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Re-Evaluation of Individual and Combined Garlic and Flaxseed Diets on Hyperlipidemic Rats

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Abstract: The aim of this study was to re-evaluate the effect of individual garlic and flaxseed and for the first time, we studied the effect of combined consumption of 10% of flaxseed and garlic, for 30 days on biochemical and histological factors of hyperlipidemic rats. The feeding trial was conducted on rats with high levels of serum cholesterol and triacylglycerol. Histological results showed a marked improvement of kidney tissues responding to garlic alone and a combined flaxseed and garlic diet but a slight histopathological change were noticed in flaxseed diet group. Garlic results showed no histopathological changes in aorta, kidneys and liver that may illustrate the healing effect of fresh garlic on tissues. Biochemical results indicated that the mean of blood total cholesterol, triacylglycerol were reduced, as the effect of fresh garlic (FGD), flaxseed (FD) and combined fresh garlic and flaxseed diet (FFGD) but HDL-C was increased in fresh garlic diet only. Best results were obtained from flaxseed diets that reduced cholesterol levels markedly to 115% over negative control group. Slight reduction of serum levels of LDL-C has noticed in flaxseed (FD) and fresh garlic diets (FGD). These results may support the Mediterranean diet consumption that is rich in fresh food such as fresh garlic and seeds that may protect from heart disease.

Key words: Flaxseed, garlic, hyperlipidemia, histology, rats, mediterranean diet

Introduction

Ancient records show that the human race has consumed flaxseed and garlic since the beginning of civilization. Before 5,000 BC, Egyptians carried flaxseed in their medicine bags. Garlic and onions were used regularly to promote good health and found in their tombs (Nicholson, 2000). An increased serum cholesterol and triacylglycerol levels have been implicated as important risk factors for the development of coronary artery disease. Cardiovascular disease is the leading cause of death in Arab countries for example, United Arab Emirates (UAE) recorded 41 percent and conditions relating to heart disease are prevalent throughout the Middle East. The prevalence of hypertension for example, is 45.3 percent in Egypt compared to Kuwait 26.3, percent, 32.1 percent in Qatar and 33 percent in Oman (The 4th Partners International Cardiovascular Conference Charts Path to Reduce Heart Disease). It is estimated that 55% of Americans have cholesterol levels greater than (200 mg/dL) (5.17 mmol/L) and are at increased risk for the development of coronary artery disease.

The well known term "the Mediterranean Diet" was first popularised in the 1970s when studies showed that Mediterranean countries have diets associated with low incidences of cardiovascular disease with later studies showing that these countries also enjoy a low incidence of cancers of the colon and breast. The Mediterranean diet is characterised by consumption of fresh vegetables, such as garlic, dietary fibre, seeds and low

intakes of meat and saturated fats. It not only produces favourable effects on blood lipids but also protects against oxidative stress, the latter being thought to represent one of the mechanisms leading to chronic diseases such as atherosclerosis and cancer (Abdel-Moemin, 2004). Flaxseed has a scientific name of *Linum usitatissimum*. Whole flaxseed contains approximately 41% fat and 21% protein of the seed weight (Obermeyer *et al.*, 1995). There is an evidence that whole flaxseed may lower serum cholesterol in both normal (Cunnane *et al.*, 1995) and hyperlipidemic (Stuglin and Prasad, 2005) subjects. Flaxseed may be slightly helpful for improving cholesterol profile, according to some but not all studies. (Hallund *et al.*, 2005). Purified alpha linolenic acid or lignans alone have not consistently shown benefits (Zhang *et al.*, 2007). It may be the generic fiber and not the other specific ingredients in flaxseed that benefit cholesterol levels. Some but not all human studies have found that flaxseed improves cholesterol profile (Banerjee and Maulik, 2007).

Garlic *Allium sativum* has been considered as one of the blood lipids lowering agents and various studies have been carried out, some of them confirmed this effect of garlic and some did not. Garlic's current principal medicinal uses are to prevent and treat cardiovascular disease by reducing platelet aggregation by inhibiting prostaglandin E2 and the release of fibrinogen degradation products, lowering blood pressure and cholesterol, as an antimicrobial and as a preventive agent for cancer. Many clinical trials showed

Table 1: Basal diet, Ain-93M

| Ingredient | g/kg Diet |
|-------------------------|-----------|
| Cornstarch | 465.692 |
| Casein | 140.000 |
| Dextrinized corn starch | 155.000 |
| Sucrose | 100.000 |
| *Corn oil | 40.000 |
| Fiber | 50.000 |
| Mineral mix | 35.000 |
| Vitamin mix | 10.000 |
| L-cytesine | 1.800 |
| Cholin bitartarate | 2.500 |
| Tert-butylhydroquinone | 0.008 |

*Soybean oil was replaced by corn oil

a positive effect of garlic on cardiovascular diseases, however a number of negative studies have recently shed doubt on the efficacy of garlic specially its cholesterol lowering effect (Wendland *et al.*, 2005).

