All animals were maintained in plastic cages having husks lining at 25 ± 20C with 12 hours cycles of light and darkness. The rats were fed with standard rat chow (Nutrivet Life Sciences, Pune, India) and purified water *ad libitum*. After acclimatization, rats were distributed into eight groups with six animals in each and named as group I normal control rats, group II to IV rats treated with *Arjunarishta*, group V control rats with ISO, group VI to VIII *Arjunarishta* pretreated rats with ISO. According to the Ayurvedic Pharmacopeia of India, the

human dose of arjunarishta is 15 to 30 ml twice daily. The animal dose was calculated as per dose translation guidelines of the FDA and Reagan-Shaw et al.[22], assuming 60 Kg adult human body weight and *Km* factor 37 and 6 for humans and rat, respectively. The maximum dose of *Arjunarishta* (30 ml) was considered for the calculation. Animals from group II-IV as well as group VI- VIII were administered with 3, 2 and 1 ml/kg body weight of novel *Arjunarishta*, respectively, for 14 days. The specified formulation dose was diluted with water and orally administered twice daily using an intragastric tube. No medicine treatment was given to the rats in Groups I and V. All animals had free access to standard feed pellets and purified water *ad libitum*. At the end of the experimental period for the last 2 consecutive days, that is on the 13th and 14th day, animals of Group V-VIII treated with ISO (85 mg/kg, once a day, subcutaneously) to induce myocardial infarction [23]. Animals from all groups were starved on the final day, blood was obtained the next morning, serum was made, and several biochemical parameters were estimated using this serum. Activities of marker enzymes Creatine phosphokinase (CK-MB), Lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as serum lipids, total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL) were measured in the serum using commercial kits purchased from Coral Clinical Systems (Verna, Goa, India) on a Coralab 3000 biochemical analyzer. Then the amount of very low-density lipoprotein cholesterol (VLDL) and low-density lipoprotein cholesterol (LDL) were calculated using following formulae [1,23].

VLDL = TG/5

LDL = TC – (HDL + VLDL)

Antioxidant Activity

The antioxidant potential of *Arjunarishta* formulation was determined by the free radical scavenging assay and ferric reducing antioxidant power (FRAP) assay.

Free radical scavenging DPPH assay

The free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay process given by Rahman et al. and Tunalier et al. modified to determine antioxidant capacity[24, 25]. The *Arjunarishta* formulation was filtered through 0.45 μm syringe filter, weighed and suitably diluted with methanol to get 3.9, 7.8, 15.6, 31.2, 62.5, 125 and 250 μg/ml concentration. A 2 ml aliquot of each dilution was mixed with 2 ml of 0.004% methanolic DPPH solution and allowed to stand for 30 min at room temperature. The absorbance of resultant reaction mixture recorded at 517 nm. Gallic acid and ascorbic acid were used as standards for positive control [24,26]. Dilutions of standard drugs prepared and processed similarly as like *Arjunarishta* solutions. The negative control was prepared without formulation or standard; instead of sample only methanol was used. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity [27]. The experiment was performed in duplicate and percentage of scavenging of DPPH was calculated using following formula.

DPPH scavenging effect (%) = $[(A_0 - A_1)/ A_0] \times 100$

where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample.

Then the % DPPH scavenging effect was plotted against respective concentrations. Ascorbic acid was used as a positive control [24]. The amount of arishta at which the absorbance decreases to half its initial value was considered as its EC_{50}.

Ferric reducing antioxidant power (FRAP) assay

Process described by Huang et al., [28] was used to determine ferric reducing antioxidant power of arishta. The arishta formulation was filtered through 0.45 μm syringe filter; the filtrate was weighed and suitably diluted with 0.2M phosphate buffer (pH 6.6) to get 3.9, 7.8, 15.6, 31.2, 62.5, 125 and 250 μg/ml solutions. A 2.5 ml aliquot of each dilution was mixed with 2.5 ml potassium ferricyanide (1% w/v) solution and incubated at 500C for 20 min. Subsequently, 2.5 ml trichloroacetic acid (1% w/v) solution was added to the mixture and centrifuged at 3500 rpm for 10 min. Then the 2.5 ml supernatant liquid was mixed with equal volume of distilled water and 0.5 ml ferric chloride (0.1 %w/v) solution. Finally absorbance was recorded at 700 nm. Gallic acid and ascorbic acid were also used as standard for FRAP assay. Their dilutions were prepared in 0.2M phosphate buffer (pH 6.6) and processed similarly. Greater absorbance of the reaction mixture indicated higher reducing activity. An ascorbic acid equivalent value of arishta was calculated for comparative study.

