The Impact of Four Potential Herbal Foods on Modifying Metabolic Parameters in Hypercholesterolemic Rats Model

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Abstract: The present investigation was performed to examine the effect of some herbal food plants (black seeds, curcumin roots, flax seeds and garlic at a level of 20% on liver enzymes function and lipids profile in hypercholesterolemic rats model on modifying some metabolic parameters. Forty two male male adult Wistar albino rats weighing initial mean body weight of approximately 120 ± 10g were used. The rats were divided into six groups of seven rats each with similar mean total weight. Group 1 served as normal control fed the balance diet at 14% protein level. The five groups rats were fed the the balance diet and high fat high cholesterol diet (HFHCD) at 1% cholesterol with 5% lump fat. Group 2 served as positive control. Groups 3, 4, 5 and 6 fed (HFHCD) with different types of tested herbal food plants (black seeds, curcumin roots, flax seeds and garlic at 20% level) for five weeks. Food and water were provided ad-libitum. At the end of the experiment, rats were sacrificed under ether anesthesia and blood sample was taken from orbitoocular vein from each rat. Blood samples were subjected to the determination of fasting blood glucose, serum lipid profile, serum protein and albumin, serum ALT, AST, GGT ,bilirubin, creatinine and urea. The results indicated that different types of tested herbal plants had a great effect in all parametars studied. Each of the herbal food proved to be effective to a greater extent in modifying the metabolic parameters, as compared with positive control group. Diets containing flax seed could be scored as the highest in term of modification of the metabolic parameters, from the qualitative and quantitative point of view

Key words: Hypercholesterolemic rats - Black seed-Curcumin- Flax seed- Garlic, Serum lipid profile, HFHCD, serum protein and albumin, serum ALT, AST, GGT, bilirubin, creatinine and urea.

INTRODUCTION

The effects of dietary components to the development of systemic pathologies such as hypercholesterolemia and atherosclerosis have received much attention (Cordain et al., 2005). One of the major constituents of the "western" diet (WD) is saturated fat; which correlates positively with the incidence of several co-morbid conditions (Law et al., 1994, Pietinen 1996, Hu et al., 1997, Clarke, et al., 997, Law et al., 1994 and Mozaffarian et al., 2004).

The third millennium is associated with increased life expectancy and greater media coverage of health care issues, and support for disease control or prevention. Hypercholesterolaemia and hypertriaclylglycerolaemia are independent risk factors of coronary vascular disease (CVD). Therapeutic measures designed to reduce blood lipid parameters can decrease the risk for CVD (Levine et al., 1995). The need for safe and effective lipid-lowering therapies directs significant attention towards adopting modern strategies for combating with emphasis on botanicals with history of use in folk medicine. The current research interest all over the world is directed towards innovative dietary compounds with specific effects on health promotion and reducing the risk of diseases. Selected phytochemicals may act as a modifying factor in the pathogenesis of metabolic syndrome (Minich, 2008) This is driving research interest, the pace of which has been greatly facilitated by the development of new molecular technologies.

The seeds of Nigella sativa Linn commonly known as black seed or fennel is an annual herb and is believed to be indigenous to the Mediterranean region with thymoquinone, being the main active component Ali and, Blunden, 2003).

It has wide application as a flavouring agent in bread and natural remedy for wide spectrum of diseases , (Randhawa MA 2008).

