Proceedings VI World Avocado Congress (Actas VI Congreso Mundial del Aguacate) 2007.
Viña Del Mar, Chile. 12 – 16 Nov. 2007. ISBN No 978-956-17-0413-8.

COMPARATIVE STUDY OF THE HYPOLIPIDEMIC EFFECT INDUCED BY DIFFERENT MONOUNSATURATED AVOCADO OILS

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In Mexico, the ischemic heart diseases are the first cause of death among men and the second one among women. Recently, in the area of vegetable oils of clinical and nutritional importance, the monounsaturated avocado oil has gained a great importance because of its oleic acid content; which is similar to the olive oil. It is also important because of its carotenoids and phytosterols content. These molecules are associated with the cholesterol reduction. The consumption of monounsaturated and polyunsaturated fatty acids could have a beneficial effect as well as an important oxidative resistance of the LDL, decreasing the atherogenic risk at the same time. The goal of this study was to evaluate and compare the hypocholesterolemic effect of the extra virgin avocado oil obtained by a clean technology, developed by Dorantes and Ortiz, along with different types of commercial monounsaturated oils on an induced hypercholesterolemic murine model. The groups were composed of eight mice each, and fed *ad libitum* for a month with a normocholesterolemic diet and an enriched cholesterol diet supplemented with either refined Hass avocado oil or extravirgin avocado oil or extravirgin olive oil or oleic oil. At the end of the treatment the serum concentrations of total cholesterol, LDL-chol, HDL-chol and triglycerides were determined by using an analyzer (Selectra II, Wiener Lab). The results showed a positive tendency of HDL-chol, and an increase of LDL and total cholesterol because of the high combination of fats and oil provided in diet. This suggests that an atherogenic effect could be generated when modulating the lipid intake.

Key words:
Hypercholesterolemic, diet, cholesterol, lipoprotein, triglycerides.

ESTUDIO COMPARATIVO DEL EFECTO HIPOLIPIDÉMICO INDUCIDO POR ACEITES MONOINSATURADOS DE AGUACATE

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Las enfermedades isquémico cardíacas son la primera causa de muerte en México. Recientemente, dentro del rubro de aceites vegetales de interés clínico y nutricional, el aceite monoinsaturado de aguacate ha cobrado gran importancia por su alto contenido de ácido oleico similar al aceite de oliva, además de carotenoides y fitosteroles, estos últimos asociados con la reducción plasmática de colesterol. El consumo preponderante de ácidos grasos monoinsaturados y poliinsaturados, puede ejercer un efecto benéfico, así como mayor resistencia oxidativa de las lipoproteínas de baja densidad (LDL) diminuyendo el riesgo aterogénico. Nuestro propósito fue evaluar y comparar el efecto hipocolesterolemiante del aguacate y el aceite de aguacate extravirgen producido con tecnología limpia desarrollada por las Doctoras Dorantes y Ortiz, con diferentes tipos de aceites monoinsaturados comerciales en un modelo murino hipercolesterolémico inducido. Se emplearon lotes de animales con 8 individuos cada uno. Los grupos fueron alimentados *ad libitum* durante un mes con una dieta normocolesterolémica y una rica en colesterol complementadas con aceite de aguacate Hass refinado, extravirgen, aceite de oliva extravirgen y con aceite oleico. Al término del tratamiento se determinaron las concentraciones séricas de colesterol total, col-LDH, col-HDL y triaciltriglicéridos, mediante un autoanalizador Selectra II Wiener Lab. Los resultados mostraron una tendencia positiva del colesterol HDL, y un incremento de las LDL y colesterol total, debido a la combinación elevada de grasas y aceites proporcionada en la dieta. Esto sugiere que al moderar la ingesta de lípidos se podría tener un efecto aterogénico.

Palabras clave:

Hipercolesterolémico, dieta, colesterol, lipoproteínas, triglicéridos.

1. Introduction.

Overweight and obesity are the main risk factors to undergo different heart diseases such as hypertension, non-insulin-dependent diabetes mellitus, gallbladder cancer and other types of cancer.

