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Comparative Physiological Study on the Effect of Rosemary, Tarragon and Bay Leaves Extract on Serum Lipid Profile of Quail, *Coturnix coturnix*

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Abstract

The potential health benefits of various herbs in relation to protect atherosclerosis and heart diseases are currently receiving considerable attention. The recent popularity in use herbals can be tied to the belief that herbs can provide some benefit over and above allopathic medicine and allow users to feel that they have some control in their choice of medication. Investigation of possible effect of rosemary, tarragon and bay leaves extract supplementation on serum cholesterol, triglycerides, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) of quail, *Coturnix coturnix*, was aimed in the present research. Administration of rosemary leaf extract daily for 3 weeks caused significant declines in the blood levels of triglycerides (45.47%), cholesterol (29.54 %), LDL cholesterol (76.98%) and VLDL cholesterol (45.47%), and increase HDL cholesterol (19.86%). Rosemary leaf extract had a comparatively greater hypolipidemic potential than tarragon and bay leaves extract. These extracts may act through several mechanisms to provide hypolipidemic role.

Key words: Lipid profile, rosemary, tarragon, bay, *Coturnix coturnix*

Introduction

Atherosclerosis and coronary heart disease are the major health problem in developed and modern societies (Braunwald, 1997; Breslow, 1997; Law, 1999). A number of epidemiological investigations have shown a clear association between dietary saturated fat and atherosclerosis (Shekelle *et al*.,1981; Posner *et al.,* 1991). The composition of human diet plays an important role in the management of lipid and lipoprotein concentrations in the blood (NCEP, 2001). The importance of serum lipoprotein disturbances and abnormal lipid metabolism characterized by hyperlipidaemia or hyperlipoproteinmia as etiological factors in the development of coronary heart diseases and potentiating of arteriosclerosis is now supported by a considerable body of evidence amassed from epidemiological and population studies (Arteriosclerosis, 1971; Coronary Drug Project Research Group, 1975; Turpanen, 1979; Lipid Research Clinics Programme, 1984). Moreover, many studies have now shown that elevated concentration of total or Low density lipoprotein (LDL) cholesterol in the blood are powerful risk factors for coronary heart disease (Law, 1999), whereas high concentrations of high density lipoprotein (HDL) cholesterol or a low LDL (or total) to HDL cholesterol ratio may protect against coronary heart disease (Sheten *et al.,* 1991; Castelli *et al.,*1992). Although attention has focused mainly on ability of HDL to participate in the removal of cholesterol from sites of atherosclerosis lesion development via a process termed "reverse cholesterol transport" as the mechanism likely responsible for the

observed inverse relationship between blood HDL cholesterol levels and incidence of heart disease (Von Eckardstein *et al.,*2001), HDL also exhibit a number of other potentially cardioprotective properties. These include preservation of vascular endothelial function, inhibition of platelet activation, anticoagulant and profibrinolytic activities, and protection of LDL from oxidation (Nofer *et al.,* 2002).

