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Cardioprotective Activity Of Herbal Formulation In Experimental Animal

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ABSTRACT

The present study was designed to investigate the cardioprotective effects of herbal formulation in rats with isoproterenol-hydrochloride (ISPH) induced myocardial infraction. Adult male Wistar Albino rats were pre-treated with herbal formulation daily for a period of 4 weeks. After the treatment period, ISPH (85 mg/kg) was subcutaneously injected into the rats at 24 h intervals for 2 days. ISPH induced myocardial damage indicated by cardiac marker enzyme activities including creatine kinase-MB, lactate dehydrogenase, SGOT, angiotensin converting enzyme. The activities of antioxidant enzymes such as superoxide dismutase were significantly decreased in hearts after ISPH-induced myocardial infraction. However, pre-treatment of ischemic rats with herbal formulation brought the biochemical parameters to near normalcy, indicating the protective effect of herbal formulation against ISPH-induced ischemia in rats.

Keywords: Myocardial infraction, Isoproterenol hydrochloride, tissue death, herbal formulation, Inflammation

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INTRODUCTION

Historically, herbs have been used for medical purpose, but their usage continues even nowadays. The global burden of disease has driven a broad shift from communicable, maternal, neonatal, and nutritional causes to non-communicable disease. Medicinal plants play an important role in the development of potent therapeutic agents. Herbal medicines are currently in demand and their popularity is increasing day by day. World Health Organization (WHO) has distinct herbal drugs as complete, labelled medicinal products that have vigorous ingredients, aerial or secretive parts of the plant or other plant material or combinations.[1]

Worldwide, ischemic heart disease (IHD) leading to myocardial infraction is a major cause of morbidity and mortality. Isoproterenol (ISP), a synthetic β-adrenergic agonist, causes severe myocardial damage in rats. Myocardial infarction (MI) refers to tissue death (infraction) of the heart muscle (myocardium). Myocardial infarction also known as heart attack occurs when blood flows decrease or stops to a part of the heart causing damage to the heart muscle. It is a type of acute coronary syndrome which describe sudden or short-term change in symptoms related to blood flow to the heart. Blood vessels carry blood and oxygen. When a blood vessels in the heart gets blocked, blood cannot get to part of the heart. This is part of the heart does not get enough oxygen called as ischemia. When the heart muscle becomes ischemic, the ischemia often causes chest pain. This is called angina pectoris. Those phrase 'heart attack' is often used non-specifically to refer to a myocardial infarction and to sudden cardiac death. An MI is different from but can cause cardiac arrest, where the heart is not contracting at all or so poorly that all vital organs cease to function, thus causing death. It is also distinct from heart failure, in which the pumping action of the heart is impaired. However an MI may lead to heart failure. MI is characterized by chest pain or discomfort which may travel into the shoulder, arm, back neck or jaw. Acute myocardial infarction is considered more appropriately part of a spectrum referred to as acute coronary syndromes, which also includes unstable angina and non-ST-elevation. Patients with ischemic discomfort may or may not have ST-segment elevation will develop Q waves. Approximately 90% of myocardial infarction results from an acute thrombus that obstructs an atherosclerotic coronary artery. [2,3]

Bhallatak Ghrit and Shwadanshtradi Ghrit is thus selected for cardioprotective study. Charaka has mentioned that there is no such kapha disease that cannot be cured with Bhallatak. Lifestyle disorders, Metabolic syndrome like obesity, diabetes dyslipidaemia etc. are considered as kapha disorders of Srotas Avarodh Janya disorders in Ayurveda. Multiple researches has shown Bhallatak beneficial for myocardium of rats with Isoproterenol induced myocardial damage

Bhallatak is considered as Medhya, that means in some way it works on brain and nervous system in positive way. It would be interesting to see how Bhallatak helps heart during myocardial infarction when arrhythmias and conduction defects is a common step towards the death due to MI. Shwadanshtradi Ghrit is mentioned in classical treaty "Chakradatta". This Ghrit is recommended for palliation of Vata, Pitta, Hridrog, Shula, Mutrakrichra, Prameha, Arsha, Shwas, Kas, Kshaya, debility of Bala and Mamsa due to excessive practice of archery, sex, alcohol abuse, weight lifting. In present study was designed to evaluate the cardioprotective activity of Bhallatak Ghrit and Shwadanshtradi Ghrit, isoproterenol induced biochemical changes using rats.[4,5]

