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STUDIES ON ETHANOLIC EXTRACT OF BRASSICA OLERACEA L. VAR. ITALICA (BROCCOLI) AND THEIR EFFECT ON DALDA INDUCED ALBINO RATS

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ABSTRACT The present study was designed to scientifically evaluate the cardiotonic activity of ethanolic extract of Brassica oleraceae L. var. italica (Family: Brassicaceae), against hydrogenated oil (dalda) induced myocardial ischemic in albino rats. Myocardial ischemia was produced in rats with 100 mg/kg of dalda administered orally in 50 days. Effect of oral treatment of ethanolic extract of Brassica oleraceae L. var. italica at a dose (250 mg/kg body weight), was given simultaneously for 50 days. Ethanolic extract of this plant showed significant cardiotonic effect by lowering the serum levels of various biochemical parameters like Creatinine Phospho Kinase (CPK), Lactate Dehydrogenase (LDH), Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were estimated in both the serum and heart tissues; antioxidant parameters viz., catalase (CAT) and malondialdehyde (MDA) were assayed in heart homogenate. The selected model in prophylactically and curatively manner was evaluated against dalda induced myocardial ischemia. The results also suggests that the biologically active phytoconstituents such as flavonoids, glycosides, alkaloids present in the ethanolic extract of plant which is confirmed from the qualitative analysis may be responsible for the significant cardiotonic activity. However, it suggests that a dose adjustment may be necessary to optimize the effects in clinical settings.

KEYWORDS : hydrogenated oil, *Brassica oleraceae L.* var. *italica,* cardiotoxicity, enzyme activity, Biochemical studies.

INTRODUCTION

The heart is a muscular organ in humans and other animals, which pumps blood through the blood vessels of the circulatory system. Blood provides the body with oxygen and nutrients, as well as assists in the removal of metabolic wastes. The heart is located between the lungs, in the middle compartment of the chest. In humans, other mammals and birds, the heart is divided into four chambers namely the right atrium receives blood from the veins and pumps it to the right ventricle. The right ventricle receives blood from the right atrium and pumps it to the lungs, where it is loaded with oxygen. The left atrium receives oxygenated blood from the lungs and pumps it to the left ventricle. The left ventricle (the strongest chamber) pumps oxygen-rich blood to the rest of the body. The left ventricle's vigorous contractions create our blood pressure.

Myocardial infarction or heart attack is the leading cause of death for both men and women all over the world. It occurs when blood supply is insufficient to the myocardium, death of myocardial muscle occurs, a condition known as ischemia. Prolonged ischemia of the myocardium leads to necrosis, which is referred as myocardial infarction (Whellan, 2005).

For decades, the major causes of death in many developed countries have been diseases of the heart and blood vessels (the venous system), collectively known as cardiovascular disease (CVD). The use of herbal medicines has been steadily increasing over the past decade to cure some of the disorders in human. Epidemiologists in India and international agencies such as the World Health Organization (WHO) have been sounding an alarm on the rapidly rising burden of CVD for the past 15 years. The reported prevalence of coronary heart disease (CHD) in adult surveys has risen four-fold in 40 years and even in rural areas the prevalence has doubled over the past 30 years. It is estimated that by 2020, CVD will be the largest cause of disability and death in India Ramadoss et al. (2012).

Myocardial infarction (MI) is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart (Petrich et al., 1996). MI and the resultant complication in cardiac function is one of the leading causes of death for both men and women. Due to changing lifestyles in developing countries, particularly in urban areas, MI is making an increasingly important contribution to mortality statistics (Levy and Feinleib, 1984). Antioxidant compounds, highly present in plants have shown protective effects against diseases without reducing their therapeutic efficacy. Moreover, there is a growing interest in the usage of natural antioxidants as a protective strategy against cardiovascular related problems such as ischemia reperfusion (You et al., 2007). The chemo-therapeutic agents, who inhibit the free radical formation and can reduce the risk of heart diseases, have gained imperative value in the modern medicines (Ke et al., 2009 and Paterson, 2006). Herbal medicines having antioxidant properties, may therefore, have a protective role in cardiovascular diseases (Viswanatha et al., 2010).