The use of herbs as medicines has played an important role in nearly every culture on earth, including Asia, Africa, Europe and the Americas (Wargovich *et al.,* 2001). Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. Several herbs can help to reduce high blood cholesterol concentrations, provide some protection against cancer and stimulate the immune system. Furthermore, a diet in which culinary herbs are used generously to flavor food provides a variety of active phytochemicals that promote health and protective against chronic diseases. Additionally, several commonly used herbs have been identified by the National Cancer Institute as possessing cancer-preventive properties. These herbs include members of the *Allium* sp. (garlic, onions and chives), members of the *Labiatae* (mint) family (basil, mints, oregano, rosemary, sage and thyme), members of *Zingiberacae* family (turmeric and ginger), licorice root, green tea, flax, members of *Umbelliferae* (carrot) family (anise, caraway, celery, chervil, cilantro, coriander, cumin, dill, fennel and parsley) and tarragon. Also, many herbs contain a variety of phytosterols, triterpenes, flavonoids, saponins and carotenoids which have been shown from studies of legumes, fruit and vegetables to be cancer chemoprotective (Steinmetz and Potter, 1991). A plant-based diet that is rich in fruit, vegetables and legumes and low in saturated fat, along with regular aerobic exercise program, is a typical prescription or anyone with elevated risk of cardiovascular disease. Moreover, there are a few herbs available that provide some help for persons either hyperlipidemia, an abnormal tendency to form blood clots, impaired blood flow, or other cardiovascular problems. Whereas some herbal products may be safe and may contain active constituents that have beneficial physiological effects, others may be unsafe to use. The Food and Drug Administration has classified several herbs as unsafe, even in small amounts, and hence they should not be used in foods (Larkin, 1983; Saxe, 1987). Some herbs are safe in modest amounts but they may become toxic at higher dose. Other herbs are known to be lethal (Nielsen and Pederson, 1984; Mostefa-Kara *et al.,* 1992; Chan *et al.,* 1993). Over 4000 flavonoids have been identified in plants (Hollman, 1997). These universal plant pigments are responsible for the colours of flowers, fruits and sometimes leaves (Bruneton, 1995). The commonly used herbs that provide substantial amounts of falvonoids include chamomile, dandelion, ginkgo, green tea, hawthorn, licorice, passionflower, milk thistle, onions, rosemary, sage, thyme and yarrow. Flavonoids have extensive biological properties that promote human health and help reduce the risk of disease. Flavonoids extend the activity of vitamin C, act as antioxidants, protect LDL cholesterol from oxidation, inhibit platelet aggregation and act as antiinflammatory and antitumour agents (Smith and Yang, 1994; Cook and Samman, 1996). Rosemary (*Rosmarinus officinalis* Linn.), mint (*Lbiatae*) family, is a common household plant grown in many parts of the world. It is commonly used as a spice and flavoring agent in food processing (Saito *et al.,* 2004). Also, rosemary is used as an antispasmodic in renal colic and dysmenorrhoea, in relieving respiratory disorders and to stimulate growth of hair. Extract of rosemary relaxes smooth muscles of trachea and intestine, and choleretic, hepatoprotective and antitumergenic activity. However, rosemary and its constituents have a therapeutic potential in treatment or prevention of bronchial asthma, spamogenic disorders, peptic ulcer, inflammatory diseases, hepatotoxicity, atherosclerosis, ischaemic heart diseases, cataract, cancer and poor sperm motility (Al-Sereiti *et al.,* 1999; Masuda *et al.;* 2002 Sotelo-Fleix *et al.;* 2002; Osakabe *et al.,* 2004). Tarragon, *Artemesia dracunulus* is a member of the *Asteraceae* (sunflower) family. The leaves are used as seasoning in soups, mayonnaise, butter, stews, sauces and vinegars. Tarragon is also used in perfumes, soaps and cosmetics. Tarragon is a recognized herbal treatment for many conditions and symptoms including insomnia, toothache, upset stomach, loss of appetite, intestinal worms, hyperactivity, antibacterial

properties for cuts and depression. Bay leaf , *Laurus nobilis,* belongs to the family *Lauraceae,* and it is on of the most popular culinary spices in Western countries. Bay leaf has been used as herbal medicine and has pharmacological activity which includes antibacterial, antifungal, antidiabetes and antiinflammatory effects (Fang *et al.,* 2004).

The present research was designed to evaluate the effects of rosemary, tarragon and bay leaves extract supplementation for 3 weeks on blood lipid profile of quail, *Coturnix coturnix*. Blood lipid profile includes determination of the values of cholesterol, triglycerides, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL). Quail was chosen as an experimental model, which probably allows extrapolation results to mammals.

Materials and Methods

Forty healthy mature male and female quails, *Coturnix coturnix,* average body weight 164.8 g, were used for the present study. Quails were divided into 4 groups of 10 animals in each on the basis of their average body weight. The experimental animals were acclimated for one week prior to commencement of the experiment. Birds were kept at 26±1 °C, exposed to 12h. light/ 12h. dark cycle and fed *ad libitum* with free access to water.

