

ANTIATHEROGENIC EFFECT OF *NIGELLA SATIVA* L. (KALONJI) SEEDS IN RABBITS WITH EXPERIMENTALLY-INDUCED HYPERCHOLESTEROLEMIA

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ABSTRACT

The present paper investigates the hypolipidemic effect of *Nigella sativa* L. administration on diet-induced hypercholesterolemia. Sixteen age-matched rabbits were divided into two experimental groups. Base line values of the requisite parameters were observed and animals were then administered atherogenic diet for four weeks. 100mg/kg of body weight/ day *N.sativa* seed powder were fed to these hypercholesterolemic rabbits for another four weeks. At the end of experimental period, blood samples were collected and assayed for alterations. The administration of *N. sativa* seed powder in hypercholesterolemic animals significantly decreased plasma cholesterol, triglyceride and LDL-C concentrations and increased plasma HDL-C concentration. Beneficial effects were also observed on serum ALT, AST, glucose and GSH levels. These findings suggested cardioprotective effect of dietary supplementation of *N.sativa*.

Key-words: *N. sativa* (Kalonji) seed powder, hypercholesterolemia, Atherosclerosis, Rabbits, Hypolipidemic effects.

INTRODUCTION

High levels of total cholesterol and low-density lipoprotein (LDL) cholesterol, reduction in high-density lipoprotein (HDL) cholesterol and increase in triglyceride (TG) levels predispose to coronary disease (Castelli, 1998). Genetic studies in humans and transgenic mice have established a strong link between dyslipidemia and atherosclerosis (Attie, 2001). In recent years, evidence has accumulated that a number of cardiovascular risk factors other than traditional risk factors may contribute to the development of atherosclerosis. Among these risk factors, LDL oxidation and LDL particle size have also received extensive attention for their atherogenic potentials (Oparil and Oberman, 1999; Kullo *et al.*, 2000). Atherogenic lipoprotein phenotype (ALP) syndrome has been identified as a common dyslipidemia in patients with coronary heart disease (CHD) (Swinkels *et al.*, 1989; Austin *et al.*, 1990; Griffin *et al.*, 1994). In view of the significant role of oxidative stress and dyslipidemia in the coronary artery disease, it seems appropriate to investigate the role of non-toxic and safe products, which can lower the risk of coronary artery disease (CAD) by mediating two above-given factors. Dietary supplementation with nutrients rich in antioxidants is associated with inhibition of atherogenic modifications to LDL and atherosclerosis. Based on this approach, the present study is undertaken to investigate the role of *N. sativa* (seed powder) consumption in the management of dietary hyperlipidemia. *N. sativa* L. (Kalonji) belongs to family Ranunculaceae. Its seeds are used in herbal medicine all over the world for the treatment and prevention of a number of diseases including asthma, diarrhoea and dyslipidemia (Ali and Blunden, 2003). Present study aims at identifying the exclusive role of this nutritional antioxidant in reduction of CAD risks.

MATERIAL AND METHODS

The animals:

A total of 16 female white rabbits were employed in this research. The animals were three months old at the start of experiment, and had body weight ranging from 1.5 to 2.0 kg.

Experimental Protocol:

Initially all rabbits were acclimatized for about a week in animal house of Department of Physiology (University of Karachi, Karachi). Animals were housed at 25°C with a 14-h light: 10h dark cycle. Body weights and other physical conditions were closely monitored through out the study. After an overnight, fast blood was drawn from marginal ear vein and base line values for the requisite parameters were checked. Rabbits were then randomly divided into two experimental groups. Group I (n = 8) animals were fed normal rabbit chow and served as control. Group II (n = 8) animals received an atherogenic diet (1g butter fat / 100g of daily diet) for four weeks (modified from Moghadasian *et al.*, 1999). Food and water were provided *ad libitum* during the study and food intake was recorded periodically to avoid differences between groups in the amount of feed consumed. After four weeks: Group

II animals were maintained on atherogenic diet and in addition received 100mg / kg of body weight *N. sativa* orally for four weeks (Khan and Sultana, 2005). Blood samples were collected from all animals after every dietary modification and body weight, plasma lipid profile, glucose, blood glutathione (GSH) concentration and levels of alanine aminotransferase (ALT) & aspartate amino transferase (AST) were determined.