Turmeric is the dried ground rhizome of Curcuma longa Linn often used in curry dishes as a dietary spice in Middle Eastern cuisines. Curcuminoid pigments consist of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the proportion of 75: 20: 5. Curcumin has antioxidant and cytoprotective actions with ability to lower cholesterol, and to stimulate the production of bile, which is needed to digest fat and to protect the liver from the damaging effects of toxic chemicals and pharmaceutical drugs. (Ali and, Blunden, 2003, Randhawa, 2008 and, Chattopadhyay et al., 2004). Deters et al. (2003) also, investigated the effects of...
curcumin on cyclosporine-induced cholestasis and hyperlipidemia in a subchronic bile fistula model. Male Wistar rats were daily treated with curcumin (100 mg/kg of body weight per oral) cyclosporine (100 mg/kg of body weight, intraperitoneally) and a combination of curcumin with cyclosporine. After two weeks a bile fistula was installed into rats to measure bile flow and biliary excretion of bile salts, cholesterol, bilirubin, cyclosporine and its main metabolites. Blood was taken to determine the concentration of these parameters in serum or blood. Their results indicated that cyclosporine reduced bile flow (-14%) and biliary excretion of bile salts (-10%) and cholesterol (-61%). On the other hand, cyclosporine increased serum concentrations of cholesterol and triacylglycerols by 32% and 81% respectively. Also sole administration of curcumin led to a slight decrease of bile flow (-7%) and biliary bile salt excretion (-12%), but showed no effect on biliary excretion of cholesterol and serum lipid concentration. They found that when curcumin was given simultaneously with cyclosporine, the cyclosporine induced cholestasis was enhanced but the cyclosporine –induced hyperlipidemia was not affected. Neither the biliary excretion nor the blood concentration of cyclosporine was influenced by curcumin. The blood concentration of the main cyclosporine metabolites was lowered by half while their biliary excretion was strongly increased by curcumin. Their results concluded that curcumin is not able to prevent cyclosporine induced cholestasis and hyperlipidemia after prolonged administration in bile fistula rats. Administration of 500 mg curcumin to human volunteers for 1 week did produce significant change in serum cholesterol level, with curcumin tending to lower serum cholesterol and TG levels Levine et al., (1995).

Soni and Kuttan (1992) found that consumption of curcumin over 1 month or 6 months was reported not to affect blood concentrations of TG, or TC, LDL, and HDL-cholesterol. Baum et al. (2007) investigated the hypolipidemic effect of curcumin in high-fat diet treated rats. Furthermore, to investigate the mechanism of the hypolipidemic action of curcumin and examined the effect of curcumin on the regulation of gene expression of cholesterol 7a-hydroxylase (CYP7A1), a rate limiting enzyme in bile acid biosynthesis from cholesterol.

The flax seed (Linum usitatissimum) became one of the modern miracle food. It owes its healthy reputation primarily to three ingredients: Omega-3 essential fatty acids; the richest food source of lignans which have both plant estrogen and antioxidant qualities and dietary soluble & insoluble fiber. Benefits of flax seed as shown in human clinical trials and pathological animal model include lowering total cholesterol and LDL cholesterol levels and other indices of lipid homeostasis with apparent safety (Bloeden & Szapary 2004, Felmlee, et al., 2009, Hall et al., 2006, Lucas et al., 2002, and Tarpila et al., 2006).

Flaxseed contains approximately 41% fat and 21% protein of the seed weight Whole flaxseed is rich in protein, fat and insoluble and soluble fibers (Cunnane et al. 1995 and Whole Oomah and mazza 1993).The fat in the flaxseed is particularly rich in α-linolenic acid (LNA), which accounts for approximately 57% of the total fatty acids in flaxseed oil (Cunnane et al., 1993), α-linolenic acid has intrinsic lipid-lowering properties (Dodin et al., 2005).

Other major constituents of flaxseed which may be responsible for their lipid lowering effects are the phytoestrogens, namely isoflavones and lignans. Flaxseed is the richest source of lignans, which have also been reported to have hypolipidemic effects (Prasad, 1999). Lignans are a group of plant derived diphenolic compounds structurally related to endogenous estrogen that possess a wide range of biologic activities (Setchell and Adlercreutz 1988).

Garlic (Allium sativum) is widely used as a flavoring agent in food. It is also known to have biological activity for lowering hypertension; reducing blood triglycerides;total and LDL-cholesterol; risk factors associated with (CVD), (Chi, et al., 1982 Yeh and Liu, 2001).

Garlic was found in Egyptian pyramids and ancient Greek temple. Medical applications of garlic were mentioned in India, Egypt and Rome (Rivlin et al., 2006). Interest has increased considerably in finding naturally occurring antioxidants for use in foods, cosmetics or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Sasaki et al., 2002). Garlic has strong antioxidant properties and it has been suggested that garlic can prevent different diseases (Jalal et al., 2007, Jung et al., 2008 and Abd El-Razek et al., 2011).