Broccoli *(Brassica oleraceae L. var. italica)* belongs to the Brassicaceae family and is closely related to the cabbage, cauliflower and brussels sprouts. Broccoli is an edible green plant in the cabbage family whose large flowering head is eaten as a vegetable. The word broccoli comes from the Italian plural of broccolo, which means "the flowering crest of a cabbage", and is the diminutive form of brocco, meaning "small nail" or "sprout". Broccoli is often boiled or steamed but may be eaten raw. Broccoli is classified in the Italica cultivar group of the species *Brassica oleracea.* Broccoli has large flower heads, usually green in color, arranged in a tree-like structure branching out from a thick, edible stalk. The mass of flower heads is surrounded by leaves. Broccoli resembles cauliflower, which is a different cultivar group of the same species.

Brassica oleracea var. italica is an annual herb reaching 400mm during vegetative stage and 1-2 meter at the end of flowering. Broccoli has antimicrobial and anticancer activities and has become established as an important human food crop plant, used because of its large food reserves (Survay et al., 2012 and Vasanthi et al., 2009). It is rich in, essential nutrients including quercetin-7-rutinoside, quercetin3-gluccoside-7rhamnoside, kaempferol3-glucoside etc. Because of its abundance flavonoid content and traditional use, *Brassica oleracea* was considered to be effective in treatment of variety of human disorders caused by oxidative stress and thus selected for investigation of its antioxidant & Blood Glucose lowering potential (Sibi et al., 2013).

Brassica oleraceae var italica was reported to have anticancer, antioxidant (Gawlik-Dziki et al 2014), antiseptic (Sanchez-Moreno, 2002), antiulcer (Vasanthi et al., 2009), hypoglycaemic activities (Park et al., 2012). Traditionally, it has been used in anemia but there is no scientific proof to support this claim. Hence, the study was

undertaken to evaluate the cardiotonic property of ethanolic extract of *Brassica oleraceae var italica* in dalda induced myocardial ischemia.

MATERIALS AND METHODS

For the study, the flower of *Brassica oleracea* L. var italica plenck belongs to family Brassicaceae was collected from Super market, Thanjavur, Tamilnadu, South India. The plant was identified with the help of manual of Tamil Nadu and Karnatic flora (Gample, 1967 and Matthew, 1983) with standard references (Kirtikar and Basu, 1983).

Preparation of plant powder

The *Brassica oleracea* L. var *italica* was collected, washed, cut into small pieces and dried at room temperature (28±10C) for three weeks and made into powder for further analysis. The dried powder of *Brassica oleracea* L. var *italica* (200g) was successively extracted with 1000 ml of alcohol, in a soxhlet apparatus at 60-70ºC for 10-12h consecutively. Ethanol was removed from the extract under vacuum and a semisolid mass was obtained. The yield of extracts was 22.45% w/w for ethanol extract of Brassica oleracea. The extracts were stored in sterile amber colored storage vials in refrigerator until used for experimentation.

Experimental Animals

Adult Wistar albino rats weighing of 200 - 250 gm breed in the Central Animal House, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Trichy - 21, were used in this study. They were housed in Tarson`s polypropylene cages with metal grill tops and provided with food and water ad libitum. They were maintained in a controlled environment under standard conditions of temperature and humidity with alternating light/dark (LD 12:12) cycle. In the laboratory, rats were fed with standard rat pellet diet.

