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EFFECTS OF PROBIOTIC KUMBU-YOGHURT ON CHOLESTEROLEMIA AND HISTOLOGICAL CHANGES IN CHOLESTEROL-FED RATS

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SUMMARY

Thirty two female Wistar Albino rats (average weight 100 – 120g) were selected as the experimental subjects and divided into eight groups and fed on cholesterol-enriched diet containing Kumbu-yoghurt at different levels. All rats were weighed in the beginning and by the end of the experiment and blood samples were collected from the neck and then, serum cholesterol levels were determined. Serum cholesterol levels showed a significant decrease of total cholesterol, very low density lipoprotein VLDL and low density lipoprotein LDL cholesterol in all the treated groups than that of positive control. Also, there was significant decrease in all the TG, phospholipids and NEFA levels due to increasing of the levels of added kumbucha to Kumbu-yoghurt than of that for the controls either fed on normal yoghurt or positive groups . The histological figures showed an improvement in the hepatocytes vacuolation and a decrease in the degree of destruction and desquamation in the blood vessels lining epithelium of the tunica intema. Moderate improvement of the heart muscle. The degree of improvement was correlated with feeding on kumbu-yoghurt and it reached its magntitiude value with increasing the added kumbocha to the yoghurt.

Key words: Yoghurt, serum cholesterol, rats, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, kumbucha, Histology - Kumbu-yoghurt

INTRODUCTION

Probiotic foods are now important as a medicine beside the nutrition provides in general. Commercial culture of probiotic bacteria are species of *Lactobacillus* and *Bifidbacterium* that inhibit the human hazard intestine microflora and impart through there presence (Holzapfel *et al*., 1998). Strains of probiotic bacteria can be administered to patients as probiotic yoghurt as well as a probiotic preparations.

Several studied have been indicated that consumption of certain cultured dairy products resulted in reduction of serum cholesterol, as it was decreased in men from a trible of African Maasai Warriors after consumption of large amount of milk fermented with a wild *Lactobacilli* strains (Mann and Spoerry, 1974 and Grunewald, 1982).

Yoghurt is a fermented milk, which has its origins in Eastern Europe, but is now consumed throughout the world. Consumption of yoghurt also has been shown to decrease serum cholesterol levels in humans and rabbits (Hepner *et al*., 1979, Mann, 1977, Thakur and Jha, 1981 and Suzuki *et al*., 1991). During the production of fermented milks the fermentation process is mostly caused by lactic acid bacteria. In the final stage during production of probiotic yoghurt, probiotic bacteria which have a known beneficial influence on intestine microflora and immunological state can be added.

Cholesterol was recognized as the lipid present in atheromatous plaques in the 19th century, and cause heart diseases which consider to be number one of the death in USA according to the latest U.S census. HDL-cholesterol concentration is inversely proportional to cardiovascular disease risk. High levels of HDL-cholesterol seemingly protect against cardiovascular disease and this generally attributed to the special role of HDL in delivering peripheral cholesterol to the liver for excretion from the body (Tall and Small, 1980 & Assmann and Gotto, 2004). The epidemiological association between plasma cholesterol or, more precisely, low-density lipoprotein (LDL) and coronary heart disease was well-established by the 1960s. Subsequently, studies of patients with familial hypercholesterleamia demonstrated that increased plasma concentrations of LDL, been identified within arteries. The commonality between these atherogenic lipoproteins is the presence of apolipoprotein (apo) B. The theories of how lipoproteins enter and accumulate within the artery have

remained rather consistent for decades. Lipoproteins infiltrate the artery wall, the lipid is altered to a toxic form, and this promotes an inflammatory response. Depending on their size, lipoprotein particles are able to penetrate arterial tissue ***via*** transcytosis. The liver plays an important role in the cholesterol metabolism in the body and most of the chronic liver disease are associated with the development of different types of cholesterol. (Burt, 1993).