MATERIALS AND METHOD

Experimental Animals

Male Wistar rats weighing 250-350 gms were used for study. Male Wistar rats (250-350 gm) were separately grouped housed in ambient room temperature (27±2°C) and relative humidity (50±5%), maintained at 12±1 hr dark-light cycle. Food and water were available *ad libitum*. Animals were acclimatized to the experimental condition for a period of one week before actual experimentation. The care and handling of animal were done in accordance with laboratory with the standard guidelines for animals (CPCSEA) permission and approval for animal studies obtained from Institutional Animal Ethics Committee (IAEC) of Datta Meghe Institute of Medical Sciences, Sawangi, Wardha .

Drugs and Chemicals

The assay kits for the measurement of creatine kinase –MB (CK-MB), lactate dehydrogenase (LDH), SGOT (Serum Glutamate Oxaloacetate Transminase) and Oxidative stress enzymes SOD (Superoxide Dimutase) and ACE(Angiotensin Converting Enzyme) were all purchased from Maharashtra Emporium, Wardha. Isoproterenol Hydrochloride (ISPH) was obtained from Unique Traders, Nagpur. Picric acid solution, ketamine hydrochloride, 2% formalin solution. The chemicals used and other solutions were of analytical grade. All drugs and reagents were prepared immediately for use.

Collection of materials

Bhallatak Ghrit, Shwadanshtradi Ghrit will be used for screening of cardioprotective activity and was procured from Madhavbaug, Pune.

Induction of experimental MI

ISPH was dissolved in normal saline and was subcutaneously injected into rats (85 mg/kg) at 24 h intervals for 2 days at 29th and 30th day to induce experimental MI.

Experimental Design

After a 7 days acclimation period, animals were randomly divided into 4 groups (with 24 rats in each group) and were treated as follows:

1. Group I-(Normal control) ,Rat were given distilled water for 28 days and normal saline
2. Group II- (ISO control), Rat were given distilled water for 28 days and ISO (85mg/kg) s.c.
3. Group III- Rat were given Compound A (Bhallatak Ghrit) orally for 28 days and ISO (85mg/kg) s.c. on 29^{th} and 30^{th} day
4. Group IV- Rat were given Compound B (Shwadanshradi Ghrit) orally for 28 days and ISO (85 mg/kg) s.c. on 29^{th} and 30^{th} day

During the experimental period, the body weights of the rats were weekly recorded and the doses were administered accordingly. The doses of Bhallatak Ghrit and Shwadanshradi Ghrit was also selected based on the results of recent studies, in which the treated Bhallatak Ghrit and Shwadanshradi Ghrit and showed cardioprotective effects. All rats survived until they were sacrificed. Forty-eight hours after the first dose of ISPH, all animals were sacrificed by decapitation. Blood samples were collected through retro-orbital puncture, and serum will be separated in cooling centrifuge for analysis of diagnostic marker enzymes.

Biochemical Estimation

Serum enzyme CK-MB (Creatine Kinase –MB isoenzyme) , LDH (Lactate Dehydrogenase), SGOT (Serum Glutamate Oxaloacetate Transminase) and Oxidative stress enzymes SOD (Superoxide Dimutase) and ACE(Angiotensin Converting Enzyme) were measured by appropriate methods. The estimation was done using either UV spectrophotometer or Microtitre plate reader.

CK-MB

Chemicals

Imidazole **,**D-Glucose , N-Acetyl – L – Cysteine , Magnesium acetate , NADP , Hexokinase, Creatine phosphate, ADP , AMP , Diadenosine pentaphosphate , G-6-PDH , CK-MM Antibody

Method

This was done using commercially available kit from Vital Diagnostics , enzyme reagent and buffer was mixed gently to get working reagent. After 5 minutes , 1000 µl working reagent and 50 µl of sample were pipette into clean and dry test tube. The mixture was aspirated and initial absorbance was taken after exactly 30 second at 340 nm. Subsequently the absorbance at exactly 60, 90 and 120 seconds were noted and mean Δ A / minute were calculated. The enzyme activity was calculated as per the given formula.

