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BIOCHEMICAL CHARACTERIZATION OF HYPOCHOLESTEROLEMIC ACTIVITY OF ETHANOLIC FLOWER EXTRACT OF *BRASSICA OLERACEA* L. VAR. *ITALICA*

KEYWORDS: hydrogenated oil, Brassica oleraceae L. var. italica, hypocholesterolemia, Biochemical studies of lipid profile.

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ABSTRACT The present study was designed to scientifically evaluate the hypocholesterolemic activity of ethanolic extract of Brassica oleraceae L. var. italica (Family: Brassicaceae), against hydrogenated oil (dalda) induced hypercholesterolemic rats. It is now widely accepted that atherosclerosis is a complex multicellular process involving oxidation of cholesterol and the intracellular accumulation of oxidized cholesterol. From ancient times, botanicals have played a major role in the lifestyle of people. The active phytochemicals derived from these herbs and plants have provided protection against atherosclarosis. The association of hypercholesterolemia with the development of atherosclerotic lesion has promoted widespread search for plant based compounds which effectively control the lipid profile in the blood and tissues with least or no toxic effect. Hypercholesterolemia 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. The present work was under taken to assess usefulness and toxic effects of the Broccoli extracts in dalda fed rats. Ethanolic extract of this plant showed significant hypocholesterolemic effect by lowering the serum levels of biochemical parameters like lipid, total cholesterol, phospholipid, triglycerides, LDL, VLDL cholesterol were estimated in the serum. Around eighty percent of the global population still relies on botanical drugs and herbal medicines have advanced to clinical use in modern times. Based on these findings, present work is written to identify the "Lipid-Lowering and Antioxidants Properties" of commonly used plants and herbs.

INTRODUCTION

Hypercholesterolemia is an increase in the normal concentration of cholesterol in the blood, and is one of the major risk factors for cardiovascular diseases such as atherosclerosis, stroke and myocardial infarction, leading causes of death and disability almost worldwide (Deng, 2009). Maintaining cholesterol homeostasis involves various regulatory mechanisms associated with its synthesis, absorption, metabolism, elimination. Hypercholesterolemia is a mismatch metabolic resulting from those processes. The hypocholesterolemic agents reduce the high cholesterol levels, hence, the cardiovascular risk (Maza Cave et al., 2000).

Hypercholesterolemia is a type of hyperlipidemia, ie, high levels of blood lipids, including triglycerides, cholesterol and lipoproteins that make possible the cholesterol transport in blood plasma. Cholesterol-lipoprotein complexes with very low, low, and intermediate densities are named pro-atherogenic cholesterol and are cardiovascular risk factors. In contrast, cholesterol-lipoprotein complex with high density is named anti-atherogenic cholesterol and has protective effect on cardiovascular disease (Deng, 2009). The hypolipidemic agents reduce the risk of cardiovascular diseases by lowering total cholesterol, pro-atherogenic cholesterol and triglycerides levels (García Mesa, 2014).

Hyperlipidemia and hypolipidemia (its opposite) are types of dyslipidemias. The generic term dyslipidemia refers to alterations in the synthesis, transport or metabolism of lipids, which modify the plasma concentrations of total cholesterol, transporter lipoproteins, and triglycerides (Furgione et al., 2009; García Mesa, 2014). Primary dyslipidemia is due to genetic factors, and secondary dyslipidemia is due to environmental factors (diet, sedentary lifestyle) or pathologies such as obesity, diabetes, metabolic syndrome, among others (Maza Cave et al., 2000; Alegría Ezquerra et al., 2008).

Common feature of Hypercholesterolemia associated cardiovascular pathology, including atherosclerogenesis and myocardial infarction is associated with impaired lipid and lipoprotein metabolism. Several Hypercholesterolemia effects have been described as being atherogenic, such as direct vascular actions, oxidative stress, and thrombogenic factors and secondary dyslipidemia, whereas a large number of research groups depicted increased plasma levels of atherogenic lipoproteins (LDL-C and VLDL-C) and decreased levels of antiatherogenic lipoprotein (HDL-C) in hypercholesterolemia person. Besides, total cholesterol and triglycerides were increased and phospholipids were decreased in high cholesterol food exposed rats. To modulate the lipid and lipoprotein alterations several antioxidant and hyperlipidaemic herbs has been used that may be useful adjuncts in reducing the risk of cardio vascular diseases (Kmietowicz, 2002).