Kumbucha is a tea fungus contains symbiotic culture of *Acetobacter xylium*, two yeasts *Zygosacharomycis rouxii* and *Candida* spp. and *Bifidobacteria* which consider to be the most common bacteria in this culture. This culture produce essential organic acids such as acetic, lactic, folic, gluconic, glucuronic, usinc, ascorbic, oxalic acids……*etc*. All of these acids are essential for helping the liver to detoxify especially glucronic acid which normally produced by the healthy liver and has a powerful detoxifier (Betsy and Sanford, 1996). So the aim of this study is to using of the a new type of yoghurt (Kumbu-yoghurt) which has a high probiotic and symbiotic effect and produced by (Shenana and Hafez) and :

A- Study the effect of feeding by the Kumbu-yoghurt on the different cholesterol levels in the cholesterol-fed rats.

B- Study the effect of feeding by Kumbu-yoghurt on histological changes of cholesterol-fed rats.

MATERIALS AND METHODS

1- MATERIALS

Fresh milk:

Fresh mixed buffaloes and cow's milk (1:1) was obtained from the herd of Faculty of Agriculture, Moshtohor, Benha Univ.

Starter cultures:

Yoghurt strater culture:

Dried yoghurt strater culture contains *Lactobacillus delbreukii* sub spp *bulgaricus* and *Streptocuccus salivarious* sub spp *thermophilus* was obtained from Chr. Hansen, Cobenhagen, Danmark, activated and added at a rate of 3g $100g^{-1}$ for all the treatments.

Kumbucha:

Kumbucha was prepared according to Betsy and Sanford, 1996

Animals:

Thirty two healthy female Wistar Albino rats (average weight 100 – 120g) were selected as the experimental subjects and obtained from Agric. Res., Center, Giza, Egypt.

2. Methods:

Preparation of the Kumbu-yoghurt :

The fresh mixed milk was standardized to fat ~5 /100g^{-1} and divided to six portions 5kg each. G1 was served as a control yoghurt. Kumbucha was added a levels of 0.4 , 0.6, 0.8, 1.0 and 1.2% to the prepared milk for making Kumbu-yoghurt (G2, G3, G4, G5 and G6, respectivily. Each portion was heat treated up to ~ 90°C for 5 min, cooled to 42°C, inoculated with 3g 100g^{-1} of starter culture, dispensed into plastic cups (0.5kg each) fitted with press-on lids and incubated at 40 – 42°C till the pH reached 4.6.

The produced Kumbu-yoghurt contains 5% protein, 5% fat and 17% total solids. stored at ~5°C untill used in rats diet comopsation according to the recommended amounts in Table 2.

Biological experiment:

The rats were acclimatized for 7 days with laboratory conditions at 22 - 25°C with a 12 h light/dark cycle. The rats were randomly divided into eight groups four animals each. *First group* (negative group) was fed on basal diet (cholesterol free diet Table 1) throughout the expermental perioed. *The second group* was fed on basal diet contained 0.5% cholesterol (cholesterol-enriched diet) and served as positive control. *The other six groups* were fed on a basal diet contained 0.5% cholesterol to create the hypercholesterloenic rat for one week, then they fed for 8 weeks on a cholesterol-enriched diet supplement with normal yoghurt (control) and called G1 and different types of Kumbu-yoghurt from G1 to G6 as shown in Table 2. The rats received human care in the animal house Agric. Res., Center, Giza, Egypt according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health, USA.

Blood samples were collected from the neck. Serum was obtained after centrifugation (3000 rpm, 20 min, 4°C) and stored at -20°C for biochemical assay. Rats were sacrificed by decapitation under care conditions and the organs (heart, liver, lung and aorta) were excisted from each rat and kept in 10% v/v formaldhyde for histological study.

Method of analysis:

Chemical analysis of Kumbu-yoghurt :

Protein, fat, and total solids of Kombo-yoghurt were determined according to the International Dairy Federation (IDF) Standards, 1993, 1991a and b, respectively.

Biochemical analysis:

Serum total lipdis, total cholesterol, triacylegelecerol, phospholipids and non esterefied fathy acids (NEFA) were determined by spectrophotometry methods according to the methods described by Frings *et al.*, (1972), Meiattini *et al*., (1978), Bucolo and David, (1973), Zilversmit and Davis, (1980) and DunCombe, (1964), respectively. Serum high denesity lipoprotein cheolesterol (HDLC), low density lipoprotein cholesterol (LDLC) and very low denesity lipoprotain cholesterol (VLDLC) were detremined by the methods of Finley *et al*., (1978), Friedewald *et al*., (1972) and Bauer, (1982), respetivily.