Creatine phosphate + ADP $\xrightarrow{\text{Creatine kinase}}$ **Creatine + ATP**

Glucose + ATP —Hexokinase→ **Glucose-6-phosphate + ADP**

G-6-NADP —G-6-PDH→ **Gluconate-6-Phosphate + NADP + H+**

LDH

Chemicals

Reagent A :Tris buffer, puruvate , sodium chloride; Reagent B : NADH and sodium azide

Method

LDH assay was performed using commercial kit provided by Bio systems Diagnostics. The reagents provided in the kit were properly mixed as per the given instruction to prepare the working reagent and 0.1 ml of it was mixed with 20 µL of sample in the cuvette. Absorbance after 30 second and at 1 minute intervals thereafter for 3 minutes was recorded. The difference between consecutive absorbance, and the average absorbance difference per minute (ΔA/min) was calculated. The LDH concentration in the sample was calculated using the formula.

Lactate dehydrogenase (LD or LDH) catalyzes the reduction of pyruvate by NADH, to form lactate and NAD+.

Pyruvate + NADH +H+ —LDH→ **Lactate + NAD**

SGOT

Chemicals

Reagent 1 (Enzymes): Maleate dehydrogenase, Lactate dehydrogenase, NADH, α-Ketoglutarate; Reagent 2 (Buffer): Tris buffer and L-Aspartate

Method

The kit provided by Autopak Siemens was used for the estimation of SGOT. In a test tube, 1 ml of reconstituted reagent and 100 µl of serum samples were added and the absorbance was read at 340 nm using the specified parameters in the kit. There is a decrease in absorbance at 340 nm as NADH is converted to NAD. The rate of decrease in absorbance is measured and is proportional to SGOT activity in the sample.

L- Aspartate+α-Ketoglutarate —GOT→ **Oxaloacetate + L-Glutamate**

Oxaloacetate + NADH + H —MDH→ **L-Malate + NAD**

Superoxide dismutase (SOD)

Chemicals

Methionine, Nitro Blue Tetrazolium, Riboflavin, and Triton x 100

Method

SOD in the serum was measured following the riboflavin method of Marklund and Marklund with some modification 20µl of sample or buffer was mixed with 20µl reagent mix. The mixture was

exposed to light and OD560 nm was recorded. Results are expressed as % inhibition. One unit of enzyme is defined as amount capable of inhibiting colour development by 50%.

Angiotensin Converting Enzyme (ACE)

Chemicals

HEPES HCl buffer with sodium chloride, 1 M Hydrochloric acid, ethyl acetate, angiotensin converting enzyme, Hippuryl-L-Histidyl-L-Leucine solution (HHL), ethylacetate

Method

The mixture of 20 µl HHL(Hippuryl-L-Histidyl-L-Leucine) and 5 µl of enzyme solution was incubated for 15 min at 37 C. In an aliquote of 2 ml,25 of HCl and 200 µl ethylacetate was added to this, shaken vigorously and centrifuged for 2 min. After centrifugation, 1.0 ml of supernatant was pipette out in vials and kept for boiling in water bath for 15 min. Hippuric acid formed is extracted with ethylacetate, condensed and redissolved and then read at 228 nm. The enzyme level is calculated as per the specified formula and expressed as Units/ml enzyme.

Hip-L-His-L-Leu(HHL) + H2O $\xrightarrow{\text{ACE}}$ **Hippuric acid +L-His-L-Leu**

Statistical Analysis

The data were expressed as mean ± SEM . Results were analyzed statistically by One way ANOVA followed by DUNNET's test using Graphpad instat version 3 and Graphpad prism 8. The difference was considered significant if **p < 0.05, ***p<0.001, **** p<0.0001.

RESULTS AND DISCUSSION

Determination of maximum tolerable dose by acute toxicity study:

For acute oral toxicity and determination of maximum tolerable dose, the Organization for Economic Co-operation and Development (OECD) guideline 423 was followed and the behavioral study was performed. Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method).

Wistar rats (n = 6), healthy and young adult rat having body weight 250-350 gm of either sex were selected by random sampling technique for the study. The animals were kept fasting for overnight providing only water, after which the extract were administered orally at the dose level of 50, 300 & 2000mg/kg body weight using cannula and behavior was observed for 48 hours. From the toxicity study, extract of Bhallatk Ghrit, Shwadanshtradi Ghrit did not produce any toxicity on low and mid dose. Therefore dose between 50- 300 mg/kg can be used. Hence 300 mg/kg and above were selected for screening of cardio protective activity shown in table 1.