Biochemical Analysis:

Plasma cholesterol and triglyceride levels were measured using enzymatic kit (Clonital Italy). Serum HDL-C levels were measured by dextran sulphate Mg (II) method, using enzymatic kit (QCA, France). Serum LDL-C concentration was determined with polyvinyl sulphate method using enzymatic kit (QCA, France). Blood GSH levels were measured by the method of Beutler et al., (1963). Plasma glucose concentration was determined by O-toluidine method (Winkers and Jacobs, 1971). ALT and AST were measured with Rietman-Frankel colourimetric method using enzymatic kit (QCA, Spain).

Statistical Analysis:

The data expressed as mean \pm S.E.M. and were analyzed by t-test. A value of $p < 0.05$ was chosen as the criteria of statistical significance.

RESULTS

All control and treated groups of animals that were fed with hypercholesterolemic diets and *N. sativa* during the study showed no signs of toxicity or discomfort. In control animals values of all parameters were stable throughout the experimental period.

Effect of atherogenic diet administration:

With administration of 1g butter fat /100g of daily diet for four weeks, significant ($P < 0.05$) increase in body weights (12%), plasma total cholesterol (87%), triglyceride (67%), and lipoprotein levels of animals were observed as compared to control group (Table I). Plasma ALT and AST levels increased significantly ($P < 0.05$) however a non significant 10% increase was observed in plasma glucose concentration of animals at the end of experimental period. Erythrocyte glutathione concentration also showed significant (45%) decrease as compared to control group (Table I).

Table1. Effects of *N. sativa* seed powder oral administration in rabbits with experimentally-induced hypercholesterolemia.

| PARAMETERS | | EXPERIMENTAL GROUPS | | |
|----------------|-----------------------|---------------------|---------------------------------|--------------------------------|
| | | CONTROL | HYPERLIPIDEMIC | N.SATIVA TREATED |
| BW | (grams) | 1436.12 \pm 49.21 | 1601.75 \pm 36.6*** | 1498.5 \pm 39.04* |
| TC | (mg/dl) | 60.69 \pm 7.03 | 113.53 \pm 8.66*** | 94.19 \pm 8.13*** |
| TG | (mg/dl) | 78.25 \pm 7.89 | 131.05 \pm 9.42*** | 109.14 \pm 8.31** |
| HDL | (mg/dl) | 27.07 \pm 1.49 | 42 \pm 4.47*** | 43.14 \pm 3.79*** |
| LDL | (mg/dl) | 17.96 \pm 5.21 | 45.31 \pm 8.72* | 29.21 \pm 6.02 ^{NS} |
| GLUCOSE | (mg/dl) | 114.36 \pm 17.18 | 125.97 \pm 7.14 ^{NS} | 93.12 \pm 2.65 ^{NS} |
| GSH | (nmol/g of Hb) | 0.46 \pm 0.05 | 0.25 \pm 0.05*** | 0.41 \pm 0.08 ^{NS} |
| ALT | (IU) | 4.28 \pm 0.27 | 5.43 \pm 0.28*** | 4.81 \pm 0.11 ^{NS} |
| AST | (IU) | 3.56 \pm 0.11 | 6.76 \pm 0.09*** | 3.5 \pm 0.33 ^{NS} |

BW= Body weight; TC = Total cholesterol; TG = Triglyceride; HDL = High density lipoprotein; LDL = Low density lipoprotein; GSH = Glutathione; ALT = Alanine aminotransferase; AST= Aspartate aminotransferase.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; NS = non significant.

Effect of N. sativa administration on Blood lipid profile:

100mg/kg of body weight/day *N. sativa* administration for four weeks produced significant antihyperlipidemic action. Plasma total cholesterol, triglyceride and LDL-C significantly ($P < 0.05$) decreased as compared to hypercholesterolemic group but this decreased level was still significantly higher than control group (Table I). A non significant increase was observed in plasma levels of HDL-C as compared to hypercholesterolemic group but this increase was significantly different ($P < 0.05$) different from that of the control group.

Effect of N. sativa administration on liver enzymes:

Oral administration of *N. sativa* failed to produce significant change in plasma ALT levels, however, plasma AST level showed significant ($P < 0.005$) decrease in treated animals (Table I).

Effect of N. sativa administration on plasma glucose and erythrocyte glutathione concentration:

Plasma glucose concentration significantly decreased ($P < 0.05$) in treated animals where as no significant change was observed in blood glutathione concentration (Table I).