Histological study

These were done according to the methods of Bancroft *et al.*, (1996).

Statistical analysis:

The obained results were statistically analysed according to the methods of Clarke and Kempson, (1997)

Table (1) Composition of basal diets

Ingredients	% of total diet
Casein	10
Corn oil	0.75
Vitamin mix*	1.00
Mineral mix*	4.00
Starch	64.75
Tallow	9.75

***Acording to AOAC, (1990).**

Table (2) Composition of the Expermental Diet

	Group	Expermental diet
Cholesterol free diet	**Negative group**	100g basal diet + 47.1 ml water
Cholesterol-enriched diet	**Positive group**	99.5g basal diet + 0.5g cholesterol + 47.1 ml water
	G1	31.22g basal diet + 0.5g cholesterol + 68.28g yoghurt
	G2	31.22g basal diet + 0.5g cholesterol + 68.28g kumbu-yoghurt
	G3	31.22g basal diet + 0.5g cholesterol + 68.28g kumbu-yoghurt
	G4	31.22g basal diet + 0.5g cholesterol + 68.28g kumbu-yoghurt
	G5	31.22g basal diet + 0.5g cholesterol + 68.28g kombo-yoghurt
	G6	31.22g basal diet + 0.5g cholesterol + 68.28g kumbu-yoghurt

RESULTS AND DISCUSSION

Body weight:

The changes of body weight in the experimental rats fed on cholesterol-enriched diet with Kumbu-yoghurt are presented in Table (3). The average of initial body weight recorded a range of 133.00 to 136.50g for G6 and G4, respectively. The body weight increased gradually in all groups where, it recorded 185.53g in the negative group and 208.61g for the positive group which, recorded higher daily body weight gain by the end of the experimental. These results are in accordance with that obtained by Ibrahim 2002 and Ibrahim *et al.*, 2005.

Blood serum lipids profile levels mg/dl:

Tables (4 and 5) show the results of serum lipids profile concentrations (mg/dl) in hypercholesterolaemic rats and their controls after 2 and 8 weeks of feeding on kumbu-yoghurt , respectively. The obtained data reveled that feeding of hypercholestrmic diet to the normal rats allover the experimental period (8 weeks) showed very high significant increase *($P< 0.05$)* in serum total lipids, total cholesterol, triacyleglycerol, phospholipids, low density lipoproteins (LDL), very low density lipoproteins (VLDL) and high density lipoproteins (HDL) concentration. This increase may

be due to the reduced catabolic rate of serum cholesterol or reduced activity of hepatic cholesterol 7-alph-hydroxylase. Also, may be attributed to the increase of 3-hydroxy-3methyl glutaryle - CoA reductase activity in liver of animals fed on hypercholestermic diet (Zult *et al*., 1999). These results are in agreement with those of Vlad *et al*., 1995, Okazaki *et al*., 1998 and Ismail *et al*., 1999. On the other hand, Zult *et al.*, (1998) reported that the intake of increased cholesterol diet with the passage of time, lead to decrease in the activity of LDL receptors of hepatocytes which would reduce the synthesis of bile acids.

Marked hypertriglycerdemia might be a consequence of either over production of VLDL by liver or defective removal of TG rich lipoprotein from circulation or both, this may be explained through lipoprotein lipase, an insulin dependent enzyme involved in TG removal (Yost *et al*., 1995).

During feeding of rats on hypercholesterolaemic diet supplied with kumbu-yoghurt there was a significant decrease in total lipids, total cholesterol, triglyceride, phospholipids, NEFA, HDL, LDL and VLDL after 2 weeks of feeding. However, this decrease became very highly significant by the end of the experiment (8 weeks). Similar results were observed by Fukushima and Nakano (1995) and Ibrahim *et al*. (2005) in rats fed on high cholesterol diet containing probiotics. This decrease may be attributed to lower activity of hydroxymethyl glutoryle Co-enzyme E in the liver, which responsible for cholesterol syntheses.