Experimental design

The animals were randomly divided in to four groups, each containing three animals. Four groups (Group I, Group II, Group III and Group IV) of rats, six rats in each group were taken. Group – I: Served as normal, which received, feed and water only. Group – II: Animals of this group were orally administered 100 mg/kg of body weight of dalda along with formulated feed for 50 days. Group – III: Animals of this group were orally administered 100 mg/kg of body weight of dalda along with formulated feed. Then the animals were treated with the alcoholic flower extract of *Brassica oleracea* L. *var italica* daily for 50 days at concentration of 250mg/kg of body weight. Group – IV: Animals of this group were orally administered 100 mg/kg of body weight of dalda along with formulated feed. Then the animals were treated with Atorvastatin for 50 days at concentration of 10 mg/kg of body weight. On 51st day the treated animals were fasted for 12hours after the last dose of drug treatment and were scarified cervical decapitation method under Xylazine + Ketamine (16 + 100 mg/kg i.m.), blood samples were collected via abdominal aorta puncture using sodium citrate (3.8%w/v) as anticoagulant and the serum separated were used for the determination of diagnostic marker enzymes.

Biochemical assay in the serum and heart tissue Estimation of serum enzymes: lactate dehydrogenase (LDH) by the method of King (1965), creatine phosphokinase (CPK) by the method of Okinaka et al. (1961), aspartate transaminase (AST) and alanine transaminase (ALT) by the method of Bergmeyer and Bernt (1974) and creatine phosphokinase-MB (CK-MB) in serum were estimated using commercially available kit (Beacon assay kit).

The membrane bound enzymes assay of tissue homogenate pallet obtained after centrifugation was resuspended in ice cold Tris buffer (10mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of Na^+/K^+ ATPase was assayed by Bonting et al. (1970), Ca^{2+} ATPase was assayed using the method of Hjerken and Pan (1983) and Mg^{2+} ATPase was assayed using the method of Ohinishi et al. (1982). Protein was estimated according to the method of Lowry et al. (1951) were analyzed.

RESULTS

Natural therapy for various human ailments purified with plant products has gained much attention now a day's *Brassica oleracea* L. var *italica* is frequently used for the food. The use of herbal preparations in the treatment of diseases is very common in the rural communities of world. *Brassica oleracea* L. var *italica* is frequently used for the treatment of nausea, skin disease, and juice is useful in urine related disorders and urinary tract infections. It is also useful in stopping haemorrhages occurring in body. This medicinal plant is believed to be an important source as well as promising pharamacological properties were verified in our laboratories.

Table 1 shows the activities of marker enzymes of cardiac function (LDH, AST, ALT, CK-MB and CPK) in the serum of control and experimental rats. The administration of dalda resulted in significant ($p<0.005$) increase in the serum levels of heart marker enzymes in group II rats. Pretreatment with *Brassica oleracea* L. *var italica* (250 mg / kg b.w.) extract in Group III rats showed a significant ($p<0.05$) decrease in the activities of above mentioned cardiac marker enzymes when compared with Group II rats. Group IV rats also pretreated with Atorvastatin (10 mg / kg b.w.) showed the values near normal to control rats (group I). The results were observed in dose dependent manner when compared with treated rats.

Effect of *Brassica oleracea* L. var *italica* on membrane bound enzymes. The levels of membrane bound ATPase (Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase) in the heart of the control and experimental rats are represented in table 2. It was evident that, a significant ($p<0.05$) decrease in the activity of Na^+/K^+ ATPase whereas a significant ($p<0.05$) increase in the activities of Ca^{2+} ATPase and Mg^{2+} ATPase in dalda administered rats when compared to control group. Pretreatment with Brassica oleracea L. var italica (250mg /kg b.w.) showed a significant ($p<0.05$) increase in the activity of Na^+/K^+ ATPase and a significant ($p<0.05$) decrease in the activities of Ca^{2+} ATPase and Mg^{2+} ATPase as compared to dalda treated group. This effect is due to the membrane stabilizing properties of *Brassica oleracea* L. var *italica.*