Comparison of Physical Parameters

Treated group significantly increase body weight as compared to vehicle control group. Weight was gradually increased in group (G3, G4) from 1st week to 4th week (P<0.05) as compared to control group. No significant change was observed in ISO-treated group and control group on week 4. However, on week 4th a significant increase in weight was observed in Bhallatk Ghrit and Shwadanshtradi Ghrit group as compared to control group(P<0.05 on week 4). The results of weight variation are depicted in Figure no.1. and the values are listed in table 2.

Comparison of Biochemical Parameters

The activities of serum CK-MB, LDH, SGOT, SOD and ACE in the plasma of control and experimental rats are given in table no.3. Increased activities of CK-MB, LDH, SGOT, ACE were observed in ISO-induced rats. Treatment with Bhallatak Ghrit and Shwadanshtradi Ghrit lower the above parameters significantly. The treatment drug improved the activities of CK-MB, LDH, SGOT, SOD, ACE to near normal levels. This results are depicted in below table no.3.

ISO treated group significantly decreases SOD activity as compared to control group (**<0.05, ***p<0.001). Treatment with Bhallatak Ghrit and Shwadanshtradi Ghrit significantly increased the activity of SOD as compared to ISO treated. However, activity was more significantly in Bhallatk Ghrit group (G3) . Interestingly, no significance difference was observed Bhallatak Ghrit and Shwadanshtradi Ghrit. This results are depicted in fig and values listed below in table no.4.

Table 1: Observation table of acute toxicity study

Sr. No.	Parameter	Animals 1	2	3	4	5	6
1.	Lacrimation	NO	NO	NO	NO	NO	NO
2.	Salivation	NO	NO	NO	NO	NO	NO
3.	Piloerection	NO	NO	NO	NO	NO	NO
4.	Drowsiness	NO	NO	NO	NO	NO	NO
5.	Tremors	NO	NO	NO	NO	NO	NO
6.	Convulsion	NO	NO	NO	NO	NO	NO
7.	Fur	Normal	Normal	Normal	Normal	Normal	Normal
8.	Food consumption	Normal	Normal	Normal	Normal	Normal	Normal
9.	Water consumption	Normal	Normal	Normal	Normal	Normal	Normal
10.	Morality	NO	NO	NO	NO	NO	NO

Table 2: Effect of Bhallatk Ghrit, Shwadanshtradi Ghrit on Body Weight of the rats

Weeks	Weight variation Groups			
	G1	G2	G3	G4
Week 1	345.83 ±7.27	317.37±5.86	323.37±8.06	330.5±8.31
Week 2	345.91±7.26	317.95±5.89	326.70±8.41	337.45±8.09
Week 3	347.37±7.25	319.875±05.87	327.37±8.38	338.25±8.15
Week 4	348.25±7.24**	321.04±5.85	330.66±8.02	340.54±8.03**

Results are expressed as the mean± SEM, n=10. Data was analyses by one way analysis of variance (ANNOVA) followed by Dunnet test ** indicates <0.05 when compared to control and ISO treated group.