There was noticeable decrease of NEFA levels in rats fed on kumbu-yoghurt as a probiotic for treating hypercholesterolaemic. This may be due to the decrease of lipocytic effect of lipase activity which decrease lipolysis of triglycerides into glycerol and free fatty acids (FAA) (Emara, 1999). It was clear from the obtained results that increasing of kumbucha level in yoghurt caused a highly significant decrease in serum lipids profile of hypercholesterolaemic rats.

The histological View

Control group

The heart was consisted of branching cardiac muscle and central located multineuclated basophilic nuclei (Fig 1). The aorta was consisted of three tunics; tunica intema which lined with endothelial cells and subendothelial connective tissues (CT), while the tunica media was consisted of fenestrated elastic membranes and the tunica

adventitia was consisted of loose CT (Fig 2). The liver was consisted of cords of hepatocytes, which were polygonal in shape with basophilic centrally located nuclei (Fig 3). The lung was appeared as spongy shape alveoli which were lined with simple squamous cells (Fig 4).

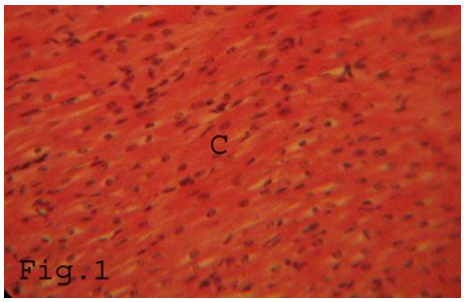

Fig 1: Photomicrograph of the heart showing, cardiac muscle (C). H&E …… .X100

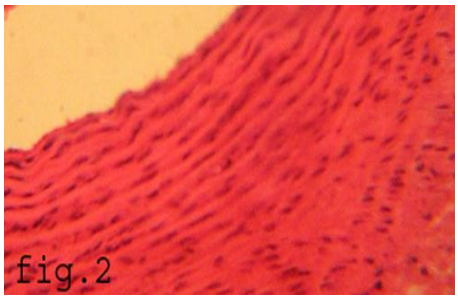

Fig 2: Photomicrograph of the aorta showing, tunica intema. H&E ……. X100

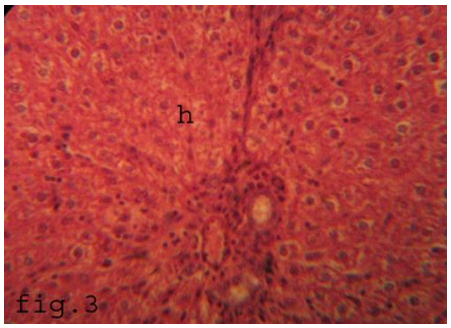

Fig 3: Photomicrograph of the liver showing, hepatic cords (h). H&E ……. X400

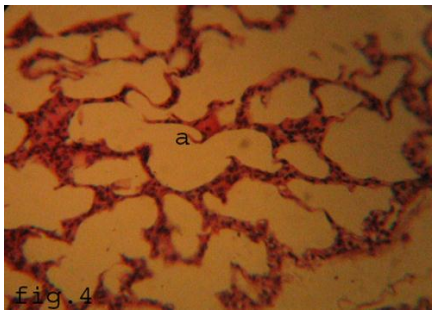

Fig 4: Photomicrograph of the lung showing, alveoli (a). H&E ……. X10

Hyper Cholesterolemia group

The heart showed intramuscular hemorrhage with destruction in some muscle fibers (Fig 5). The aorta showed hemorrhage and desquamation in the endothelial cells of the tunica intema (Fig 6). The liver cells showed some degenerative changes include destruction in the liver cells , vacuolation in the hepatocytes and accumulation of some lymphocyte cells in the destructed area of the liver (Fig 7). The lung showed thickening in the interalveolar wall and hemorrhage in the blood vessels (Fig 8).