DISCUSSION

Traditionally used medicinal plants have always remained a major tool for drug development. The medicinal values of traditional medicinal plants cannot be ignored and studies have been carried out in order to investigate various active principles of the extracts with intensive follow up studies to establish their exact mechanism of action. One of the most important area in which compounds from plant sources have contributed successfully, is the cardiovascular research. Myocardial infarction remains a leading cause for death worldwide and prompt treatment for a heart attack is indispensable to save the life. In the traditional Indian medicinal system, a major role has been played by the plants, especially, in the aspect of cardio protection. Several herbs and herbal products have been recommended for prophylactic and therapeutic effects in reducing cardiovascular diseases (CVDs) and that have been reviewed by Radhika et al. (2011). These include *Allium sativum* (garlic) (Sharma et al., 2012), *Allium cepa* (onion) (Neha Nausheen et al., 2014), *Daucus carota* (wild carrot) (Muralidharan et al., 2008), *Ocimum sanctum* (tulsi), *Withania somnifera* (ashwagandha) and *Zingiber officinalis* (ginger) (Rohini et al., 2013). In this context, there is a need to reveal the cardio protective activity of extract of the *Brassica oleracea.* Myocardial ischaemia results from the reduction of coronary flow to such an extent that supply of oxygen to the myocardium does not meet the oxygen demand of myocardial tissue. When this ischaemia is prolonged and irreversible then myocardial cell death and necrosis occurs which is defined as myocardial infarction (Nigam, 2007). Recent studies suggest that increased free radical formation and subsequent oxidative stress associated with the occurrence of a relative deficit in the endogenous antioxidants, may be one of the mechanisms for the development of heart failure after myocardial infarction (Singal et

al., 1983). In our study, dalda is used to induce myocardial ischemia and it has been found to cause a severe stress in the myocardium resulting in necrosis of the heart muscle. It is also well known to generate free radicals and stimulate lipid peroxidation, which may be a causative factor in irreversible damage to the myocardial membrane in experimental myocardial infarction (Kumar et al., 2001). The rat model of dalda induced myocardial infarction offers a reliable non-invasive technique for studying the effects of various potential cardioprotective agents (Korkmaz et al., 2009).

Cardiac glycosides and catecholamine have been used as the main therapeutic drugs in the treatment of congestive cardiac failure (Kitada et al., 1987). However, the dangers of cardiac glycosides intoxication are well documented (Bellar et al., 1971) and doubts have been expressed about their long term effectiveness. The use of catecholamine is limited by their in-sufficient differentiation between positive ionotropic and chronotropic action, their potential arrhythmogenic properties and tachyphylaxis due to receptor down regulation (Kitada et al., 1987). From the above observation we investigate cardiotonic principles in ethanolic flower extracts of *Brassica oleracea* L. var *italica* by different experimental methods.

The serum enzymes namely LDH, AST, ALT, CPK and CK-MB serve as sensitive indices to assess the severity of myocardial infarction (Sheela-Sasikumar and Shyamala-Devi, 2000). The increased activities of these enzymes following injection of IP as observed in this study confirmed the onset of myocardial necrosis (Paritha-Ithayarasi and Shyamala-Devi, 1997). Pretreatment with the extract of *Brassica oleracea* L. var *italica* lowered the elevated activities of the enzymes comparable to the control. This is an indication of the protective action of the extract in reversing cardiac damage. Similar observation was reported by Vishal et al. (2010) using *Lagenaria siceraria* fruit powder in IP-induced myocardial injury in rats. The reversal of these enzyme activities by pretreatment with the extract indicates its therapeutic potential against myocardial infarction.

Membrane bound enzymes play a significant role in maintaining ion levels within the myocytes. Any alteration in the properties of these enzymes is known to affect the function of heart. Failure of the cell membrane to maintain normal trans-membrane ionic distribution through ion pumps is considered to be a major event in pathogenesis of ischemia and arrhythmia (Vajreswari and Narayanareddy, 1992). Na^+K^+ ATPase are the 'SH' group containing enzyme that is responsible for the active transport of Na^+ and K^+ across cell membrane. Reduction in activity levels of Na^+K^+ ATPase in IP treated rats might be due to enhanced lipid peroxide at ion by free radicals as reported by Upaganlawar et al. (2009). Also, reduced activity levels of Mg^{2+} ATPase and Na^+K^+ ATPase in IP treated group may be responsible for creating ionic imbalance and eventually damage the membrane proteins. Cardiac cytosolic calcium level is a key factor involved in maintaining normal activity levels of many enzymes (Hamet, 1995). IP induced myocardial necrosis has been reported to enhance adenylate cyclase activity, resulting in increased formation of cAMP (Dhavan et al., 1978). During IP induced β-adrenergic stimulation, cAMP phosphorylates several sites on the C terminal chains of the calcium channels resulting in the channel opening up (Varadi et al., 1995). This may be the reason for enhanced activity of Ca^{2+} ATPase in dalda induced myocardial ischemiea rats in the present study. Intra cellular Ca^{2+} overload is known to generate various reactive oxygen species (ROS) and Ca^{2+} surge in combination with ROS is responsible for contractile dysfunction of the ischemic myocardium (Jan and Shu, 2005). In our study, Brassica oleracea L. var italica treatment could effectively prevent dalda induced reduction in activity levels of Mg^{2+} and Na^+/K^+ ATPases and elevate Ca^{2+} ATPase activity. This result may be due to the membrane stabilizing properties of B. oleracea L. var italica.