The present work aims to assess the effectiveness of the above mentioned four herbal botanicals namely black currant; curcumin; flax seeds and garlic on modifying lipids profile and other risk factors in pathological animal model. Results coming out of the study may provide novel targets for prevention and/or treatment of the metabolic syndrome and the associated chronic inflammatory- mediated diseases

**MATERIAL AND METHODS**

**Materials :**

The black seed [Nigella sativa] was obtained from the local market and was ground in an electric mill and was saved frozen for subsequent use in biological trials

Curcumin Turmeric (Curcuma longa). The roots with their vibrant yellow color were purchased from the local retail market and were ground to fine flour and saved frozen for subsequent use in biological trials
Flax seeds *(Linum usitatissimum)* The seeds were ground in an electric mill and saved frozen in air tight containers for subsequent use in biological trials.

Fresh *garlic lobes* were purchased from the local retail market. Two kilogram of the fresh garlic was homogenized in an electric chopper and the resulting paste was saved frozen in air tight containers for subsequent use in biological trials. The incorporation of the botanicals was added at the expense of starch. The composition of the (6) diets is presented in Table (1). The composition of the vitamin mixture was according to that of AIN – (1993) and the mineral mixture was according to AOAC (2000).

**Biological Evaluation:**

**Design of the Experiment:**

Male adult *Wistar albino* rats were derived from the Animal House, Faculty of Medicine, King Saud Univ, Riyadh. The animals were housed individually in metabolic stainless steel cages in an environmentally controlled room at ± 22°C with a 12/12 h light: dark cycle and experiments were performed according to the guidelines of the Institutional Animal Ethics committee. They were given commercial diet for three days for acclimatization. On the day of the experiment, the rats were divided into six groups of 7 rats each with initial mean body weight of approximately 120 ± 10g. Each group received one of six different diets group (1) rats fed balanced control diet, its composition is presented in table (1). Pure cholesterol 1% was incorporated with 5% lump fat in the other 5 diets at a final concentrations to induce hypercholesterolemia by the (HFHCD). Group 2 served as positive control fed HFHCD (control +). Groups 3, 4, 5 and 6 were fed (HFHCD) with different types of tested herbal food plants (black seeds, curcumin roots, flax seeds and garlic at 20 % level respectively). The body weights were recorded weakly and the weight of diet residue remaining from the previous day was recorded and discarded. The feeding experiment lasted for 5 weeks. At the end of the experiment, the rats were fasted for 12 hr. with free access of drinking water then anaesthetized with diethyl ether for the blood collection from the orbitoocular vein. Serum was separated by centrifugation at 3000 rpm for 20 min. and aliquots of serum stored frozen at –20°C for subsequent biochemical analysis.

**Serum biochemical parameters:**

Blood glucose was assayed enzymatically after deproteinization using commercial kit (Glucose Assay Kit, Cayman USA). The concentration of triacylglycerols were assayed by the enzymatic endpoint method according to the instruction of the Kit’s manufacturer (Randox Laboratories, Antrim, UK) according to AOAC. Total cholesterol and high – density lipoprotein cholesterol (HDL) were analyzed using the automated Reflotron, according to Friedewald *et al.* (1972). The serum low –density lipoprotein cholesterol (LDL) was determined by calculation according to the equation >, Serum alanine amino transferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT); enzymes of liver function test were assayed using commercial kits according to instructions of the manufacturer (Randox Laboratories, Antrim, UK) Robertson (1981). The levels of serum total protein, albumin, total bilirubin (Randox Laboratories, Antrim, UK) were assayed by method of Owen *et al.* (1954), creatinine and blood urea, Richards *et al.*(1984) were measured by standard methods.

**Statistical analysis:**

Quantitative data were expressed as arithmetic means and standard error. One way analysis of variance (ANOVA) test was used to compare between groups. P-value = 0.05 or less was taken to signify statistical significance. (Snedecor and Cochran 1967).

**Results:**

The results obtained in this study are presented in Table (2, 3 and 4). Feeding the rats for 5 weeks the control (+) diet inducing hypercholesterolemia led to statistically significant increases in the mean level of nine biochemical parameters in the serum compared with respective mean values obtained among the group fed the control (-) diet.