Leaves extract preparation

Fine quality leaves of rosemary and tarragon (McCormick Com., Inc. Hunt Valley, USA), and bay (American garden products seaford, NY.USA) were obtained from a commercial market. The aqueous extracts of these leaves were prepared daily by boiling 2g in 100 ml water for 3min and cooling down to room temperature. After separation of undissolved residue, the solutions were used for experiment.

Experiment

After acclimation period, the experimental animals were treated for 3 weeks as following:

Group 1: Experimental animals were untreated and served as control.

Group 2: Birds were administrated of 0.5ml rosemary leaf extract, daily, by intragastrric intubation.

Group 3: Animals were fed with 0.5ml of tarragon leaf extract, daily.

Group 4: quails were given 0.5ml of bay leaf extract, daily.

At the end of treatment period, all quails were sacrificed and blood sera were obtained and stored at 4°C prior to determination the levels of cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol by using BM/Hitachi system 717 Automatic Analyzer. Low density lipoprotein (LDL) cholesterol and very Low density lipoprotein (VLDL) cholesterol were calculated by the following formulas:

$$\text{LDL Cholesterol} = \frac{\text{Triglycerides}}{5} + \text{HDL}$$

Cholesterol - Total Cholesterol

$$\text{VLDL Cholesterol} = \frac{\text{Triglycerides}}{2.175}$$

Statistics

Computerized one-way Analysis of Variance (ANOVA) was used for statistical analysis. The chosen level of significance is $p \leq 0.05$.

Result

Table 1. Summarizes the quantitative data of blood lipid profile of the experimental animals, *Coturnix coturnix,* administrated of rosemary, tarragon and bay leaves extract. In comparison with control, treatment of quails with rosemary leaf extract (Group 2) showed a significant reduction in the levels of blood triglycerides (45.47%), cholesterol (29.54 %), LDL cholesterol (76.98%) and VLDL (45.47%), whereas the value of HDL cholesterol was increase (19.86%). The level of LDL cholesterol was decline (46.63%) and HDL cholesterol was significantly increase (29.43%) in quails treated with tarragon leaf extract (Group 3). Also, The levels of triglycerdies, cholesterol and VLDL cholesterol were significantly unchanged. Notably, animal exposed to bay leaf extract (Group 4) showed reduction in the values of blood cholesterol (10.13%) and LDL cholesterol (51.22%), whereas the level of HDL cholesterol was enhance (16.65%). The levels of triglycerides and VLDL cholesterol were

Table 1. Blood lipid profile (X±S.D.) of quail, *Coturnix coturnix* (n=6) treated with rosemary, tarragon and bay leaves extract.

	Treatment			
Parameter	**Control**	**Rosemary**	**Tarragon**	**Bay**
Triglycerides (mg/dl)	163.57±28.80	89.20±6.89* (-45.47%)	156.76±26.88 (-4.16%)	130.36±21.08 (- 20.30%)
Cholesterol (mg / dl)	225.30±14.67	158.75±23.13* (- 29.54%)	226.50±15.26 (+.53%)	202.48±19.21* (- 10.13%)
HDL Cholesterol (mg /dl)	113.63±17.04	136.20±17.04* (+ 19.86%)	147.07±18.27* (+29.43%)	132.55±6.13* (+16.65%)
LDL Cholesterol (mg/dl)	90.08±9.15	20.74±12.13* (-76.98%)	48.08±21.00* (-46.63%)	43.94±23.38* (-51.22%)
VLDL Cholesterol (mg /dl)	75.20±13.25	41.01±3.17* (-45.47%)	72.07±12.36 (-4.16%)	59.98±9.69 (-20.24%)

* Statistically significant at $P \leq 0.05$ One-Way Analysis of Variance (ANOVA).
+ or - indicates increase or decrease percentage.

significantly unchanged in the fourth group.