Hyperlipemias and hyperlipoproteinemias are other diseases which can be found associated with the conditions mentioned above. These are elevations of the plasmatic concentrations of cholesterol and/or triglycerides. Dyslipemias are also in this group and are alterations of the plasmatic lipids, including an elevation of the total cholesterol and the one which is transported by the low density lipoprotein cholesterol (LDL-cholesterol), also called "bad cholesterol". The latter condition leads to an increase of the triglyceridemia and a decrease in the cholesterol transported by the high density lipoprotein cholesterol (HDL-cholesterol), also called "good cholesterol" (Carmena, 1999)

Hypercholesterolemia is the form of dyslipidemia with the greatest atherogenic potential (Villa Poza, 2001)

In order to counteract the damage that this plasmatic lipids alterations might produce, there are different pharmacological treatments that aim to prevent and stop the atherosclerosis, a vascular and brain coronary disease which is also a silent process that begins at early stages with the appearance of fibrous plaques and continues developing in a progressive and asymptomatic way for several decades leading to a narrowing of the arterial light due to the deposition of cholesterol rich lipoproteins, mainly low density ones, and their subsequent alterations. The fore mentioned causes atherosclerotic lesions because of the weakening of the middle endothelial layer which in turn causes an aneurism (Angelin *et al*., 1996)

Diet is one of the main causes of these diseases; for example, the population who consume a lot of red meat, milk products and carbohydrates show a higher mortality rate because of ischemic heart diseases compared to the population who feed on fruit, vegetables, unsaturated oils and fish (Caggiula *et al*., 1997; Sanhayne and Solá, 1998). The consumption of monounsaturated and polyunsaturated fatty acids such as the oleic and linoleic acids can have a hypocholesterolemic effect and a higher oxidative resistance of the low density lipoproteins which leads to a lower atherogenic risk (Bonanome *et al*.,1992; Fitó *et al*., 2000)

It is being reevaluated the population's way of eating in order to improve their health. For this reason, many natural and healthy products have received much attention because they help to the people's wellbeing and they prevent different diseases, too (Ramaa *et al.,* 2006)

At the same time, different kinds of fruit and vegetables are being reevaluated and classified as excellent sources of nutraceutics (food or food components which has a recognized physiological effect (Feugang, 2006)

The avocado (*Persea Americana Mill)* is one kind of fruit which is gaining a lot of importance because of its content of different components that are considered as nutraceutics. The avocado contains vitamin K, folic acid, ascorbic acid, carotene, flavonoids, tocopherol, beta sitosterol, phosphorus and iron among other components. It is a source of fiber and it is rich in proteins compared to other fruits. The oleic acid is the main fatty acid contained in the avocado, followed by the palmitic and linoleic acids (Alonso, 2004)

Vitamin E, lutein and beta sitosterol are three of the most important nutraceutics contained in the avocado that we want to highlight. Vitamin E works as an antioxidant and diminishes the formation of free radicals which can cause hear damage and different kinds of cancer. Lutein is a carotene that prevents the eyes from developing cataracts. Beta sitosterol can inhibit the absorption of cholesterol by the intestine leading to a lower concentration of cholesterol in blood. Studies directed with animals have showed that the latter can induce the inhibition of malignant tumors (O'toole, 2000).

Vitamin E, lutein and beta sitosterol are three important nutraceutics that we want to highlight. Vitamin E works as an antioxidant and diminishes the formation of free radicals which can cause both damage to the heart and several kinds of cancer. Lutein is a carotene which protects the eyes damages such as the cataracts. Beta sitosterol can inhibit the cholesterol absorption by the intestine, leading to a lower concentration of cholesterol in blood. Studies directed with laboratory animals have shown that this compound affects the inhibition of malignant tumors (O'Toole, 2000).

The great amount of active nutraceutics and their properties make the avocado a fruit with a high potential to obtain food and food supplements that help us to improve an avoid risk of diseases.

Recently, in the area of vegetable oils with clinic and nutritional importance, the monounsaturated avocado oil has gained a great importance because of its oleic acid content; which is similar to de olive oil. It is also important because of its carotenoids a phytosterols content. These molecules are associated with the cholesterol reduction (Berger *et al*; 2004)

The goal of this study was to evaluate the possible hypocholesterolemic effect of the extra virgin avocado oil obtained by a novel technique developed at the IPN along with other monounsaturated oils on an induced hypercholesterolemic murine model.

2. Material and methods.

Male NIH strain mice donated by the Instituto Nacional de Virología BIRMEX (Secretraría de Salud, Ciudad de México) with a weight from 25 to 28 g were used. The animals were randomized in 6 different groups. The groups were fed on different diets: The control group was fed on standard food provided in pellets (Harlan 2018S); another group was fed on a hypercholesterolemic diet and six groups were fed on an enriched cholesterol diet supplemented with either refined Hass avocado oil or IPN extra virgin avocado oil or New Zeland extra virgin avocado oil at a concentration of 2.5% or 5%. The composition of the diet is shown on chart 1.