Overall mean rise in total cholesterol was 118.2 %; in LDL cholesterol 393. %; in VLDL cholesterol 22.7 %. On the other hand, the concentration of serum HDL cholesterol was reduced significantly by 15.1 % An overall significant rise of 23.3 % was also found in the level of serum triglycerides and 14.3 % in fasting blood glucose concentration.

Furthermore, the activities of the serum enzymes of liver function test were increased by 32.1 % for ALT; 418.2 % for AST and by 25.8 % for GGT. The concentration of serum albumin was reduced by 21.9 % in the control (+) diet; whereas the respective overall mean rise in serum globulins averaged 12 %. The indicators of the kidney function; creatinine and blood urea also showed an overall rise of 38.9 % and 58.8 %, respectively in the serum of the group fed the control (+) diet.
The incorporation of black seed at 20% in the diet-induced hypercholesterolemia [HC] modified the levels of (Levine et al., 1995), biochemical parameters i.e., fasting blood glucose, triglyceride, VLDL cholesterol, albumin, globulin, bilirubin and creatinine to be overlapping with the respective mean levels of the control (-) (P>0.05).

The incorporation of curcumin at 20% in the [HC] diet modified the mean levels of Ali and Blunden (2003), biochemical parameters; whereas, the incorporation of flax seeds in the [HC] diet modified the mean level of Chattopadhyay et al., (2004). Biochemical parameters and garlic containing diet modified the mean level of (Randhawa, 2008).

Biochemical parameters to be overlapping with the respective mean values of the control (-) (P>0.05)

**Discussion:**

High blood cholesterol level is a major risk factor for cardiovascular disease Girard (2010). Recent investigations have focused on elucidating the contribution of diet to the development of systemic pathologies. Cholesterol containing diets had been widely used as a model to induce pathologies in animal models, (Ogawa et al., 2010). Animal models have revealed that diet-induced hypercholesterolemia (HC) in vivo leads to an increased adhesion of monocytes to the endothelium of the vessel wall that results in atherosclerosis, (De Grujtier et al., 1991). According to the same investigators, an increased level of plasma triglycerides is the major determinant, since high cholesterolemia alone does not induce this alteration. Furthermore, it had been hypothesized that individuals with combined hypercholesterolemia-hypertriglyceridemia (CHH) are at risk to develop premature atherosclerosis. This hypothesis was supported by extensive epidemiologic findings that lowering the total plasma cholesterol level would lead to a reduction in the progression of atherosclerosis.

In the present study the incorporation of 1% cholesterol + 5% saturated fat in the control diet (+) led to an overall rise in serum cholesterol, LDL cholesterol, triglyceride and the atherogenic index. Modern strategies for disease control or prevention put more emphasis on botanicals with history of use in folk medicine. The current research interest all over the world is directed towards innovative dietary compounds with specific effects on health promotion and reducing the risk of diseases, (Che et al., 1982). Clinical and experimental studies have demonstrated protective and therapeutic effects of the black seed, curcumin, flax seed and garlic against atherogenic, hepatotoxicity and nephrotoxicity induced by either disease or chemicals.

A recent study showed that when rabbits with diet-induced hyper-cholesterolemic were fed powder Nigella sativa at a dosage of 1g per kg body weight for 6 weeks, their serum cholesterol level was reduced from 10.7 down to 1.57 mmol/l, their serum LDL cholesterol from 9.3 to 1.16 mmol/l and serum triglycerides from 0.72 down to 0.5 mmol/l. In addition, the plaque area (cm²) formation in the abdominal aorta shranked from 0.8 down to 0.16, Al-Naqeep et al. (2010).

In the present study, feeding the cholesterol positive control (+) diet showed an overall rise in the fasting blood glucose of 14.3%, Only the black seed containing diet successfully modified the mean blood glucose concentration to its respective normal level. Diets containing curcumin, flax seed and garlic failed to lower the mean blood glucose levels to the respective normal control (-) diet.

Our result is in good agreement with earlier reports, showing that a plant mixture containing N. sativa produced a significant hypoglycemic effect in alloxan-induced diabetic rats, (Eskander et al., 1995).