Discussion

Coronary heart disease and acute myocardial infraction, a leading cause of death, is due to weakening of the muscle of the heart or cardiac dystrophy, as it is commonly referred to. Cardiac dystrophy is the result of reduced blood (oxygen) transport to the heart muscle, due to the narrowing (stenosis) of the blood vessels, including the arteries of the heart. Stenosis results from the formation of the so-called atherosclerotic plaque in the blood vessels and arteries. Atherosclerotic plaque formation is the result of deposition of, among other things, foam cells, platelets and cholesterol. Cholesterol deposition, although partly genetic, is also affected by diet. Low density lipoproteins (LDL), the main cholesterol carriers in blood circulation, deposit cholesterol in arterial wall, thereby contributing to atherosclerotic plaque formation. On the other hand, high density lipoproteins (HDL) take up deposited cholesterol from the arterial wall and transfer it back to the liver for excretion, thereby inhibiting the formation of atherosclerotic plaque. For these reasons, LDL cholesterol is considered atherogenic ("bad cholesterol") and HDL cholesterol antiatherogenic ("good cholesterol"). The present result of oral administration of rosemary leaf extract caused significant declines in the blood levels of triglycerides, cholesterol, LDL cholesterol and VLDL cholesterol, and increase HDL cholesterol. Moreover, it seemed that rosemary leaf extract had a comparatively greater hypolipidemic potential than tarragon and bay leaves extract. This is may be an indication of progressive metabolic control of rosemary leaf extract on mechanisms involved in elimination of the lipids from the body. Hypolipidemic properties have been confirmed in many plant species and plant products in medicinal use (Kono *et al.*, 1992; Naidu and Thippeswamy, 2002; Devi and Sharma, 2004). The most important constituents of rosemary are caffeic acid and its derivatives such as rosmarinic acid. These compounds have antioxidant effect (Al-Sereiti *et al.,* 1999). A variety of phenolic compounds, in addition to falvonoids, are found in fruit, vegetables and many herbs. The phenolic compounds (such as caffeic, ellagic, and ferulic acids, sesamol, and vanillin) inhibit atherosclerosis (Decker, 1995). In addition to a well-documented role in reverse cholesterol transport, HDL have recently been recognized to have several other important cardioprotective properties including the ability to protect LDL from oxidative modification (Nofer *et al.,* 2002). Also, Parthasarathy *et al.* (1990) suggested that HDL may play a protective role in atherogenesis by preventing the generation on an oxidatively modified LDL and the mechanism action of HDL may involve exchange of lipid peroxidation products between the lipoproteins. HDL is the major carrier of cholesteryl ester hydroperoxides, but more than this it appears to have the prolonged capacity to decrease the total amount of lipid peroxides generated on LDL during oxidation while the quantity