DISCUSSION

The major cause of CHD is atherosclerosis with reference to major and minor etiological and pathogenetic factors associated with atherosclerosis, hyperlipidemic states especially hypercholesterolemia have been widely studied (Ahmed *et al.*, 1992). Present studies were undertaken to investigate the antiatherogenic effectiveness of *N. sativa* (seed powder) administration in cholesterol fed rabbits. We found beneficial effects of *N. sativa* seed powder, orally administered at the rate of 100 mg /kg of body weight, on plasma lipid profile in hypercholesterolemic rabbits. Plasma total cholesterol, triglyceride and LDL concentrations were found to be reduced significantly whereas HDL levels showed insignificant increase with *N. sativa* administration. These anti-hyperlipidemic effects are consistent with many previous studies (Zaoui *et al.*, 2002; Dahiri *et al.*, 2005). Lipid lowering effects of *N. sativa* may be hypothesized to be due to the presence of monounsaturated fatty acids as diets containing monounsaturated fatty acids (like olive oil) have been known to increase serum HDL and decrease LDL levels (Sundram, 1992). Thymoquinone and polythymoquinone that are active components of *N. sativa* are reported to increase bile excretion in rats, dogs and guinea pigs and that in turn could be related with decreased plasma cholesterol concentration (El-Dakhkhani, 1982). Hyperglycemia is another important cardiovascular risk factor (Balkau *et al.*, 1998; Wei *et al.*, 1998) possibly because of non-enzymatic glycation of proteins (Witztum and Koschinsky, 1989) and lipoproteins, which increase their atherogenic potency. *N. sativa* administration in present study was found to be associated with significant decrease in plasma glucose concentration of animals and this hypoglycemic action of *N. sativa* appeared to be due to inhibition of hepatic gluconeogenesis (Al Awadi *et al.*, 1991).

Role of oxidative stress in the development of atherosclerosis has been evident from many previous studies (Diaz *et al.*, 1997; Frei, 1999). GSH is a cysteine containing tripeptide found in mammalian cells (Bray and Taylor, 1993). Lower glutathione and elevated lipid peroxidation concentrations are considered as risk factors for the development of pathological states such as retinopathy, neuropathy, cataract and atherosclerosis (Comporti, 1987; Bayness, 1991; Giugliano *et al.*, 1996). Burits and Bucar (2000) found that *N. sativa* essential oil and its four constituents (thymoquinone, carvacrol, t-anethol and 4-terpineol) had anti-oxidant effect in different chemical assays, like diphenylpicrylhydrazyl assay for non-specific hydrogen atom or electron donating activity. 100mg/kg of body weight *N. sativa* administration in present study showed a non significant increase in erythrocyte glutathione concentration in hypercholesterolemic animals. Fayed *et al.* (1998) in his study on diabetic rats also showed that antioxidant defenses including superoxide dismutase (SOD), GSH and total SH groups increased after food supplementation with *N. sativa* or fish oil to the diet of diabetic rats. These antioxidant effects of *N. sativa* may be due to inhibition of reactive oxygen species production (Ozugurlu *et al.*, 2005).

The most widely used pharmacological agents for the treatment of dyslipidemia in patients of CAD are "statins". Hepatotoxicity has been described with all statins and usually manifests as asymptomatic elevation of serum transaminases (aminotransferases). Persistent elevation greater than three times the upper limit of normal are considered significant and treatment should be discontinued if this occurs (Zhao *et al.*, 2003). As elevated ALT and AST levels is a specific index of liver cell damage, plasma levels of these two parameters were checked in this study to identify the effect of 100mg/kg of body weight/day *N. sativa* administration on liver cell functioning. Decreased plasma AST and ALT levels were observed with *N. sativa* consumption in present study. *N. sativa* administration has been shown to protect against liver damage in many other animal experiments. Daba and Rehman (1998), reported protective effect of thymoquinone on tertbutylhydroperoxide induced hepatotoxicity. Recently Mahmoud *et*

al., (2002) have also reported protective effect of 2.5 and 5ml *N. sativa* oil/kg, orally for two weeks on liver damage induced by *Schistosoma mansoni* infection in mice.

It may be concluded that oral administration of *N. sativa* seed powder at the rate 100mg/kg of body weight per day for four weeks shows significant anti-hyperlipidemic, anti-diabetic and antioxidant activity. It is beneficial in reducing CAD risks in rabbits with hypercholesterolemia.

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