RESULTS AND DISCUSSION

The arjunarishta is a classical ayurvedic formulation prepared by fermenting decoction of *T. arjuna* bark. In traditional formulations the inoculum of yeast required for fermentation comes from herbal source usually *W. fruticosa* [9]. The yeast isolated from flowers of *W. fruticosa* has been effective for manufacturing of arishta. Manwar et al. also made an attempt to isolate and characterize yeast from *W.*

fruticosa for production of fermented medicine [29]. Chandra et al., concluded that fermentation induced by commercially available yeast is no substitute for the ethnopharmacological and traditional fermentation prescribed in the traditional Indian system of medicine [30].

Physicochemical Properties

The arjunarishta formulation prepared by novel approach was acidic in nature, brown in colour with aromatic odour and acrid taste. It demonstrated production of 7.68% v/v alcohol. The amounts of reducing and non-reducing sugar were found to be 5.92 and 0.19% w/v respectively. Arishta consist high amount of sugar and nitrogen-rich constituents making it susceptible to microbial attack but production of ethanol improves its stability by preventing microbial spoilage. Self generated alcohol itself acts as preservative. The arishta formulations have infinite shelf life. Alcohol also facilitates extraction of some key bioactive molecules from crude herbal drug contributing for the activity of formulation. The total phenolic content was found 1.03 mg/ml tannic acid equivalent. Quantity of gallic acid present in arjunarishta was determined by HPTLC. It consist 0.313 mg/ml gallic acid which exhibited peak with retention factor starting at 0.32, maximum at 0.37 and end at 0.41. Gallic acid is the most ubiquitous plant phenolic compound known for antioxidant potential and its concentration can also be used to correlate the total phenolic content of herbs. Antioxidant potentials are important for strengthening cells.

Fermentation Kinetics

Fermentation is a process of production of product by mass culturing of cells. During the fermentation a wide variety of nutrients are required for microbial cell growth. Fermenting material provide such vital components which include carbohydrates, polypeptides, vitamins, etc. Carbohydrate is one of the key constituent fulfilling carbon requirement of the microorganism. In arjunarishta yeast cells utilizes nutrients and forms metabolites like alcohol. These essential nutrients come from jaggery and *M. indica* flowers. Jaggery is composed of 65-85% w/w sucrose, 10-55% w/w reducing sugars, 0.4% w/w proteins, 0.1% w/w fats and 0.6-1% w/w minerals [31]. Dried *M. indica* flowers consist around 41.6 – 47.3% w/w total sugars and 28.1 to 36.3% w/w reducing sugars. Along with sugars it also consist 6 to 7% w/w crude proteins [32,33]. Usually total sugar content was found very high at the beginning of fermentation in arjunarishta manufacturing process but subsequently it decreases with rapid rate. Tremendous fall in carbohydrate level indicate extensive metabolism due to quick growth of yeast population. Reducing sugars may support yeast cell growth during initial adaptive phase while sucrose gets metabolized in later stages and forms alcohol. This is also reflected in alcohol production rate. As the sugar gets metabolized, a steady increase in alcohol detected. Phenolic compounds or polyphenols are the most abundant secondary metabolites present in plants. Tannase present in yeast may catalyse the hydrolysis of tannins and produces gallic acid. Furthermore, concentration of gallic acid increases with incubation time. Progressive increase in gallic acid level from 0.143 to 0.296 mg/ml during fermentation demonstrates extraction of chemicals from herbal drugs in presence of alcohol. There was no any significant change in the pH of formulation. Gradual reduction in pH signifies accumulation of acidic components. Such components might get released as metabolic bi-product along with alcohol.

Antioxidant Activity

The DPPH free radical scavenging assay and FRAP assay were found efficient for determination of antioxidant activity of Arjunarishta. The effect of antioxidants on DPPH radical was thought to be due to their hydrogen donating ability [34]. A DPPH radical can accept an electron or hydrogen radical from antioxidant. Thus, free radical character of DPPH gets neutralized making it a stable diamagnetic molecule [27,35]. The reduced form of the radical is generated accompanied by loss of colour. The novel Arjunarishta had exhibited EC50 value 9.397 µg/ml; which were comparably higher than the reference gallic acid and ascorbic acid solutions (Table 1). The FRAP assay mechanism involves reduction of Fe (III) to the blue-coloured Fe (II) form due to reducing agent (antioxidant) present in the sample [36]. Higher absorbance at 700 nm interpreted as higher reducing capability. Ascorbic acid equivalent values of arishta were also determined on the basis of slope obtained from different concentration levels of product and standard ascorbic acid. Greater ascorbic acid equivalent value represents higher antioxidant power. The novel Arjunarishta had 2.17 ± 0.05 mg/ml (12.329 mM) ascorbic acid equivalent. The standard gallic acid showed ascorbic acid equivalent value 2.46 or 13.977 mM indicates it has 2.46 fold stronger antioxidant potential as compared to ascorbic acid. Self-generated alcohol during fermentation facilitates extraction of phenolic compounds from herbal drugs contributing for enhanced antioxidant activity. Antioxidants help organisms to deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contain one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Reactive oxygen species (ROS) formed in vivo, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damages transient chemical species. These are

continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase [37]. Depressed immunocompetence and poor nutrition may result from excess generation of ROS due to the down regulation of the enzymes [38]. These free radicals are capable of reversibly or irreversibly damaging compounds of all biochemical classes including nucleic acids, amino acids, lipids and proteins. Such damage increases the risk of various diseases like neurodegenerative disorders, ischemic heart diseases, cancer, ageing process, diabetes, etc. [39]. Thus, antioxidants defense systems have coevolved with aerobic metabolism to counteract oxidative damage from ROS [40].

Cardioprotective effect

The ISO induced myocardial ischemia model is widely used *in-vivo* technique for study of beneficial effects of drug on cardiac function. The ISO generates free radicals may attack polyunsaturated fatty acids within membranes causing a chain reaction of lipid peroxidation. The lipid hydroperoxide end products are also harmful which may be responsible for tissue and organ damage [11]. Tissue damage makes cell membranes permeable, resulting in the leakage of enzymes that easily identified in blood. Especially, high amount of CK-MB detected due to cardiac injury. Tissue damage also increases the levels of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) in the blood, which in turn leads to the build-up of harmful deposits in the arteries, thus favouring coronary heart disease [12,23]. The novel Arjunarishta was found to be efficient for regulating cardiac biomarker enzyme in rats preventing the chances of oxidative damage. The rats of group V treated with ISO exhibited myocardial cell injury. This is evident by increase in serum CK-MB, LDH, AST and ALT level (Table 2). There elevated levels of serum biochemical triglycerides (TG), total cholesterol (TC), LDL-cholesterol and VLDL-cholesterol, while, here were decreased concentration of HDL-cholesterol in group V animal both indicating myocardial cell damage (Table 3). The amount of biomarker and biochemical in rats of novel Arjunarishta treatment groups II, III and IV was nearer to the levels of group I normal control rats. Indeed the product had control over oxidative stress which is evidenced by slight decrease in levels of biomarker in comparison with control animal. Whenever, rats of group VI, VII and VIII under treatment with different doses of novel Arjunarishta administered with ISO to induce myocardial injury then cell damage was quite lesser as compared to directly ISO treated group V animal. The CK-MB level of group VI treated with 3 ml formulation dose was $p<0.05$; whereas, group VII treated with 2 ml formulation dose and group VIII treated with 1 ml formulation dose had $p<0.001$ compared with control animal. The LDH level of group VI treated with 3 ml formulation dose had $p<0.05$, group VII treated with 2 ml formulation dose had $p<0.01$ and group VIII treated with 1 ml formulation dose had $p<0.001$ compared with control animal. All the groups pretreated with Arjunarishta and administered with ISO exhibited $p<0.001$ for CK-MB and LDH compared to ISO control group V. This indicates 3 ml dose of Arjunrishta was effective as a cardioprotective agent. But still, it did not reduce ALT level to a greater extent. Noteworthy increases in the amount of total cholesterol and LDL-cholesterol as well as a decrease in HDL-cholesterol have been evident in animals due to ISO-induced myocardial damage. The administration of 3 ml Arjunarishta significantly reduces the chances of injury ($p<0.001$) by restraining changes in total cholesterol and LDL-cholesterol. Thus, Arjunarishta formulations might counteract with oxidative stresses incurred due to ISO. Besides, the increase in duration of arishta treatment may strengthen the myocardial cells and will provide greater efficacy. This is the first attempt to demonstrate the cardioprotective effect of Arjunarishta. The animal studies of the butanolic fraction of arjuna bark ethanolic extract performed on doxorubicin induced cardiotoxicity by Singh et al.,[41] also resulted in significant decrease in serum CK-MB levels and reduction in lipid peroxidation above 1.7 mg/kg extract. Shukla et al.,[7] reported 100 mg/kg of hydroalcoholic extract also significantly reduces CK-MB in isoproterenol induced myocardial infarction. The cardiotonic action is primarily attributable to the aqueous extract of bark rather than its organic extracts [4]. Consequently, hydro-alcoholic extraction of active constituents during fermentative process of Arjunarishta improves the cardioprotective effect.

Table 1: DPPH free radical scavenging activity of novel arjunarishta

Particular	Novel arjunarishta	Gallic acid	Ascorbic acid
Slope	2.030	3.181	1.242
Intercept	30.927	13.580	20.967
Regression	0.786	0.978	0.913
EC_{50} (μg/ml)	9.397	11.448	23.385

Table 2: Effect of novel arjunarishta on serum cardiac biomarker in myocardial infarction rat model.