accumulating on HDL itself reaches an early plateau. These effects are not explained by chain-braking antioxidants present in HDL and are likely to involve an enzymic mechanism. Several enzymes are present on HDL: paraoxonase (an enzyme normally resident on HDL), lecithin:Cholesterol acyl transferase, platele activating factor acetylhydrolase, phospholipase D and protease. Apolipoproteins, such as apolipoprotein AI, could also have enzymic activity (Mackness and Durrington, 1995). Mackness *et al.* (1993) suggested that a direct role for HDL in preventing atherosclerosis probably by an enzymic process which prevents the accumulation of lipid peroxides on LDL. They reported that paraoxonase is an example of an enzyme which might possibly be involved. Also, Bonnefont-Rousselot *et al.,* (1999) reported that the oxidative hypothesis of atherosclerosis classically implies a central role for LDL oxidation. However, new antiatherogenic properties have been recognized for HDL, apart from their ability to reverse cholesterol transport. Indeed, native HDL could protect LDL from oxidation, thereby minimizing the deleterious consequences of this process. Several mechanisms have been suggested to explain this protective role. Two HDL-associated enzymes, paraoxonase and platelet activating factor acetylhydrolase (PAF-acetylhydrolase), detoxify oxidized phospholipids produced by lipid peroxidation. In addition, HDL could reduce hydroperoxides to their corresponding hydroxides. It has also been suggested that HDL could inhibit oxidized LDL-induced transduction signals. However, in vivo HDL oxidation in the subendothelial space would favor the atherosclerotic process. Indeed, atherogenic properties of these oxidized HDL partly result from some loss of their cholesterol effluxing capacity and for an inactivation of lecithin-cholesterol acyltransferase, which is a HDL-associated enzyme involved in reverse cholesterol transport. Finally, oxidized HDL could induce cholesterol accumulation in macrophages. However, the mechanism by which rosemary, tarragon and bay leaves extract, especially rosemary, exhibit hypolipidemic role is unknown. It is probably that rosemary leaf changed the rate of fatty acids oxidation in the liver and reduced the rate of triglycerides biosynthesis in quails. The increased availability of triglycerides may be required for the formation of VLDL as a vehicle to transport the excess cholesteryl ester out of the liver. Also, it can not excluded that the possibility that all leaves extract, particularly rosemary, decreased LDL-cholesterol production and LDL oxidation, changed LDL receptor activity and uptake of LDL-cholesterol by hepatocytes and Kupffer cells. Fuhrman *et al.* (2000) reported that polyphenols glabridin (derived from licorice), rosmarinic acid or carnosic acid (derived from rosemary), as well as garlic (which contains a mixture on natural antioxidants) inhibited LDL oxidation in a dose-dependent manner. Moreover, several studies showed that plant extracts lowered LDL oxidation (Ramirez-Tortosa *et al.,* 1999; Doi *et al.,* 2000; Naidu and Thippeswamy, 2002). However, the present data demonstrated that consumption of these leaves may be can lead to reduction in the risk of hyperlipidemic symptoms and heart diseases.

It can be concluded from presented results that rosemary, tarragon and bay leaves extract expressed hypolipidemic role. Moreover, additional investigation will be needed to purify the bioactive constituents in the extract of these leaves and use the purified constituents for bioassy-directed experiments either in hyperlipidemic or non- hyperlipidemic organisms.

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دراسة فسيولوجية مقارنة عن تأثير مستخلص أوراق إكليل الجبل و الطراجون و الغار على مظهر دهون السيرم في طيور السمان

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ملخص
تلقى حديثاً المنافع الصحية العالية للأعشاب المختلفة ذات العلاقة بوقاية تصلب الشرايين و أمراض القلب اهتماماً على نحو اعتباري. ترتبط العادات الشعبية الحديثة في استخدامها الأعشاب مع الاعتقاد بأن الأعشاب تستطيع أن تمدها ببعض الفوائد الناجعة علاوة على استخدامها في عدة أغراض طبية و بذلك تسمح للمستخدمين الشعور للحصول على بعض التحكم في اختيارهم للمداواة و التطبيب.
هدف البحث الحالي إلى استقصاء إمكانية تأثير مستخلص أوراق إكليل الجبل و الطراجون و الغار على كوليسترول و الجليسريدات الثلاثية و الليبوبروتينات عالية الكثافة و الليبوبروتينات منخفضة الكثافة و الليبوبروتينات منخفضة الكثافة جداً في سيرم طيور السمان. أحدث مستخلص ورقة إكليل الجبل بعد ثلاثة أسابيع تناقصاً معنوياً في مستوى الجليسريدات الثلاثية (٤٧ ,٤٥٪) و الكوليسترول (٥٤ ,٢٩٪) و الليبوبروتينات منخفضة الكثافة (٩٨ ,٧٦٪) و الليبوبروتينات منخفضة الكثافة جداً (٤٧ ,٤٥٪) و زادت الليبوبروتينات عالية الكثافة (٨٦ ,١٩٪). أظهر مستخلص ورقة إكليل الجبل إمكانية عالية كمخفض للدهون على نحو مقارن بالنسبة لمستخلص أوراق الطراجون و الغار. يحتمل أن هذه المستخلصات تؤثر من خلال عدة آليات لتظهر دورها كمخفض للدهون.