The role of combined dietary flaxseed and garlic on hyperlipidemia has not been examined. For the first time we investigated the effects of dietary flaxseed and garlic diet on serum lipids and histopathological changes in the liver, kidneys and aorta in rats.

Materials and Methods

Animals and diets: This study was performed on 63 male Sprague-Dawley rats, weighed in the beginning of the experiment 120 g (± 5) (eight weeks of age). The animals were housed and bred as approved by the Animal Ethics of Ophthalmology Institute Research, Egypt. The animals were housed in conventional wire-mesh cages in a room temperature regulated at 21 \pm 1°C, humidity 45-50 %, and light/dark cycles (12h). Environmental conditions such as humidity, heat, light, and ventilation were kept constantly for 24 hours daily during the period of the study. The animals were kept on rodent chow for a week after this washout period; rats were divided into seven groups of 9 each. Seven diets were prepared; Basal diet Ain-93M diet formulated for maintenance of adult rodents negative control (C-) [11]; positive control (C+) that high in fat and supplemented with 1% cholesterol, flaxseed (FD), fresh garlic (FGD), dry garlic (DGD), flaxseed with fresh garlic (FFGD) and flaxseed with dry garlic (FDGD) all supplements were added to the basal diet at 10%.

Experimental design: Rats were divided into seven groups, negative control lived on basal diet only and 6 groups were received high fat diet with 1% cholesterol; after 10 days the lipid profile come to cut off point that over 100mg/dL for total cholesterol and triacylglycerols. Rats were then received the following diets. Group II: Positive control group, rats carry on feeding high fat diet with 1% cholesterol. Group III: Ground flaxseed diet group (FD), the hyperlipidemic rats were fed on diet with 10% flaxseed mixed with basal diet. Group IV: Dry garlic diet group (DGD). The rats were fed on diet with 10%

Table 2: Mean weight (g) changes in rats treated with hyperlipidemic diets

| Group | Initial weight | Final weight | Weight gain(g) | % weight gain |
|------------------|----------------|----------------|----------------|---------------|
| Negative Control | 125 \pm 2.30 | 223 \pm 2.74 | 88 | 78 |
| Positive Control | 125 \pm 2.59 | 233 \pm 2.90 | 108 | 86 |
| FD | 125 \pm 0.35 | 208 \pm 4.63 | 83 | 66 |
| DGD | 125 \pm 0.55 | 198 \pm 1.23 | 73 | 58 |
| FGD | 125 \pm 0.87 | 183 \pm 4.50 | 58 | 46 |
| FDGD | 123 \pm 0.85 | 204 \pm 2.81 | 81 | 65 |
| FFGD | 125 \pm 0.93 | 186 \pm 4.05 | 61 | 48 |

FD: Flaxseed diet, DGD: Dry garlic diet, FGD: Fresh garlic diet, FGD: Fresh garlic diet, FDGD: Flaxseed and dry garlic diet, FFGD: Flaxseed and fresh garlic diet FFGD, \pm Standard Error of Mean, values are the mean of 9 results, n=9.

garlic powder. Group V: Fresh garlic diet group (FGD). The rats of this group were fed on diet has 10% fresh garlic. Group VI: The rats in this group were fed on 10% of flaxseed and dry garlic (FDGD). Group VII: The rats were fed on 10% of flaxseed and fresh garlic (FFGD). Animals of the entire group fed for 30 days, after an overnight fasting, blood samples were withdrawn from the retro orbital venous plexus and removed organs such as kidneys, livers and aorta in order to carry out the biochemical analyses and histology.

Biochemical studies: Serum total cholesterol and triacylglycerol concentrations were determined enzymatically using kits from Boehringer (Germany). Serum high-density lipoprotein (HDL) cholesterol was determined by a direct method (Unimate HDL Direct, Roche Diagnostics) that uses the combined action of polymers, polyanions and detergent to solubilize cholesterol from HDL but not from VLDL, LDL, LDL-cholesterol concentration was calculated by the (Friedewald *et al.*, 1972). Non-HDL-cholesterol concentration was calculated by subtracting HDL cholesterol from total cholesterol. The coefficients of variation (CVs) were 1.7% and 2.5% and 2.6%, for total cholesterol, triglycerides, HDL cholesterol, respectively. the rats were anaesthetized with ether after which blood samples were collected immediately in sterile tubes from the retro orbital venous plexus and left to stand for 30 min. at room temperature (<20°C) to coagulate before being centrifuged for 20 min at 2000 rpm (Sorvall RT7, Newtown, MA).