Serum cholesterol, triglyceride and visible fat % significantly decreased with Nigella sativa supplementation while serum GPT level significantly increased with nigella sativa supplementation, these results are similar with the present study.

N. sativa seed supplementation were correlated with alterations in serum aspartate transaminase (AST) and alanine transaminase (ALT) activities and concentrations of total protein, albumin, globulin and cholesterol (Ali-Homidan, 2002). Further studies indicated that Black cumin seeds showed increased total plasma protein as well as also higher non significant value was noticed by the 6th N. sativa seed supplementation were correlated with alterations in serum aspartate transaminase (AST) and alanine transaminase (ALT) activities and concentrations of total protein, albumin, globulin and cholesterol (Ali-Homidan, 2002). Further studies indicated that Black cumin seeds showed increased total plasma protein as well as albumin and globulin, while the opposite was true with, plasma cholesterol, plasma total lipids, GOT and GPT (Tollbaand and Hassan 2003 and Hassan, et al., 2007).

This is likely not a concern, since studies indicate that consumption of up to 12 g of curcumin daily is not harmful or toxic to human and animals In, a diet enriched with curcumin showed a significant decrease in the levels of serum TG, TC, and LDL-C as reported by previous studies. In addition, curcumin significantly decreased the (AI) which is an important risk index for CVD, clearly demonstrating hypocholesterolemic potential of curcumin. (Ejaz et al., 2009).

Kim and Kim (2010).revealed that the curcumin diet significantly decreased serum triglyceride (TG) by 27%, total cholesterol (TC) by 33.8%, and LDL-cholesterol by 56%, respectively as compared to control group.
The curcumin-supplemented diet also significantly lowered the atherogenic index (AI) by 48% as compared to control group. Hepatic TG level was significantly reduced by 41% in rats fed with curcumin-supplemented diet in comparison with control group (P < 0.05).

The most commonly used markers of hepatocyte injury are serum albumin concentrations and serum enzymes of liver function test i.e., ALT, AST and GGT. In the present study, feeding the cholesterol diet for 5 weeks was associated with adverse effect on the liver function test presented by significant lowering in the serum albumin concentration (2.25 g/dl). Albumin has a plasma half-life of three weeks; therefore, serum albumin concentrations change slowly in response to alterations in synthesis, (Rothschild et al., 1988), but it is immediately and severely depressed if the synthetic capacity of the liver is decreased. The incorporation of the black seed in the diet effectively raised the serum albumin by 26.2 %.

An overall rise in the mean activities of ALT (35 U/l); AST (125.2) and GGT (6.29) was found after feeding the cholesterol diet for 5 weeks compared with the respective mean value of normal control (-). It is noteworthy that the enzyme ALT is cytosolic, while AST has both cytosolic and mitochondrial forms (Thapa, and Walia, 2007). Hepatocyte necrosis in acute hepatitis, toxic injury or ischemic injury results in the leakage of enzymes into the circulation. Higher AST-to-ALT ratio is often found in toxic liver injury or in liver cirrhosis, (Haber et al., 1995). The incorporation of the black seed in the diet effectively lowered the activities of the three liver enzymes by 5.0; 32.1 and 18.3 % respectively. Other investigators reported also the hepatoprotective effect of Nigella sativa (Kanter et al., 2005 and Muhtasib et al., 2006) An overall rise in indicators of kidney function; serum creatinine (37.3 %) and blood urea (58.7 %) were found in the group consuming the positive control (+) diet. The incorporation of black seed, curcumin, flax seeds or garlic in the diet effectively reduced the mean concentration of serum creatinine and urea. According to (Bayrak et al., 2008), animals that underwent renal ischaemia exhibited significant increases in serum blood nitrogen urea (BUN) and creatinine levels compared to normal animals. Whereas, Pre- and post-treatment of rats with the oil of Nigella sativa produced reduction in the serum levels of Blood urine Nitrogen (BUN), creatinine that was statistically significant.

The active bases of the black seed are mainly thymoquinone (TQ), dithymoquinone, thymohydroquinone and monoterpenes with antioxidant property that makes it useful in treating cardiovascular disorders. In addition, the black seed contains on the average 149 mg proanthocyanidin (PA) per 100 g. Black seed PA is highly polymeric with 88.4% having more that (≥7 pymeric units).