Hence, it can be summarized that pre-treatment with ethanolic flower extract of *Brassica oleracea* L. var *italica* elicited dose dependent positive effect on normal and hypodynamic heart. This shows that *Brassica oleracea* L. var *italica* produces cardiotonic activity. Dalda a non selective beta blocker block the responses produced by B. *oleracea* L. var *italica* indicating that it may elicit the mechanism of action through receptors. Also, pre-treatment improvement the status of enzymatic antioxidants that further contributes to its overall cardio protective property. Further studies are needed to determine the mechanism by which plant acts on the myocardium to beneficially affect the cardiovascular system. Further evaluation is warranted to explore the possibility of mechanism of action and some more pharmacological actions for therapeutic gain of *Brassica oleracea* L. var *italica* in future. The search for new pharmacological-active compounds for drug development is an important issue, as the trend toward using standardized plant extracts of high quality, safety and efficacy will continue. Therefore, all efforts have to be targeted to reveal the chemical-pharmacological profiles of extracts and fixed combinations and to rationalize their therapeutic application.

Table 1. Effect of Brassica oleracea extract on the marker enzymes levels in dalda stimulated rats

S. No	Treatment group	SGOT (IU/L)	SGPT (IU/L)	CKMP (U/L)	CPK (U/L)	LDH (IU/L)
1	Normal saline	78.5 ± 0.65	40.2 ± 0.06	118 ± 0.06	80.3 ± 0.32	117 ± 0.12
2	Positive control (100mg/kg of dalta)	98.2 ± 0.53	52.5 ± 0.19	329 ± 0.15	293 ± 0.18	163 ± 0.17
3	100mg/kg of dalda + 250 mg/kg of *Brassica oleracea*	81.8 ± 0.24	44.1 ± 0.52	197 ± 0.33	132 ± 0.29	125 ± 0.51
4	100mg/kg of dalda + 10mg/kg of Atorvastatin	80.8 ± 0.36	43.5 ± 0.15	192 ± 0.44	121 ± 0.52	116 ± 0.19

Table 2. Effect of Brassica oleracea extract on heart membrane bound enzymes activities in dalda stimulated rats.

S. No	Treatment group	Na+-K+ ATPase (µmoles of phosphorus liberated/mg protein)	Ca^2+- ATPase (µmoles of phosphorus liberated/mg protein)	Mg^2+ - ATPase (µmoles of phosphorus liberated/mg protein)	Protein (g/dl)
1	Normal saline	4.03 ± 0.129	1.82 ± 0.128	0.422 ± 0.196	7.2 ± 1.12
2	Positive control (100mg/kg of dalta)	2.15 ± 0.512	3.25 ± 0.341	0.126 ± 0.125	4.8 ± 1.19
3	100mg/kg of dalda + 250 mg/kg of *B.oleracea*	3.82 ± 0.657	1.98 ± 0.257	0.386 ± 0.352	6.6 ± 1.35
4	100mg/kg of dalda + 10mg/kg of Atorvastatin	3.73 ± 0.153	1.85 ± 0.631	0.397 ± 0.249	6.8 ± 1.14

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