Histology: Specimen from organs; liver, kidney and aorta were fixed in 10% neutral buffered formalin. Sections were routinely processed for light microscopy with formalin fixation, embedded in paraffin and stained with H&E according to (Bancroft *et al.*, 1996). All sections were coded and analyzed blindly by the pathologist without knowledge of related characteristics or diet. Histological results have been graded by a scale from 0 to 4 according to severity of histological sections. 0 shows no histopathological results, 1 shows slight degree of severity, 2 mild, 3 moderate and 4 severe histopathological results.

Liver Histology

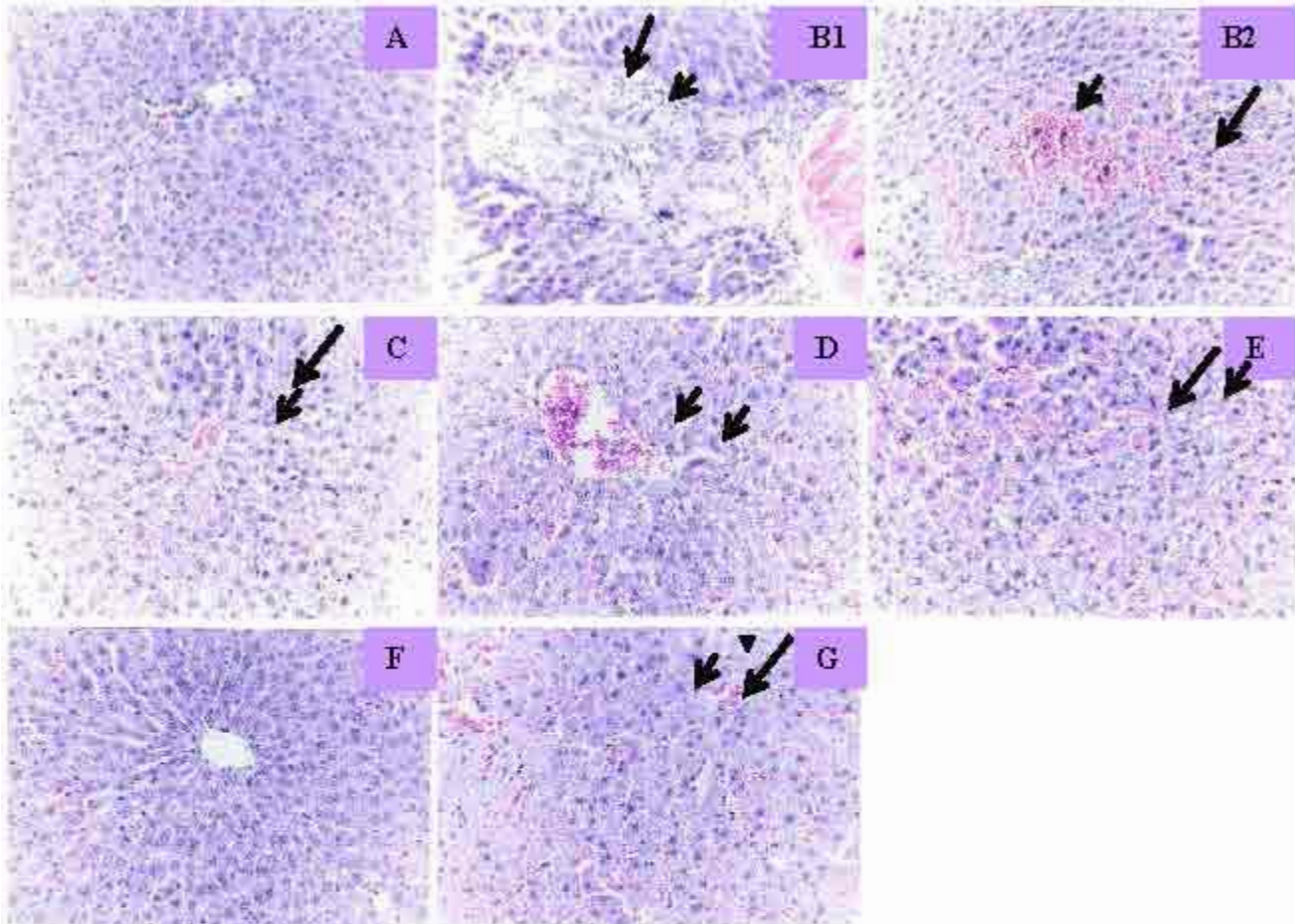


Fig. 1: liver of rats of control group showing no histopathological signs (Hand EX 200) (A) (Grade 0). Liver of rats of positive control showing marked thickening in the wall of bile duct (arrows) (B1) and marked dilatation and congestion of hepatic sinusoids (small arrows) atrophy of hepatocytes and pyknosis of their nuclei (large arrows) (B2) (Grade 4). Liver of rat from dried garlic and flaxseed group showing vacuolar degeneration of hepatocytes (small arrow) (C) (Grade 2.0). Liver of rat from fresh garlic and flaxseed group showing vacuolar degeneration of hepatocytes (small arrow) (D) (Grade 1.5). Liver of rats of flaxseed group showing vacuolar degeneration of hepatocytes (small arrow) and congestion of hepatic sinusoids (large arrow) (E) (Grade 1.0). Fresh garlic showing no histopathological changes (F) (Grade 0). Liver of rats of dried garlic group showing vacuolar degeneration of hepatocytes (small arrow) and congestion of hepatic sinusoids (large arrow) (G) (Grade 3).

Statistical analysis: Results are expressed as mean \pm standard error of the mean. Comparisons between negative and positive groups for the data in Tables 3 were made by using one-way analysis of variance (ANOVA).

Results

All rats were generally healthy throughout the feeding experiment period. Due to the general unpalatability of the diet, specifically FGD and FFGD that have shown low percentage in weight gain; 46 and 48 respectively compared to negative control group (Table 2). In

contrast, positive diet group showed the highest percentage of weight gain compared to other diet groups.