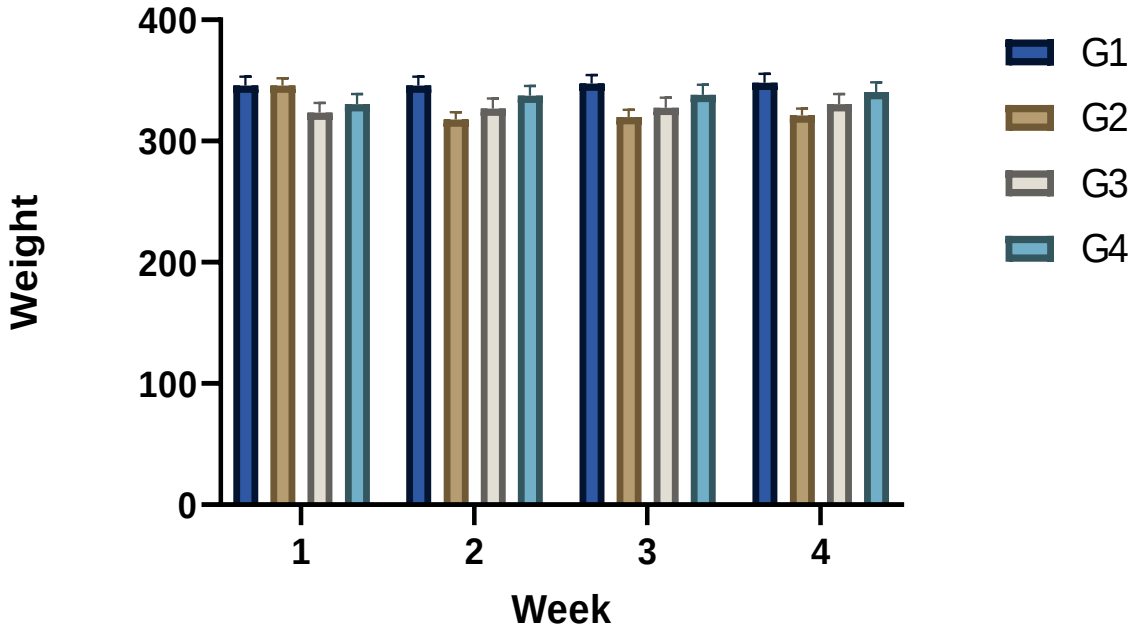

Figure 1: Effect of Bhallatk Ghrit, Shwadanshtradi Ghrit on Body Weight of the rats

Table 3: The Cardioprotective effects of Bhallatk Ghrit, Shwadanshtradi Ghrit on activities of CK-MB, LDH, SGOT, ACE on rats.

Groups	CK-MB (IU/L)	LDH (IU/L)	SGOT (IU/ml)	ACE (µL)
Control (G1)	22.288±2.81	162.09±15.6	56.105±4.16	62.203±14.2
ISO treated (G2)	105.346****±16.3	273.112****±32.9	100.318****±11.5	273.01****±38.5
Bhallatak Ghrit+ ISO(G3)	17.946**±3.49	113.325****±10.8	35.964***±6.64	94.7**±9.50
Shwadanshtradi Ghrit + ISO(G4)	23.616±2.83	120.444±10.8	42.311**±3.82	85.936***±24.8

Results are expressed as the mean±SEM, n=10. . Data was analyzed by one way analysis of variance (ANNOVA) followed by Dunnet test ** , ***,**** indicates <0.05, <0.001, <0.0001 when compared to control and ISO treated group.

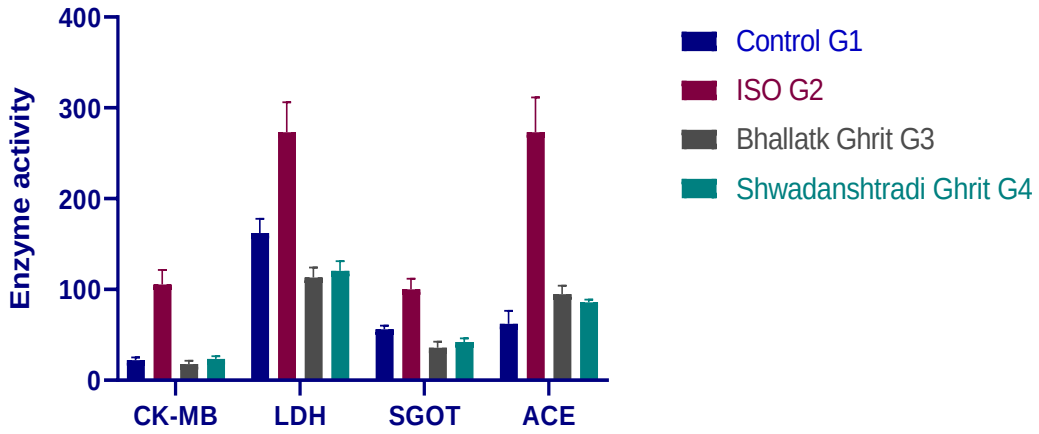

Figure 2. Effect of Bhallatk Ghrit, Shwadanshtradi Ghrit on activities of CK-MB, LDH, SGOT, ACE on rats.