In curcumin the respective (PA) content averaged 74.2 mg; 43.7 % is in dimer and trimer form and the rest (56.3 %) in 4 – 6 pymeric units (Gu et al., 2004).

All the 4 studied herbs reduced the enzyme AST by one third, which was still significantly higher than the respective mean value of 24.2 Units/L obtained in the serum of the group on the control (-) diet.

Diets containing 20 % Flaxseeds showed the highest potential efficiency in modifying the lipid profile in the serum. In this group, the atherogenic index dropped down to 1.2. This is due to the protective effect of flax seed alpha-linolenic acid against cerebrovascular stroke and atherogenic carotid plaque formation. Flaxseed is also the richest food source of lignans; one of the major groups of phytoestrogens with antioxidant properties and the ability to reduce hypercholesterolemia. Soluble fiber from flaxseed mucilage might also be responsible in improving glycemic control and reducing serum cholesterol.

Flaxseed oil exhibited a considerable decrease in serum glucose concentration in comparison with control, (Hunt et al., 1994).

There is evidence that, flaxseed is the richest food source of lignans, one of the three major classes of phytoestrogens (Adlercreutz, 1993). Lignans have been shown to modulate activities of 7α- hydroxylase and acyl CoA cholesterol transferase, two of the key enzymes involved in cholesterol metabolism (Sanhvi et al., 1984). The reduction of blood cholesterol may be due in part to linoleic and α-linolenic acid present in flaxseed or an increase in bile acid excretion (Sam 2003).

Vijaimolan et al. (2006) suggest that flaxseed oil participate in the normal regulation of plasma lipid concentration and cholesterol metabolism in liver, and this is due to its contents of alpha linolenic acid. No adverse effect of FO on growth parameters and plasma lipids in rats fed with fat-free diet. The results of the present study demonstrate that FO present in flaxseeds may be developed as a useful therapy for hyperlipidemia through reducing hepatic lipids, thereby proving its hypolipidemic activity, these results was agreement with finding by (EL- Kirsh & EL-Razek, 2010).

The hypolipemic and hypocholesterolemic effects of diet containing garlic confirm previous animal studies (Chi et al., 1982, Yeh and Liu, 2001). The water-soluble sulfur compounds of garlic, especially allyldisulfide and diallylcycteine were reported to inhibit hepatic cholesterol synthesis (Kritchevsky et al., 1980). In another study, the supplementation of aged garlic extract (AGE) in animal diets was reported to reduce plasma concentrations of total cholesterol and triacylglycerol by 15 and 30%, respectively compared to the respective control values (Yeh and Liu, 2001).

A previous report by Bhuyan and Bhuyan (1984) and Babizhayev (1996) found a close relationship among cataract and hyperlipidemia. Also lipid peroxidation has been associated with the cataracts formation. Intake of
garlic powder reduced total cholesterol, LDL-C and triacylglycerol and increased HDL-C (Liu and Yeh, 2001; and Kojuri et al., 2007). Elkayam et al. (2003) suggested that garlic prevents hypercholesterolemia. Similarly Pourkabir et al. (2010) showed that supplementation with 5% garlic powder for 8 weeks had effect on the lipid profile in the cataractous rats. Similar results by Abd El-Razek et al., (2011), who indicate that consumption of garlic in the diet has a beneficial effect in maintaining the serum lipids at low or normal levels and prevent lipid peroxidation.

Results coming out of the study may provide novel targets for prevention and/or treatment of the metabolic syndrome and the associated chronic inflammatory- mediated diseases.

The present study provided scientifically based evidences that incorporation of the herbs in the atherogenic diet, each herb demonstrated satisfactorily benefit to more than one target function in the body, beyond adequate nutritional effects in a way that is relevant to an improved state of health and well-being and reduction of risk of disease.

The working definition of Functional food is that it is a concept of modern food related nutrition and nutritionally adapted foods and this applies to the present situation.

Acknowledgement: The author would like to thank Prof Laila Hussein, Depart Nutr, National research center, Giza, Egypt for revising the manuscript.

Table 1: Composition of the experimental diets.