In Table 3, the level of serum cholesterol in the positive group diet was significantly rose lipid profile compared with the negative control diet group (106 ± 1.30 vs. 77 ± 6.36). Cholesterol was reduced to 115, 110 and 103% over negative control in the presence of FD and FGD and FFGD respectively. Slight reduction has been noticed in LDL concentrations for FD and FGD over negative control group. Triacylglycerol was improved over negative control group in FFGD, FGD, FD and

Table 3: Mean serum lipid levels in hyperlipidemic rats after 4 weeks of treatments

| nGroup of Rats | TAG (mg/dL) | % | TC (mg/dL) | %* | LDL (mg/dL) | % | HDL (mg/dL) | % |
|------------------|-------------|--------|------------|------|-------------|--------|-------------|-------|
| Negative control | 73 ±0.21 | 0 | 77±1.36 | 0 | 29±1.20 | 0 | 34±0.89 | 0 |
| Positive control | 120±0.31 | | 106±1.30 | | 41±0.70 | | 27±0.75 | |
| FD | 71±0.24 | 103* | 67±0.80 | 115* | 27±0.85 | 107.4* | 33±0.70 | 97 |
| DGD | 100±15 | 73 | 90±0.4 | 86 | 33±0.62 | 88 | 34±0.56 | 100 |
| FGD | 70±0.70 | 104.3* | 70±1.2 | 110* | 28±0.76 | 103.6* | 36±0.26 | 106** |
| FDGD | 71±0.50 | 103* | 80±0.86 | 96 | 30±0.34 | 97 | 31±0.92 | 91 |
| FFGD | 68±0.74 | 107.4* | 75±0.89 | 103* | 29±0.65 | 100 | 32±0.13 | 94 |

FD: flaxseed diet, DGD: dry garlic diet, FGD: fresh garlic diet, FGD: fresh garlic diet, FDGD: flaxseed and dry garlic diet, FFGD: flaxseed and fresh garlic diet, ± Standard Error of Mean, values are the mean of 9 results, n=9. *Percentage of reduction over negative control, ** percentage of increasing over negative control.

Table 4: Effect of different diets on AST and ALT serum levels

| Groups of rats | AST (U/L) | ALT (U/L) |
|------------------|-----------|-----------|
| Negative Control | 82±0.60 | 61±0.17 |
| Positive control | 137±0.65 | 72±0.33 |
| FD | 95±0.70 | 66±0.23 |
| DGD | 148±0.38 | 85±0.52 |
| FGD | 89±0.46 | 62±0.35 |
| FDGD | 117±0.50 | 68±0.83 |
| FFGD | 112±0.34 | 65±0.80 |

AST = aspartate aminotransferase, ALT = alanine aminotransferase, ± SEM, values are the mean of 9 results, n=9.

FDGD; 107.4, 104.3, 103 and 103% respectively. On the other hand no improvements have been noticed in HDL-c compare to negative control apart from FGD.

Generally liver enzymes have been lowered in the presence of individual and combined garlic and flaxseed diets compared to the positive control group (Table 4). The same table shows a slight improvement in AST when rats were fed FGD and ALT when rats fed on FGD and FD respectively compared to negative control group. Dry garlic diets have increased AST and ALT compared to negative and positive control diet groups.