Atherosclerosis is the most frequent cause of morbidity and mortality in the entire world. Atherosclerosis is a multi-factorial disease and about 250 different risk factors have been recognized. It is thought that atherosclerosis is caused by a response to damage to the endothelium from high cholesterol, high blood pressure and cigarette smoking (Joshi et al., 2005 and Kabiri et al., 2010). There are the several main issues to be addressed in atherosclerosis, viz., hyperlipidemia, clotting factors, oxidation of low-density lipoproteins (LDL) and inflammation (Hansson, 2005). These factors collectively contribute to the development and rupture of atherosclerotic plaque (D''Souza et al., 2007). It can also be related to a hormonal disease such as diabetes mellitus, hypothyroidism and Cushing`s syndrome; or to the use of certain medication such as birth control pills, hormone therapy, some diuretics (i.e., water pills), or beta-blockers to treat cardiovascular diseases (Kreisberg and Reusch, 2005). In blood plasma, cholesterol is transported by lipoproteins, which can be mainly categorized into five classes, based on the size of cholesterol-lipoprotein complexes: chylomicrons, the very-low-density lipoproteins (VLDL), the intermediate density lipoproteins (IDL), LDL, and the high-density lipoproteins (HDL) (Steinberg, 2005). Experimental and clinical studies have shown that the amount of cholesterol transported in the Chylomicrons, VLDL, IDL and LDL classes of lipoproteins, known as pro-atherogenic cholesterol, is a risk factor for the occurrence of cardiovascular disease (Rudenko et al., 2010). Chylomicrons transport exogenous lipids to liver, adipose, cardiac and skeletal muscle tissue, where their triglyceride (TG) components are unloaded by the activity of lipoprotein lipase (LPL). Epidemiologic studies have reported that Triglyceride-rich particles such as chylomicrons and chylomicron remnants that carry dietary derived fats may play a role in the early stages of developing arteriosclerosis (Wilhelm and Cooper, 2003). VLDL is produced by the liver and some VLDL remnants seem to promote atherosclerosis similar to LDL (Vander Laan et al., 2009).

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The underlying mechanism of atherosclerosis involves the deposition and retention of serum lipids consisting of LDL cholesterol in the coronary arteries, resulting in decreased blood flow to heart muscles (Rudling, 2006). The oxidative modification of LDL plays a pivotal role in the progression of atherosclerosis and plaque formation. It is believed that modification of LDL in the arterial wall, particularly by oxidation, is crucial to the cellular uptake of LDL in the first stages of atherosclerotic plaque development (Hamad et al., 2010). Therefore, by preventing the oxidation of LDL, it may be possible to reduce the incidence of atherosclerosis. Lowering plasma cholesterol concentrations reduces the availability of atherogenic lipoproteins and also, presumably, the accumulation of cholesterol in the intima of arteries (Joshi, 2006). In contrast, cholesterol transported in HDL particles, known as anti-atherogenic cholesterol, has protective effect on cardiovascular disease (Fernandez and Webb, 2008).

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 hypocholesterolimic activity of ethanolic extract of *Brassica oleraceae* var *italica* in dalda induced hypercholesterolemic rats.

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±1^{0}C) 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 plasma were analyzed for total lipid (Chaudhary, 1989), total cholesterol (Varley, 1980), triacylglycerol (Henry et al., 1974), phospholipid, free fatty acid (Nath, 1990). Protein was estimated according to the method of Lowry et al. (1951) were analyzed.

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).

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 as mentioned in lipid profile, it has been observed that rats feed on dalda diet consecutively for 50 days resulted in a marked increase in the level of lipids, characterized by elevated levels of total lipids, phospholipids, fatty acid, total cholesterol, triglycerides, LDL, VLDL and reduced levels of HDL when compared to normal control, that is, rats receiving the normal feed. An increased level of LDL indicates hypercholesterolemia. No significant increase was found on the 50th day. However, treatment with *Brassica oleracea* for 50 days reversed the hyperlipedimic effect produced by high-fat diet significantly (< 0.001). Similar results were obtained with the

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standard drug Atorvastatin. Further, there was a significant increase in the atherogenic index in rats feed on dalda. Treatment with Atorvastatin (250mg/kg) significantly reduced the atherogenic index. The decrease in dietary hyperlipemia may partly be due to lipotropic effects of *Brassica oleracea* extracts, as evident from rise in FFA in treated rats as compared to control group of rats. Thus it can be concluded that hypolipidemic effects of *Brassica oleracea* extracts may be due to the unsaturated nature of aliphatic disulphide.

Table 2 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 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.