The animals were kept in individual cages under controlled air and temperature conditions with 12 hour cycles of light/dark and with food and water *ad libitum*.

After the animals were given the different diets for four weeks, they were submitted to a twelve-hour fasting period. After this time, blood samples were obtained from each animal.

Chart 1. Diet compositions supplied to the different groups.

Diet Ingredients	Hypercholesterolemic diet g/100g	Diet at 2.5 % of avocado oils g/100g	Diet at 5.0 % of avocado oils g/100g
Cholesterol	1.0	1.0	1.0
Sodium Cholate	0.5	0.5	0.5
Casein	10	10	10
Sucralose	30	30	30
Butter	5	2.5	0
Avocado oil*	0	2.5	5
Ground standard food	53.5	53.5	53.5

*IPN extra virgin avocado oil or Mexican refined Hass avocado oil or New Zeland extra virgin avocado oil.

The samples were centrifuged (Micrifuge 11, Beckman) for 15 min at 13000 rpm to separate the serum from the cells. Total cholesterol, HDL-cholesterol, triglycerides and blood sugar were evaluated in the serum.

The cholesterol associated to the LDL-cholesterol was calculated by using the Friedwald formula:

LDL = (Total cholesterol – HDL- cholesterol) – (0.45 X Triglycerides)

To calculate the atherogenic index, which is a risk indicator for the atherosclerosis progression, the next formula was used.

AI = (Total cholesterol – HDL-cholesterol) / LDL-cholesterol.

Necropsy was performed to all the animals treated in orther to make a macroscopic analysis of the animals' livers.

3. Results and discussion.

According to the results shown in Chart 2, a significant difference in the total cholesterol was found among the groups fed on the different diets containing avocado oil in various concentrations compared to the control group. The highest increase in the cholesterol concentrations in serum was found in the group fed on the diet including the New Zeland oil, which shown a concentration even higher than the hypercholesterolemic diet group.
It is important to remark that the diet with 2.5% concentration of IPN extra virgin avocado oil led to a decrease in the concentration of the triglycerides in serum compared to the control group. However, any significant differences were found in the triglycerides concentration among the other groups. The mentioned above indicates that the serum triglyceride concentration stays constant even when a carbohydrate rich and high cholesterol diet is supplied to the animals.

The highest increase in the HDL-cholesterol was found in the group fed on IPN extra virgin avocado oil at both 2.5% and 5% concentrations.

The lowest LDL-cholesterol elevation was found in the group fed on the IPN extra virgin avocado oil diet at 2.5% concentration. A significant increase was found in all the other groups compared to the control group. The LDL-cholesterol increase caused by the New Zeland extra virgin avocado oil at 5% concentration diet was even higher than the increase caused by the hypercholesterolemic diet. This suggests that a total substitution with this oil might increase the concentration of these lipoproteins in blood, provoking a higher risk to undergo heart diseases.

The diet at 2.5% concentration of IPN extra virgin avocado oil provoked the lowest atherogenic index. Nevertheless, the atherogenic index (LDL/HDL) in all the other groups was similar to the hypercholesterolemic group. This indicates that eating a diet rich in cholesterol and carbohydrates along with the consumption of monounsaturated avocado oils do not prevent from the formation of the atheromic plaque, all the contrary, it may increase.
Any significant differences were found compared to the control group and Blood sugar values stayed constant.

Chart 2. Lipidic profile of the eight groups of study.

	Total Cholesterol	*Triglycerids*	*HDL Cholesterol*	*LDL Cholesterol*	*Atherogenic Index*	*Blood Sugar*
Control	31.76±3.88	0.665±0.131	26.35±6.37	5.11±2.96	0.238±0.171	124.32±22.96
Hypercolesterolemic	68.93±12.95*	0.608±0.127	26.38±3.32	42.28±10.70*	1.610±0.316*	128.38±26.47
Mexican refined avocado oil at 5% concentration	93.08±28.48*	0.624±0.312	31.75±6.51	61.04±23.33*	1.900±0.509*	98.69±22.13
Mexican refined avocado oil at 2.5% concentration	82.18±29.21*	0.619±0.208	29.20±4.73	52.70±25.00*	1.760±0.574*	97.13±27.10[a]
IPN extravirgen avocado oil at 5% concentration	92.96±31.03*	0.659±0.213	32.69±6.78	59.98±25.15*	1.809±0.437*	114.88±9.65
IPN extravirgen avocado oil at 2.5% concentration	81.64±21.87*	0.552±0.180	31.08±5.33	50.32±17.23*	1.594±0.403*	118.40±34.07
New Zeland extravirgen avocado oil at 5% concentration	104.53±28.58*[a]	0.686±0.213	34.79±6.41*[a]	69.44±23.88*[a]	1.983±0.482*	109.71±39.34
New Zeland extravirgen avocado oil at 2.5% concentration	88.28±34.17*	0.730±0.313	28.37±6.80	59.59±28.64*	2.051±0.638*	126.25±17.32