Discussion

Atherosclerosis with subsequent manifestations of cardiomyopathies is one of the major causes of morbidity and mortality in the world. The primary objective of the study was to evaluate the beneficial effects of individual and combined flaxseed and garlic diets on the hyperlipidemic rats.

Histology

Effect of flaxseed and garlic: Arterial fulfilment represents the volume-pressure relationship within the arterial circulation or the distensibility or elastic properties of the proximal arterial system to changes in volume and pressure. Thus, stiffness increases and fulfilment decreases with clear structural changes, but arterial wall properties are also influenced by functional characteristics such as the tonicity of the artery or changes in pressure. Garlic results showed no histopathological changes in aorta that may illustrate the powerful effect of fresh garlic on arteries (Fig. 3). The components in garlic that influence vascular tone are not well characterized. Much interest has focused on the

numerous sulfur-containing compounds that appear to be important for a number of the medicinal actions of garlic (Block, 1985). Cysteine sulfoxides such as allicin, which are transformed into thiosulfinates, comprise a significant portion of these sulfur compounds (Reuter *et al.*, 1996). Much of the LDL-C cholesterol is manufactured by our own bodies Garlic lowers LDL by suppression of its biosynthesis. Therefore, the garlic function may play a vital role by having a healthier ratio between the two cholesterol that can dissolve some of the plaque inside arteries. Recent work (Das *et al.*, 1995) demonstrating that garlic administration may result in activation of calcium-dependent endothelial nitric oxide synthase in certain tissues and has additional direct effect on vascular smooth muscle cells. This finding is compatible with studies in isolated vascular strips that demonstrate that garlic can induce vasodilatation via smooth muscle cell membrane hyperpolarization and/or inhibition of the opening of calcium channels (Siegal *et al.*, 1992).

Kidney results showed that experimented diets achieved grades 0, with minimal histopathological changes in all groups a part from a slight histopathological change in flaxseed diet group which showed eosinophilic cast in the lumen of some renal tubules (Fig. 2 D). Combination between flaxseed and fresh garlic showed no histopathological changes in kidney. Other results found that (Clark *et al.*, 2001) showed that flaxseed was suggested to improve lupus nephritis.

Histologically, livers of negative control rats showed no histopathological changes, in contrast, livers from positive control (hyperlipidemic) rats showed marked thickening in the wall of bile duct [Fig. 1 (B1)] and dilatation and congestion of hepatic sinusoids atrophy of hepatocytes and pynosis of their nuclei (B2). Aorta, kidney and liver results indicate that fresh garlic showed no histopathological changes (Fig. 1, 2 and 3).

These results indicate that flaxseed and garlic diets have no favourable effect on both liver histology and AST and ALT. Generally, the effect of ground flaxseed on histological results was not satisfied in most hyperlipidemic. Other studies showed that flaxseed oil was improved compliance indicate an effect on the functional components that determine compliance.