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 dyslipidemia research. Further, dyslipidemia is another important hallmark in the pathogenesis of obesity characterized by hypertriglyceridemia with decreased level of LDL and VLDL (Haley et al., 2013 and Klop et al., 2013). Chronic dyslipidemia has been characterized as a major risk factor for cardiovascular risk, including atherosclerosis (Martins and Redgrave, 2004). In the present study observed that rats feed on dalda diet consecutively for 50 days resulted in a marked increase in the level of lipids, characterized by elevated levels of total lipids, phospholipids, fatty acid, total cholesterol, triglycerides, LDL, VLDL and reduced levels of HDL when compared to normal control, that is, rats receiving the normal feed. An increased level of LDL indicates hypercholesterolemia. The increase in the level of HDL was found to be in a dose dependent manner; that is, supplementation with *Brassica oleracea* at a dose of 250mg/kg shows a better effect in comparison to control. Similar results were obtained by Ghasi et al. (2000) where treatment with crude extract of *Moringa oleifera* led to an increased serum HDL level and decreased levels of total cholesterol, LDL, and triglyceride.hus, it can be concluded that leaves of M. oleifera possess cardioprotective potential (Nandave et al., 2009). Further, atherogenic index is regarded as a marker for various cardiovascular disorders; the higher the value, the higher the risk of developing cardiovascular disease and vice versa (Takasaki, 2005 and Altunkaynak, 2005). High-fat diet exposure resulted in the increased atherogenic index. Treatment with *Brassica oleracea* for 50 days reversed the hyperlipedimic effect produced by high-fat diet significantly (< 0.001). Similar results were obtained with the standard drug Atorvastatin. Further, there was a significant increase in the atherogenic index in rats feed on dalda. Treatment with Atorvastatin (250mg/kg) significantly reduced the atherogenic index. The decrease in dietary hyperlipemia may partly be due to lipotropic effects of *Brassica oleracea* extracts, as evident from rise in FFA in treated rats as compared to control group of rats. Thus it can be concluded that hypolipidemic effects of *Brassica oleracea* extracts may be due to the unsaturated nature of aliphatic disulphide.

In order to supplement the results, 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 marker enzymes in group II rats. The literature review revealed that high fat diet-induced obesity and abnormal lipid metabolism all collectively are associated with inflammation, congestion and nonalcoholic fatty liver disease (NAFLD) leading to hepatic failure causing a boost in SGOT, SGPT and total bilirubin level in the serum (Conkova et al., 2001 and Kameshwaran et al., 2013). Our results showed that consumption of dalda diet may play a crucial role in the pathogenesis of fatty liver or hepatic steatosis associated with obesity depicted via ballooning degeneration. Elevated levels of liver enzymes are a monitor of hepatocellular damage and correlate with increased liver weight (Reddy and Rao, 2006). The results obtained in the present study established that dalda diet causes hepatocellular damage, as clearly seen by the marked elevation of serum enzymes (SGOT, SGPT and ALP) activities and histopathological studies of liver exaggerated with hepatic steatosis.

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.

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 hypercholesterolemic 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 hypercholesterolemic property. 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 lipid profile in dalda stimulated rats.

S. No	Treatment group	Normal saline	Positive control (100 mg/ kg of dalta)	100mg/kg of dalda + 250 mg/kg of B. oleracea	100mg/kg of dalda + 10mg/kg of Atorvastatin
1	Lipid (mg/dl)	1.17 ± 1.18	6.12 ± 1.24	2.13 ± 1.09	1.81 ± 1.13
2	Fatty Acids (mg/dl)	73 ± 2.12	156 ± 2.34	86 ± 2.21	81 ± 2.38
3	Phospholipid (mg/dl)	105 ± 2.22	218 ± 2.72	122 ± 2.43	112 ± 2.28
4	Cholesterol (mg/dl)	84 ± 1.19	192 ± 1.14	98 ± 1.27	92 ± 1.16
5	Triglyceride (mg/dl)	62 ± 2.39	135 ± 2.14	85 ± 2.48	70 ± 2.19
6	HDL (mg/dl)	25.8 ± 2.15	14.2 ± 2.29	22.5 ± 2.48	23.7 ± 2.42

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7	LDL (mg/dl)	41.3 ± 2.18	85.2 ± 2.24	53.9 ± 2.17	43.1 ± 2.29
8	VLDL (mg/dl)	11.5 ± 2.12	19.1 ± 2.28	13.9 ± 2.31	12.4 ± 2.72
9	Protein (g/dl)	7.2 ± 1.12	4.8 ± 1.19	6.6 ± 1.35	6.8 ± 1.14

Table 2. 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

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