<table>
<thead>
<tr>
<th>Diet ingredients</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Black seed</th>
<th>Curcumin</th>
<th>Flax seeds</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>140</td>
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<tr>
<td>Cellulose</td>
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<td>50</td>
<td>50</td>
<td>50</td>
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<td>50</td>
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<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Corn oil</td>
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<td>50</td>
<td>50</td>
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<td>Mineral mix</td>
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<td>10</td>
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<tr>
<td>Hump Fat</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Methionine</td>
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<td>3</td>
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<td>3</td>
<td>3</td>
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<td>Choline chloride</td>
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<td>0.5</td>
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<tr>
<td>Bile salts</td>
<td>0</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>Cholesterol</td>
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<tr>
<td>Black seed</td>
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<td>Curcumin</td>
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<td>Total, grams</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
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</tbody>
</table>

*Vitamin mix (g/kg): Nicotinic acid 3.0; Calcium pantothenate 1.6; Pyridoxine –HCl 0.7; Thiamin – HCl 0.6; riboflavin 0.6; folic acid 0.2; vitamin B12 (0.1 %) 2.5; Vitamin A (500000 IU/g) 0.8; vitamin E (500 IU/g) 15; vitamin D3 400000 IU/g) 0.25; vitamin K 0.075; vitamin Biotin 0.02; choline powder sucrose to 1000 grams (AIN-93M, Reeves et al., 1993)

* Composition of Mineral mixture g/kg Di Potassium hydrogen Phosphate 389; Calcium Carbonate, Anhydrous 381.4; Sodium Chloride 139.3; Magnesium Sulfate, Anhydride 57.3; Ammonium Ferric Sulfate 49.57; Manganese Sulfate 4.01; Potassium Iodate 0.79; Zinc sulfate 0.548; Copper sulfate 0.477; Cobalt chloride 0.023 (AOAC 2000)

Table 2: Effect of different herbal plants on serum lipids profile (Mean values ± SE).

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Diets</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Black seed</th>
<th>Curcumin</th>
<th>Flax seeds</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides mmol/l</td>
<td>1.06±0.025</td>
<td>1.30±0.05</td>
<td>1.17±0.04</td>
<td>0.79±0.025</td>
<td>0.62±0.032</td>
<td>0.60±0.035</td>
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<tr>
<td>Total Cholesterol mmol/l</td>
<td>1.8±0.04</td>
<td>3.95±0.065</td>
<td>3.0±0.074</td>
<td>2.19±0.084</td>
<td>1.36±0.065</td>
<td>1.98±0.092</td>
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<tr>
<td>HDL Cholesterol mmol/l</td>
<td>0.53±0.03</td>
<td>0.45±0.034</td>
<td>0.68±0.029</td>
<td>0.62±0.026</td>
<td>0.50±0.041</td>
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<tr>
<td>LDL Cholesterol mmol/l</td>
<td>0.60±0.026</td>
<td>2.96±0.134</td>
<td>1.96±0.074</td>
<td>1.24±0.026</td>
<td>0.60±0.043</td>
<td>1.17±0.039</td>
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<tr>
<td>VLDL Cholesterol mmol/l</td>
<td>0.44±0.04</td>
<td>0.54±0.043</td>
<td>0.38±0.048</td>
<td>0.33±0.038</td>
<td>0.26±0.034</td>
<td>0.25±0.029</td>
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<tr>
<td>Atherogenic index [AIX]</td>
<td>1.14</td>
<td>6.53</td>
<td>2.88</td>
<td>2.00</td>
<td>1.20</td>
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</tbody>
</table>

Control (-) diet without cholesterol; Control (+) diet contained 1 % cholesterol
Mean value ± standard error of 7 rats, duration of the feeding experiment = 5 weeks
Within the same row, mean values are significantly different (P<0.05), if they don't share the same alphabet (Student's t –Test)
Table 3: Effect of different herbal plants on liver enzymes activity and serum Bilirubin Creatinine and Urea. (Mean values ± SE).