Kidney Histology

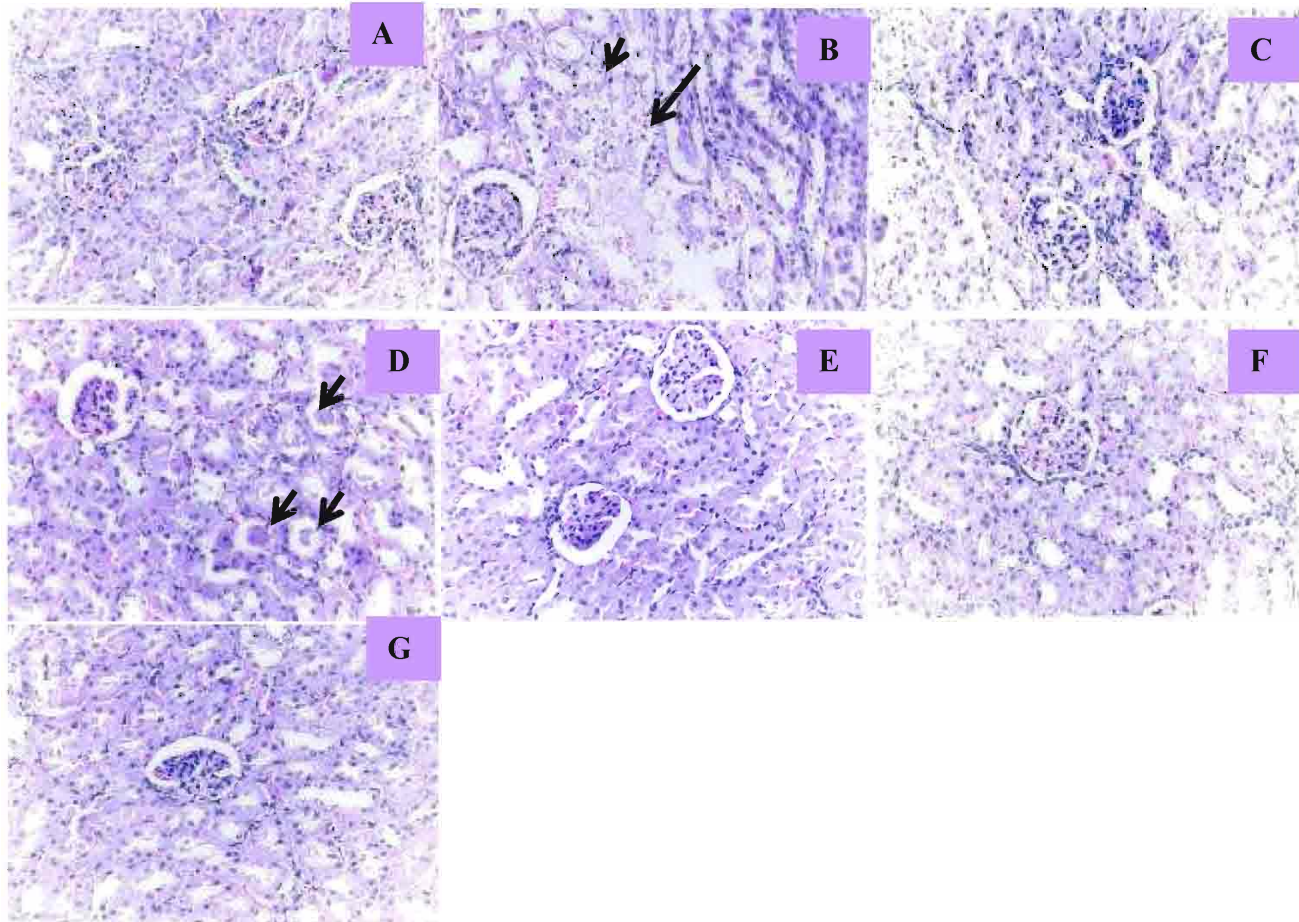


Fig. 2: Kidney of rats of negative control group showing normal histology of renal parenchyma. (H and E X 200) (A) (0 Grade). Kidney of rats of positive control group showing nephrosis (small arrows) associated with focal renal hemorrhage (large arrows) of renal parenchyma. (B) (4 Grade). Kidney of rats of fresh garlic group showing no histopathological changes (C) (0 Grade). Kidney of flaxseed group showing eosinophilic cast in the lumen of some renal tubules (arrows) (D) (1 Grade). Kidney of rat of dried garlic group showing no histopathological changes. (E) (0 Grade). Kidney of rats of dried garlic and flaxseed groups showing no histopathological changes (F) (0 Grade). Kidney of rats of fresh garlic and flaxseed groups showing no histological changes (G) (0 Grade).

Biochemical analyses

Effect of flaxseed and garlic

Flaxseed: Flaxseeds have received increasing attention for their potential role in preventing lipid disorders. However, relatively few data are available regarding the impact of flaxseed on blood lipids. Table 3 shows a significant reduction of total cholesterol, triacylglycerol and LDL-C in all diet groups specifically flaxseed, fresh garlic and combined flaxseed with fresh garlic diets respectively over negative control diets. The reduction of blood cholesterol by flaxseed in this study may be due to the hypocholesterolemic effects of whole flaxseed and can also be attributed to its α -linolenic acid and fiber

components (Jenkins *et al.*, 1999). The hypocholesterolemic effects of α - linolenic acid have been reported in both animals and humans (Chan *et al.*, 1991). Garg *et al.* (1989) demonstrated that feeding an α -linolenic acid-rich diet to rats lowered serum cholesterol levels more effectively than a diet rich in linoleic acid. Ratnayake *et al.* (1992) showed that a 20% and higher flaxseed diet given for 90 days in rats decreased serum total cholesterol. However, Babu *et al.* (2000) obtained different results in their studies of young female Sprague-Dawley rats. Hence, the mode of action of flaxseed is unclear and needs to be investigated in future studies.

