

## STUDY REGARDING THE HIPOCHOLESTEROLEMIANT EFFECT OF ISOFLAVONES SUPPLEMENTS OBTAINED FROM *TRIFOLIUM PRATENSE*

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**Abstract.** In this study has been evaluated the effect of some isoflavones supplements obtained from *Trifolium pratense* (Red Clover) on blood cholesterol. It was performed at the Department of Cardiology, Rehabilitation Hospital, Cluj-Napoca, Romania, in January-March, 2005, and it included 40 persons with slightly hypercholesterolemia. The first group (*Group A-isoflavones*) included 15 subjects with total cholesterol= $226.56 \pm 15.01$  mg/dl and LDL cholesterol =  $153.06 \pm 6.98$  mg/dl; all of them have received a daily dose of 26 mg isoflavones from a red clover supplement called Flavosin. The second group (Control group B-diet) which has included 25 subjects with total cholesterol =  $222.21 \pm 26.36$  mg/dl and LDL cholesterol =  $151.61 \pm 61.26$  mg/dl, followed a diet that is recommended in hypercholesterolemia; caloric needs have been individually established and the average was  $1450 \pm 268$  kcalories /d. At group A, it was founded a significant reduction ( $p = 0.02$ ) regarding total cholesterol after three month of isoflavonoids intake according to initial level, the percentage reduction being 7.1%. It was also founded a significant increase of HDL cholesterol ( $p = 0.055$ ) which is a protective factor from atherosclerosis in the case of hypercholesterolemia. The LDL cholesterol and triglycerides reduction was non significant ( $p = 0.135$ , respectively  $p = 0.055$ ). At Group B it was founded a paradoxical, but non significant, increase of all lipid parameters. In conclusion, the results showed that red clover supplement was efficient in this short term study in order to reduce total cholesterol and in consequence for the prevention of atherosclerotic cardiovascular diseases.

**Key words:** isoflavones, cholesterol, cardiovascular diseases.

### INTRODUCTION

The study of plasma lipids in relation to the cause, progress or regression of the atherosclerotic process has led to the development of cardiovascular epidemiology and represents a major research and intervention element of preventive cardiology, according to Expert Panel on Detection Evaluation, and Treatment of High Blood Cholesterol in Adults, JAMA 2001.

According to Zdrengea et al. (2003), few concepts in medicine have resulted in such extensive research work as the ones testing “the lipid hypothesis of atherosclerosis”.

Isoflavones represent a class of biological substances with beneficial action in the reduction of cholesterol, LDL cholesterol, increasing of HDL cholesterol and reduction of triglycerides as well as other risk factors of atherosclerotic cardiovascular diseases.

Keys (1980), reported that the cholesterol-reducing effects of isoflavones has been demonstrated by numerous studies that started from the finding that the Japanese, consumers

of much more proteins of vegetal origin than the Americans, present a lower incidence of ischemic heart disease.

Subsequent studies conducted by Anderson (1995), were carried out in patient groups with high, average, small, or normal cholesterol levels, adults or children, all of them reporting that a diet containing vegetal isoflavones led to the reduction of cholesterol levels.

Frank et al. (2006) published an overview of all the studies regarding the effects soy protein on the lipid profile. Most of these studies are focused on soy isoflavones: genistin and daidzin that have gained a considerable attention for their potential role in improving risk factors for cardiovascular disease.

In this study has been evaluated the effect of some isoflavones supplements obtained from *Trifolium pratense* (Red Clover) on blood cholesterol. Red clover is a legume that is a rich source of isoflavones. Some of the isoflavones found in red clover are not present in soy. These are: formononetin (methoxy daizein) and Biochanin A (4'-methyl ether genistin). Formononetin and Biochanin A are transformed under the action of intestinal glucosidase enzymes in daizin and genistin, respectively. Red clover also contains small quantities of genistin and daidzin.

Several authors (Howes et al., 2000; Husband, 2001; Kuhn et al., 2001; Nachtigall, 2001), have reported that the total quantities of isoflavones from red clover vary between 1.0%, 2.5%, to 3% of dried plant.

## MATERIALS AND METHODS

This study has been performed at the Department of Cardiology, Rehabilitation Hospital, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, in January-March, 2005, and the study group included 40 persons, men and women, selected by the following criteria:

- dislipidemia: total cholesterol (TC) and LDL cholesterol ("bad cholesterol") moderate elevated (TC >190mg/dl, LDL cholesterol >115mg/dl);
- HDL cholesterol ("good cholesterol") and triglycerides (TGL) normal or moderate elevated (HDL >30mg/dl, TGL <300mg/dl);
- normal blood pressure;
- normal anthropometric indicators (corporal mass indicator, abdominal circumference);
- Framingham cardiovascular risk <10%;
- no hipolipemiant therapy before the study.

The subjects were divided in two groups:

- the first group (*Group A-isoflavones*) included 15 subjects with slightly hypercholesterolemia (total cholesterol=226.56 ± 15.01mg/dl, LDL cholesterol = 153.06 ± 6.98 mg/dl); all of them have received a daily dose of 26 mg isoflavones from a red clover supplement called Flavosin.