Table 4. : The Cardioprotective effects of Bhallatk Ghrit, Shwadanshtradi Ghrit on activity of SOD of experimental animals.

Groups	SOD Activity
Control (G1)	1.931±0.53
ISO treated(G2)	0.819±0.32**
Bhallatak Ghrit+ ISO(G3)	1.63±0.320***
Shwadanshtradi Ghrit + ISO(G4)	1.493±0.28***

Results are expressed as the mean± SEM, n=10. . Data was analyzed by one way analysis of variance (ANNOVA) followed by Dunnet test ** , *** indicates <0.05, <0.001, when compared to control and ISO treated group.

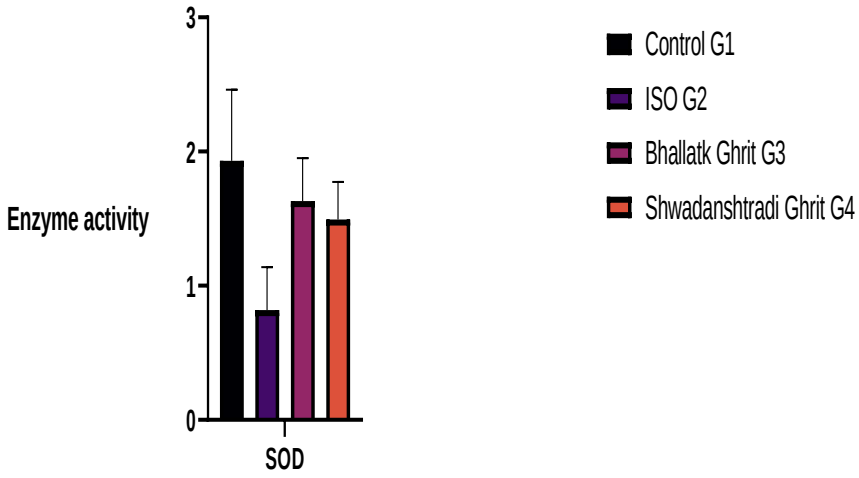

Figure 3. Effect of Bhallatk Ghrit, Shwadanshtradi Ghrit on activity of SOD on rats.

Myocardial infraction is a condition in which tissue death of heart muscle. MI is characterized by chest pain or discomfort which may travel into the shoulder, arm, back neck or jaw. Acute myocardial infarction is considered more appropriately part of a spectrum referred to as acute coronary syndromes, which also includes unstable angina and non-ST-elevation. Patients with ischemic discomfort may or may not have ST-segment elevation will develop Q waves. Approximately 90% of myocardial infarction results from an acute thrombus that obstructs an atherosclerotic coronary artery.

Isoproterenol, a potent synthetic catecholamine, when administered to animals at high doses produces 'infraction-like' lesions in the heart, which are similar to those found in myocardial infraction in humans. It is a β-adrenergic agonist. Administration of ISPH causes oxidative stress mainly via the β-1 adrenergic receptor stimulation. Stimulation of these receptors β-1 especially present in the heart muscle is responsible for the acute positive inotropic and chronotropic effects on heart, causing an imbalance between energy intake by the blood flow and increased oxygen demand leading to ischemia. If ischemia is severe and prolonged, it can induce cardiac cells death, and the release of their enzyme content in the extra cellular medium. Ischemic muscle quickly generates the radical species derived from oxygen (ROS) when the capacity of cellular antioxidant enzymes decreased. Myocardium contains an abundant concentration of marker enzymes like, CK-MB, LDH, SGOT, Catalase, ACE of MI and once metabolically damaged, it releases its contents into the extracellular fluid. In this study, increased activity of CK-MB, LDH, SGOT, ACE were observed in the plasma of ISO-induced rats. Myocardial infraction can be defined from a number of different perspectives related to clinical, biochemical, and pathologic characteristics. In our study, ISO-administered rats also showed an increase in the levels of biochemical markers in the plasma and heart. Bhallatak Ghrit, Shwadanshtradi Ghrit treatment of the ISO-induced rat decreased the CK-MB, LDH, SGOT, ACE level.