Aorta Histology

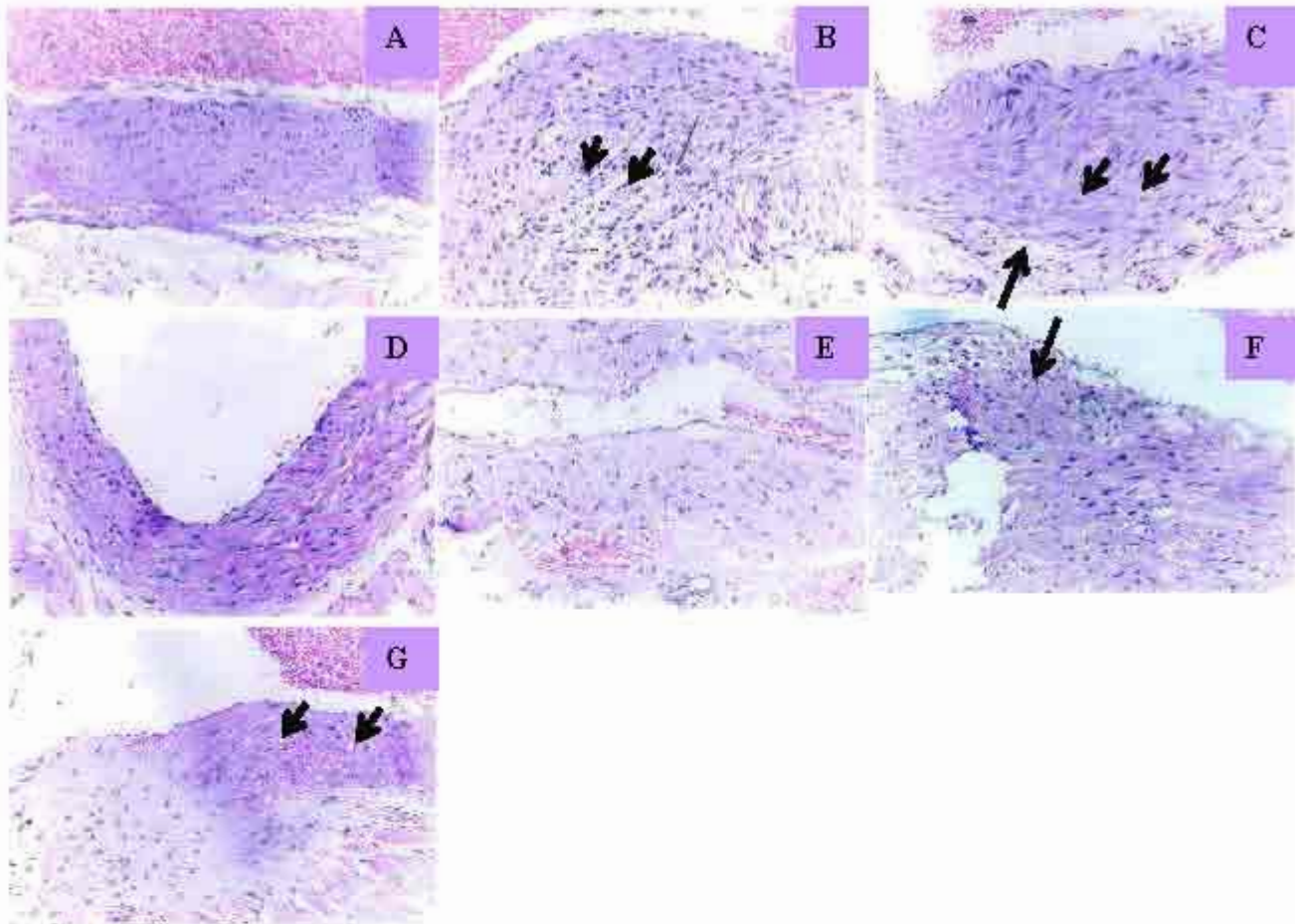


Fig. 3: Aorta of rats of control group showing no histopathological changes (H and E X 200) (A) (0 Grade). Aorta of positive control group showing vacuolation in tunica media associated with leucocytic cells infiltration (B) (4 Grade). Aorta of rats from flaxseed group showing hyalinosis the wall (small arrow) together with slight edema in the tunica adentitia (large arrow) (C) (2.5 Grade). Aorta of rats from group fresh garlic showing no histopathological changes (D) (0 Grade). Aorta of rat from group dried garlic and flaxseed showing vacuolation of some muscle fibers in the in the tunica media wall (arrows) (E) (1.5 grade). Aorta of rat from group dried garlic showing vacuolation in the tunica media (F) (2.0 grade). Aorta of rat from group fresh garlic and flaxseed showing vacuolation of some muscle fibers of tunica media (G) (1.0 grade).