Values expressed as the average ± the standard deviation; n = 6. * Significant difference regarding to the control group [a] Significant difference regarding to the hypercholesterolemic control obtained by using one way ANOVA test post-hoc LSD $p < 0.05$.

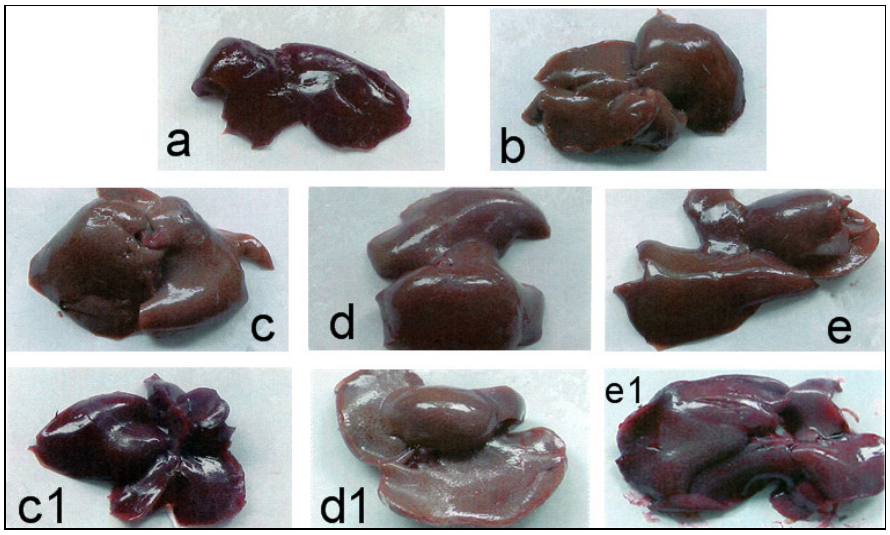

Figure 1. Pictures taken to the livers from the eight groups of study
Control diet (a),Hypercholesterolemic diet (b),Refined Hass avocado oil at 5% concentration diet (c), Refined Hass avocado oil at 2.5% concentration diet (c1), New Zeland extra virgin avocado oil at 5% concentration diet(d), New Zeland extra virgin avocado oil at 2.5% concentration diet (d1), IPN extra virgin avocado oil at 5% concentration diet (e), IPN extra virgin avocado oil at 2.5% concentration diet (e1)

The features of a normal liver are seen in picture 1a. The liver has a red color, without fatty accumulations and a normal size.

To the macroscopic analysis of the livers we found that those animals fed on the hypercholesterolemic diet shown fatty accumulations on this organ. We also observed an increase in the size of the liver compared to the liver from the control group.

The liver from the animals fed on either IPN extra virgin avocado oil or refined Hass avocado oil both at 2.5% concentration showed the least damage. The color of the livers from these animals was very similar compared to the control group and a slight increase in the size of the organ was observed.

The highest damage was found in the livers from the animals fed on the New Zeland extra virgin avocado oil at 2.5% concentration. These livers showed a discoloration and also an increase in the size of the lobes that constitute this organ.

4. Conclusions.

The model proved in this study suggests that the dietary substitution with avocado oils at both 2.5% and 5% concentrations might help to increase the HDL-cholesterol concentration and maintain a constant concentration of the triglycerides, in spite of the high caloric supply.

A dose-response relation was observed regarding the total cholesterol concentrations, the LDL-cholesterol concentration and the HDL-cholesterol concentration in all the groups fed on the different diets supplemented with the avocado oil at both 2.5% and 5% concentrations.

The livers that had the fewer alterations in the different features evaluated were those from the animals fed on both the IPN extra virgin avocado oil and the refined Hass avocado oil diets at 2.5% concentration.

The avocado oil produced at the Instituto Politécnico Nacional provoked the least increase in both the total cholesterol and the LDL-cholesterol concentrations and also had the least atherogenic index compared to the hypercholesterolemic group.

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