Particular	CK-MB (IU/L)	LDH (IU/L)	AST (IU/L)	ALT (IU/L)
Group I – Normal control	98.46 ± 3.48	152.15 ± 4.51	37.11 ± 2.10	28.75 ± 2.57
Group II - Rats treated with 3 ml Arjunarishta	94.16 ± 3.43	143.76 ± 6.59	35.83 ± 2.28	26.82 ± 3.39
Group III - Rats treated with 2 ml Arjunarishta	95.74 ± 4.23	146.28 ± 5.12	36.72 ± 2.68	27.17 ± 2.20
Group IV - Rats treated with 1 ml Arjunarishta	97.80 ± 4.87	151.78 ± 4.63	36.83 ± 2.90	28.21 ± 3.24
Group V - Control rats with ISO	190.07 ± 7.95[a]	273.59 ± 9.93[a]	69.94 ± 4.02[a]	51.13 ± 3.49[a]
Group VI - Arjunarishta (3 ml) pretreated rats with ISO	109.21 ± 4.60[c,d]	165.92 ± 6.89[c,d]	43.19 ± 4.16[d]	37.63 ± 3.27
Group VII - Arjunarishta (2 ml) pretreated rats with ISO	138.33 ± 5.29[a,d]	170.24 ± 7.37[b,d]	50.40 ± 3.44[e]	43.99 ± 2.54[c]
Group VIII - Arjunarishta (1 ml) pretreated rats with ISO	170.52 ± 5.63[a,d]	198.09 ± 8.45[a,d]	59.23 ± 3.69[a]	48.24 ± 3.54[b]

Where, values expressed as mean ± S.E.M. (n = 6); [a] *p<0.001* for comparison of treated groups with normal control; [b] *p<0.01* for comparison of treated groups with normal control;
[c] *p<0.05* for comparison of treated groups with normal control; [d] *p<0.001* for comparison of treated groups with ISO control; [e] *p<0.01* for comparison of treated groups with ISO control.
Arjunarishta treatment is given for 14 days, while ISO treatment given on 13th and 14th day.

Table 3: Effect of novel arjunarishta on serum lipids in a myocardial infarction rat model.

Particular	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Group I – Normal control	73.02 ± 3.24	18.34 ± 1.86	22.72 ± 2.98	46.62 ± 4.37	3.67 ± 0.37
Group II - Rats treated with 3 ml Arjunarishta	69.80 ± 3.58	17.79 ± 1.89	25.15 ± 2.44	41.10 ± 3.26	3.56 ± 0.38
Group III - Rats treated with 2 ml Arjunarishta	70.98 ± 3.41	17.94 ± 2.38	24.36 ± 2.24	43.04 ± 4.39	3.59 ± 0.48
Group IV - Rats treated with 1 ml Arjunarishta	72.28 ± 3.25	18.25 ± 2.54	23.56 ± 2.81	45.07 ± 3.93	3.65 ± 0.51
Group V - Control rats with ISO	123.93 ± 6.03[a]	30.45 ± 3.32[c]	12.11 ± 1.65[c]	105.73 ± 6.56[a]	6.09 ± 0.66[c]
Group VI - Arjunarishta (3 ml) pretreated rats with ISO	80.94 ± 3.96[d]	21.86 ± 3.07	19.65 ± 2.26	56.92 ± 5.50[d]	4.37 ± 0.61
Group VII - Arjunarishta (2 ml) pretreated rats with ISO	85.74 ± 2.95[d]	23.23 ± 2.73	17.97 ± 2.05	63.12 ± 4.95[d]	4.65 ± 0.55
Group VIII - Arjunarishta (1 ml) pretreated rats with ISO	97.06 ± 5.33[b,d]	27.13 ± 2.69	17.02 ± 1.96	74.62 ± 6.01[b,e]	5.43 ± 0.54

Where, values expressed as mean ± S.E.M. (n = 6); [a] *p<0.001* for comparison of treated groups with normal control; [b] *p<0.01* for comparison of treated groups with normal control;
[c] *p<0.05* for comparison of treated groups with normal control; [d] *p<0.001* for comparison of treated groups with ISO control; [e] *p<0.01* for comparison of treated groups with ISO control.
Arjunarishta treatment is given for 14 days, while ISO treatment given on 13th and 14th day.

CONCLUSION

The current method of arjunarishta preparation involves the addition of isolated yeast culture that initiates fermentation. Standardized process controls and inability of contamination are the attributes of good manufacturing practices (GMP) in ayurvedic-fermented formulations. The prepared formulation had significant antioxidant potential and could exhibit cardioprotective effect in rats. This ensures the quality, reproducibility and efficacy of product.

CONFLICT OF INTEREST: Nil

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