Prasad (1999) reported that rabbits receiving secoisolariciresinol diglucoside, the major lignan found in flaxseed, had reduced hypercholesterolemic atherosclerosis that could be partly attributed to lower total and low-density lipoprotein (LDL)-cholesterol concentrations. In studies of hypercholesterolemia rabbits, Prasad showed that dietary flaxseed reduced total and LDL cholesterol and prevented hypercholesterolemia atherosclerosis (Prasad, 1997). flaxseed results indicated to an increase of HDL levels 106% over negative control group. Whether the hypolipidemic effects of whole flaxseed are due to a single component or the interactions among its

components remains unclear. Kuroda *et al.* (1997) evaluated the hypolipidemic properties of a series of diesters of arylnaphthalene lignans. They reported that these synthetic lignans effectively lower serum total cholesterol and LDL cholesterol while increasing HDL cholesterol. Lignans have also been shown to modulate activities of 7 α -hydroxylase and acyl CoA cholesterol transferase (Sanghvi *et al.*, 1984), two of the key enzymes involved in cholesterol metabolism. Prasad *et al.* (1998) concluded that reduction in hypercholesterolemic atherosclerosis by flaxseed is due to a decrease in serum total cholesterol and LDL cholesterol and that the antiatherogenic activity of

flaxseed is independent of its α -linolenic acid content. Soluble fibre mucilage present in flaxseed may also contribute to the observed hypocholesterolemic properties (Brown *et al.*, 1999).

As we mentioned previously that whole flaxseed used in this study was in a ground form. Some studies have shown that whole flaxseed lower serum cholesterol in animals and humans (Babu *et al.*, 2000), but other studies do not show such an effect (Bordia, 1981). Since whole flaxseed is difficult to digest, it is possible that studies not showing an effect may have used whole seed rather than ground flaxseed. Comparing the nutritional effects of this dietary source of plant protein and oil and their impact on serum lipids and other cardiovascular risk factors are lacking.

Garlic: In an experiment using rats, liver weight, total liver lipid and cholesterol was increased by feeding a high cholesterol and lard diet. Garlic supplementation lowered serum lipids by 30% by increasing bile output. The same results were obtained at low levels of supplementation as at high levels. [32]. The current study used fresh garlic for availability and price reasons; garlic results showed reduction in serum levels of triacylglycerol, total cholesterol and LDL over negative control to 104, 110 and 103.6 % respectively moreover, increasing HDL over negative control to 106%. While, dry garlic had the least improving effect on liver enzymes. Combining flaxseed with dry garlic and flaxseed with fresh garlic had improved both enzymes compare to positive control (C+).

For first time, we have shown that combination of flaxseed and garlic to control rat diets can lower the concentrations of serum total cholesterol and triacylglycerol compare to negative control group, moreover, LDL and HDL-C comparing to positive control group. It has been shown that garlic can significantly lower cholesterol in rats that have consumed a high cholesterol diet with a significant decrease VLDL and an increase in HDL (Cunnane *et al.*, 1994).

The other striking finding in the present study is that flaxseed diet supplementation also produced substantial reductions in cholesterol and triacylglycerol concentrations in rats, whereas combining flaxseed and garlic have an additive effect on lipid profile (lowered TAG to 107 % over negative control), therefore we may recommend combining both in a therapeutic product in order to lowering mild hyperlipidemia. Dry garlic had minimal or no significant effects on lipid levels in rat groups. Cunnane *et al.* showed that consumption of 50 grams of flaxseed/day for four weeks resulted in a small but significant reduction in LDL cholesterol in young healthy humans. Bierenbaum *et al.* (1993) showed that flaxseed supplementation in the form of either flaxseed-containing bread or 15 g of ground flaxseed for three months resulted in significant reductions in serum total

and LDL cholesterol with no change in HDL cholesterol in human subjects with hyperlipidemia.

The reduction of serum lipid in this study was lesser than other studies probably this due to time span of the experiment was 30 days and the concentration of supplemented foods was 10% only. The results of other studies suggest that the effects of flaxseed may vary according the experimental models, the amount or type of flaxseed preparation and the pre-existing level of serum lipids. Moreover, some of these studies have used either ground flaxseed or fresh and dry garlic, which may have confounded the results.

Conclusion: In the present study, 10% of individual flaxseed, fresh garlic or combined them were mixed with basal diet to consume by hyperlipidemic rats for 30 days. Serum lipids in hyperlipidemic rats were lowered compare to negative control. Although garlic and flaxseed had improved lipid profile, but the percentage reduction in cholesterol levels were reached to the maximum level than other lipid fractions. Flaxseed, fresh garlic and the combined of both can be used in mild hyperlipidemia or when the individuals cannot tolerate the chemical drugs. It is not clear whether the constituents of flaxseed and garlic that influence lipid metabolism such as secoisolariciresinol diglucoside, α -linolenic acid, or cysteine sulfoxides such as allicin are as bioavailable when flaxseed and garlic are consumed in its raw form. The reduction of serum lipid in this study was lesser than other studies probably this due to time span of the experiment and the concentration of supplemented foods.

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