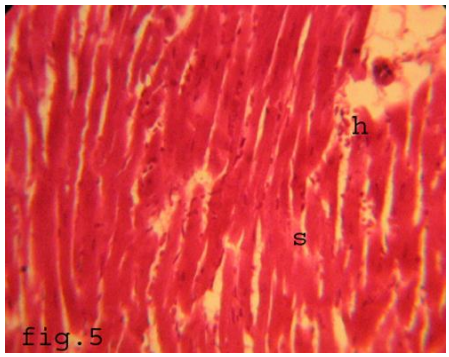

Fig 5: Photomicrograph of the heart showing, destruction of the cardiac muscle (s). Intramuscular hemorrhage (h). H&E X100

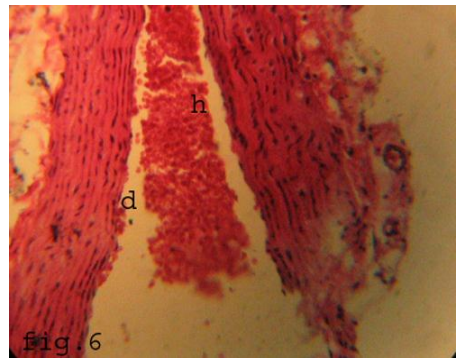

Fig 6: Photomicrograph of the aorta showing, esquamation of the endothelial cells of the tunica intema (d) and hemorrhage (h). H&E ……..X100

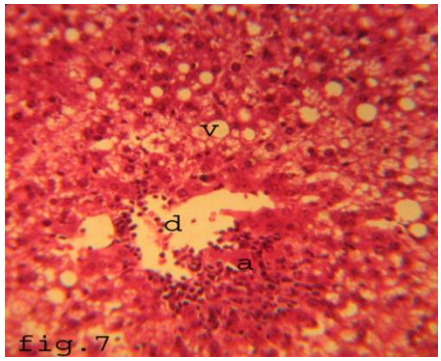

Fig 7: Photomicrograph of the liver showing, destruction in the hepatocytes (d), vacuolation in the hepatocytes (v) and accumulation of lymphocytes in the area of destruction (a). H&E ……. X100

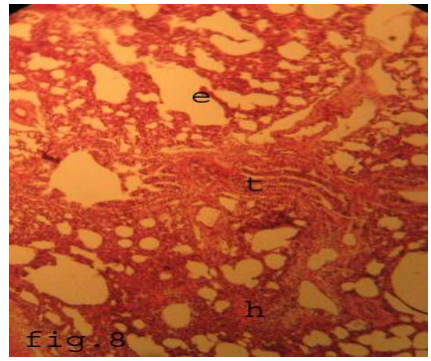

Fig 8: Photomicrograph of the lung showing, widening in the alveolar size (e), thickening in the interalveolar wall (t) and hemorrhage in the blood vessels. (h). H&E ……. X100

Treated groups

There was an improvement in all tested organs of the treated groups and the degree of improvement varied according to the concentration of the added kumbucha to the yoghurt and to the duration of the experiment.

The heart showed gradual improvement, and reaches its ultimate value in all treated groups (6 groups). The cardiac muscles almost returned back to its normal structure, but some intramuscular

hemorrhages still persist (Fig 9). The aorta was showed nearly complete improvement in the histological structure by the end of the experiment. The three tunics were appeared as normal structure (Fig 10). The liver showed partial improvement in the vacuolated hepatocytes, while some hepatic cells still suffered from some degenerative changes especially the vacuolation (Fig 11). The lung showed also incomplete improvement, there was interalveolar thickening still persist (Fig 12).

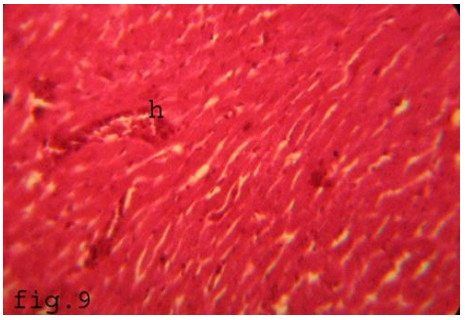

Fig 9: Photomicrograph of the heart showing, intramuscular hemorrhage (h). H&E …. X100

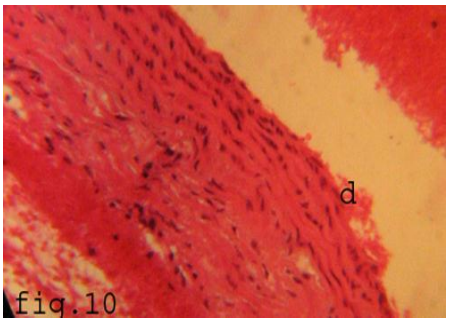

Fig 10: Photomicrograph of the aorta showing, some cellular dequamation (d). H&E . X100