High concentrations of isoproterenol administration have been reported to induce severe oxidative stress and result in necrotic lesions in the myocardium of rats. The increased production of reactive oxygen species and/or depletion of the antioxidants in the defence system may contribute to oxidative stress and affect the pathogenesis of myocardial infraction. Free radical scavenging enzymes such as SOD, Catalase are the first line of cellular defence against oxidative stress. Increased CK-MB, LDH, SGOT, ACE concentration and decreased SOD in infracted rats compared to control is a consequence of oxidative stress due to the alteration of the mechanism of dynamic balance between pre-oxidants and antioxidants and excessive ROS formation, responsible for lipid peroxidation and irreversible destruction of heart cells induced by ISPH. Probably

Bhallatak Ghrit, Shwadanshtradi Ghrit may prevent the formation of lipid peroxidase in the myocardium. It is also possible that Bhallatak Ghrit, Shwadanshtradi Ghrit cardioprotective effects may pass by inhibiting cells membrane permeability disturbance or reinforcing myocardium antioxidant activities.

It was observed in this study that cardioprotective effect of Bhallatak Ghrit, Shwadanshtradi Ghrit (996 mg/kg dose) was approximately similar to aspirin which was taken as standard drug. The level of enzyme markers first markedly increased then after the administration of treatment drug the level of enzyme markers were decreased significantly. So, doses of Bhallatak Ghrit, Shwadanshtradi Ghrit were taken and evaluated for its cardioprotective activity using different enzyme markers like , CK-MB, LDH, SGOT, ACE were found to be statistically significant and effectively decreased at dose where (****$p<0.01$). Furthermore the result suggested that Bhallatak Ghrit, Shwadanshtradi Ghrit possesses positive effect on experimental animals showing myocardial infraction induced by ISPH.

Bhallatak Ghrit, Shwadanshtradi Ghrit has been reported to exert several pharmacological properties such as , anti-inflammatory, antioxidant activity, cardiotonic, tonic, Anti-arthritic activity, antihypertensive effect and so it is said to have cardioprotective activity but appropriate research work were not done. The serum CKMB activity assay is an important and sensitive diagnostic tool due to the high abundance of this enzyme in the myocardial tissue and its virtual absence in most other tissues. CK-MB iso-enzyme activity is a useful early diagnostic index for MI or any type of myocardial injury. Cytosolic enzymes including CK-MB, LDH, SGOT which serve as diagnostic markers, leak out from the damaged tissue into the blood stream when the cell membrane becomes more permeable or ruptures. In this present study, rats exposed to ISO showed significant elevations in the levels of all of these marker enzymes in the serum. Bhallatak Ghrit showed better effects compared to Shwadanshtradi Ghrit when MI was induced by ISO at 85mg/kg body weight.

CONCLUSION

In the present study it was concluded that Bhallatak Ghrit, Shwadanshtradi Ghrit may significantly have cardioprotective activity. Cardioprotective activity using different enzyme markers like , CK-MB, LDH, SGOT, Catalase, SOD, ACE were found to be statistically significant and effectively decreased . The result suggest that Bhallatak Ghrit, Shwadanshtradi Ghrit has potential cardioprotective effect that can be explored for therapeutic advantage as an alternative treatment in medical conditions.

Increased CK-MB, LDH, SOD, SGOT, ACE concentration in infracted rats compared to control is a consequence of oxidative stress due to the alteration of the mechanism of dynamic balance between pre-oxidants and antioxidants and excessive ROS formation, responsible for lipid peroxidation and irreversible destruction of heart cells induced by ISPH. Probably Bhallatak Ghrit, Shwadanshtradi Ghrit may prevent the formation of lipid peroxidase in the myocardium. Further studies are required to confirmed the exact mechanism and isolation of bioactive compound involved in the Bhallatak Ghrit, Shwadanshtradi Ghrit.

The results of present study concluded that Bhallatak Ghrit, Shwadanshtradi Ghrit. at doses of (996 mg/Kg) showed significant cardioprotective effect in isoproterenol induced myocardial necrosis in albino Wistar rats. Cardio protective activity was confirmed by estimation of the levels of CK-MB, LDH, SGOT, SOD, ACE. Isoproterenol is a synthetic compound which alters biochemical parameters and it can be prevented significantly by administration of Bhallatak Ghrit, Shwadanshtradi Ghrit. Further studies are recommended to elucidate the mechanism of the cardioprotective action of this plant and identification of its active agents.

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