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Diets</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Black seed</th>
<th>Curcumin</th>
<th>Flax seeds</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>ALT Units/L</td>
<td>26.5 ± 1.32^a</td>
<td>35.0 ± 2.65^b</td>
<td>33.25 ± 0.66</td>
<td>24.7 ± 0.66^a</td>
<td>24.67±0.58^a</td>
<td>25.6 ± 1.22^a</td>
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<tr>
<td></td>
<td>24.17 ± 0.76^c</td>
<td>125.25 ±</td>
<td>85.00 ± 2.00^c</td>
<td>74.43 ± 1.25^d</td>
<td>87.00 ± 1.00^d</td>
<td>86.6 ± 1.15^d</td>
<td></td>
</tr>
<tr>
<td>AST Units/L</td>
<td>5.00 ± 0.26</td>
<td>6.29 ± 0.62</td>
<td>5.14 ± 0.75</td>
<td>5.83 ±0.75</td>
<td>5.90 ± 0.35</td>
<td>5.80 ± 0.46</td>
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<tr>
<td></td>
<td>62 ± 2^bc</td>
<td>67 ± 3^f</td>
<td>50 ±5^g</td>
<td>58 ± 9^ab</td>
<td>51 ± 4^h</td>
<td>51 ± 3^i</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.4± 2.3^h^c</td>
<td>48.6± 4.4^c</td>
<td>32.7 ± 2.2^j,</td>
<td>39.8 ±3.18^k^c</td>
<td>38.9± 1.5^k,c</td>
<td>35.4± 2.3^k^c</td>
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</tr>
<tr>
<td></td>
<td>8 ± 0.46^e</td>
<td>12.7±0.58^d</td>
<td>10.3±0.05^y</td>
<td>11.3±0.62^e</td>
<td>11.4±0.53^c</td>
<td>11.5±0.70^o</td>
<td></td>
</tr>
</tbody>
</table>

Control (-) diet without cholesterol; Control (+) diet contained 1% cholesterol
Mean value ± standard error of 7 rats, duration of the feeding experiment = 5 weeks
Within the same row, mean values are significantly different (P< 0.05), if they don't share the same alphabet (Student's t –Test)

Table 4: Effect of different herbal plants on Fast Blood glucose and serum protein, Albumin and Globulin (Mean values ± SE).

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Diets</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Black seed</th>
<th>Curcumin</th>
<th>Flax seeds</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Fast Blood glucose</td>
<td>5.4± 0.03^b</td>
<td>6.17±0.19^o</td>
<td>4.7±0.06^c</td>
<td>5.8±0.08^c</td>
<td>5.7±0.15^g</td>
<td>6.06±0.15^g</td>
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<tr>
<td>mmol/l</td>
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</tr>
<tr>
<td>Serum protein g/dl</td>
<td>6.18±0.27^b</td>
<td>6.00±0.26^b</td>
<td>8.28±0.25^c</td>
<td>6.56±0.09^c</td>
<td>6.08±0.07^a</td>
<td>6.17±0.06^a</td>
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</tr>
<tr>
<td>g/dl</td>
<td></td>
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<tr>
<td>Serum Albumin g/dl</td>
<td>2.88±0.07^b</td>
<td>2.25±0.05^a</td>
<td>2.84±0.05^c</td>
<td>3.11±0.08^c</td>
<td>2.74±0.02^b</td>
<td>2.73±0.04^b</td>
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</tr>
<tr>
<td>g/dl</td>
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</tr>
<tr>
<td>Serum Globulin g/dl</td>
<td>3.30±0.17^b</td>
<td>3.75±0.04^b</td>
<td>3.44±0.05^o</td>
<td>3.48±0.15^o,</td>
<td>3.34±0.06^c</td>
<td>3.44±0.03^a</td>
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<tr>
<td>g/dl</td>
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<tr>
<td>Alb : Glob ratio</td>
<td>0.87±0.03^b</td>
<td>0.60±0.06^a</td>
<td>0.83±0.02^b</td>
<td>0.90±0.10^b</td>
<td>0.82±0.01^b</td>
<td>0.79±0.03^b</td>
<td></td>
</tr>
</tbody>
</table>

Control (-) diet without cholesterol; Control (+) diet contained 1% cholesterol
Mean value ± standard error of 7 rats, duration of the feeding experiment = 5 weeks
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REFERENCES