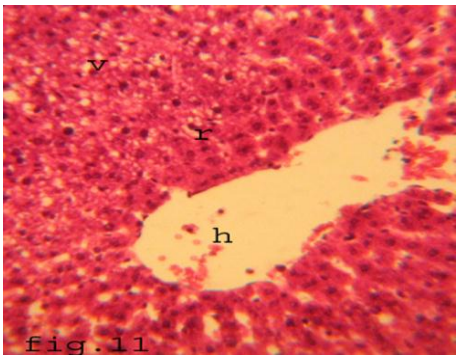

Fig 11: Photomicrograph of the liver showing, some regenerated hepatocytes (r), some vacuolated liver cells (v) and reminant of hemorrhage (h). H&E … X100

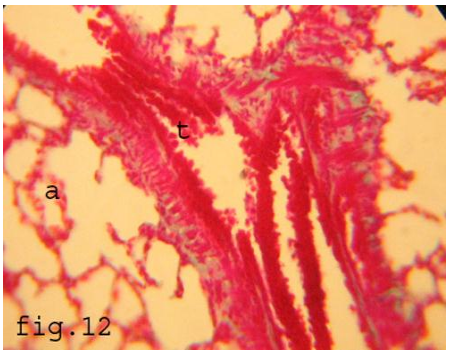

Fig 12: Photomicrograph of the lung showing, thickening in the interalveolar septum (a) and some CT in the lung tissue (t) Crossmon's trichromne …….. X100

Conclusions

From this study it could be conclude that the Kumbucha can be added for making Kombu-yoghurt successfully and used for treating the Cholesterolemia and histological changes in cholesterol-fed rats. This was clear from the significant decrease in the different serum lipid profile, NEFA and histological improvements in the hepatocytes vaculation, musculature of heart, complete improvement of aorta and some improvements in liver and lung. These improvements is correlated with increasing of added kumbucha to the yoghurt.

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تأثير الزبادي المضاف إليه الكومبوشا علي التغيرات البيوكيميائية والهستولوجية في الفئران المغذاة علي عليقة تحوي كوليستيرول

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وليد جافور*** – أمنية محمود****

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في هذه الدراسة تم دراسة التغيرات والبيوكيميائيه والهستولوجيه للفئران المغذاة علي عليقه تحتوي علي ٠.٥ % كوليستيرول ومدعمه بواسطة الزبادي المضاف كومبوشا والتي تحوي في تركيبها البفيدوبكتيريا وفيها تم تقسيم الفئران إلي ثمانية مجموعات لمعرفه التأثير الخافض للزبادي المعامل بالكومبوشا علي مستوي الدهون في سيرم الدم. (الجليسريدات الثلاثية – الفسفوليبيدات – الكوليستيرول بمستوياته) وكذلك أهم التغيرات الهستولوجيه في أعضاء الفئران (الكبد - الرئة– القلب – الأورطي) وكانت النتائج المتحصل عليها كما يلي:

في بداية التجربة كانت هناك فروق معنوية بين مجموعه الكنترول وباقي المجموعات في كل القياسات البيوكيميائيه (الجليسريدات الثلاثية – الفسفوليبيدات – الكوليستيرول الكلي – HDLC – LDL – VLDL) ونتيجه التغذيه علي الـــ Kumbu-yoghurt للمجموعات لوحظ انخفاض معنوي في كل القياسات بعد أسبوعين من التغذية وكان هذا الانخفاض شديد المعنوية في نهاية الأسبوع الثامن من التجربة.

بالنسبة للتغيرات الهستولوجيه أدت التغذية علي الـ Kumbu-yoghurt إلي تحسن بشكل واضح سواء في القلب الرئة أو الاوعيه الدموية والتي أصبحت قريبه جدا في خواصها من خواص مجموعه الكنترول السالبة. وبهذا يمكن القول بان الـــ Kumbu-yoghurt يمكن ان يستخدم غذاء يعمل علي تقليل الاصابه بتصلب الشرايين ويمكن إنتاجه للأغراض العلاجية علي نطاق تجاري.