- the second group (Control group B-diet) which has included 25 subjects with slightly hypercholesterolemia (total cholesterol = 222.21 ± 26.36 mg/dl, LDL cholesterol = 151.61 ± 61.26 mg/dl), followed a diet that is recommended in hypercholesterolemia (fat intake < 30%, with saturated fats at 1/3 from total fats, cholesterol intake < 300 mg/d); caloric needs have been individually established and the average was 1450 ± 268 kcalories /d.

Table 1 represents the most important characteristics of the study groups.

Table 1

## Characteristics of the study groups

Characteristics	Group A (Flavosin)	Control group B (diet)
Subjects number	15	25
Women/men ratio	11/4	18/7
Average	57.13 ± 8.19	56.83 ± 10.75
Smokers	4 (26.6%)	8 (32%)
HCA (Heredocolateral antecedents)	5 (33.3%)	12 (48%).
Elevated blood pressure	9 (60%)	10(40%)
Ischemic cardiopathy	5 (33.3%)	6(24%)
Mean weight (kg)	80.78 ± 19.52	77.4 ± 15.44
Systolic Blood Pressure (mmHg)	135 ± 21.23	143.77 ± 24.31
Diastolic Blood Pressure (mmHg)	82.5 ± 8.56	87.46 ± 11.59
• Total Cholesterol (TC) (mg/dl)	226.56 ± 15.01	222.21 ± 26.36
• LDL chol (mg/dl)	153.06 ± 16.98	151.61 ± 61.26
• HDL chol (mg/dl)	43 ± 8.31	53.34 ± 15.74
• triglycerides (mg/dl)	147.68 ± 53.77	139.73 ± 28.41

## RESULTS AND DISCUSSIONS

At group A, was found a significant reduction ( $p = 0.02$ ) regarding total cholesterol after three month of isoflavonoids from red clover intake according to initial level, the percentage reduction being 7,1%. The LDL cholesterol and triglycerides reduction was non significant ( $p = 0.135$ , respectively  $p = 0.055$ ) (Table 2, Figure 1).

It was also found an increase of HDL cholesterol ( $p = 0.055$ ) which is a protective factor from atherosclerosis in the case of hypercholesterolemia. In order to evaluate the red clover isoflavones benefits comparing with the benefits of the hipolipidemic diet, statistical analysis of the results has been performed.

Table 2

## Effects of red clover isoflavones at group A

Parameter	Initial	Final (3 month)	Statistical significance
TC (mg/dl)	226.56 ± 15,01	210 ± 19.47	$p = 0.02^*$
LDL chol (mg/dl)	153.06 ± 16.98	138.31 ± 16.56	$p = 0.135$
HDL chol (mg/dl)	43 ± 8.31	45.125 ± 6.03	$p = 0.055^*$
Triglycerides (mg/dl)	147.68 ± 53.77	129.56 ± 41.58	$p = 0.24$

Statistical significance has been appreciated with the „t” (Student) Test, considering  $p \leq 0.05$  as significant\*.

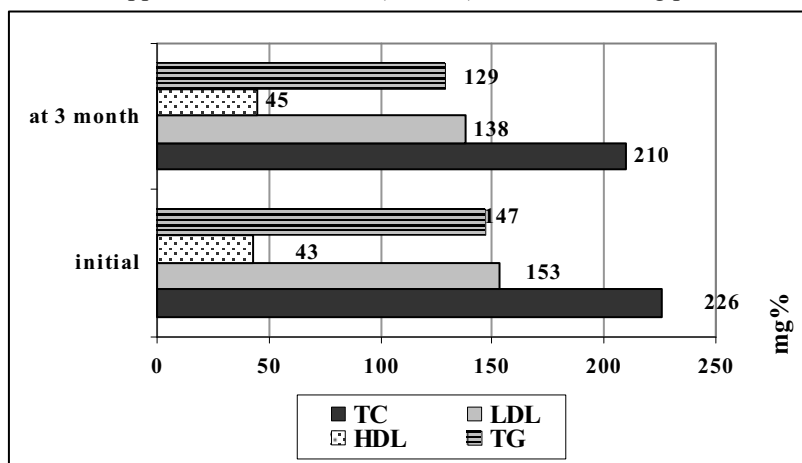


Figure 1. Effects of red clover isoflavones at group A

At Group B was found a paradoxical, but non significant, increase of all lipid parameters (Table 3, Figure 2).

Table 3

Effects of dietary recommendations at group B

Parameter	Initial	Final (3 month)	Statistical significance
CT (mg/dl)	222.21 ± 26.36	226.375 ± 25,88	p = 0.2977
LDL chol (mg/dl)	151.61 ± 61.26	158.25 ± 70.59	p = 0.269
HDL chol (mg/dl)	53.34 ± 15.74	54.375 ± 18.04	p = 0.031
Triglycerides (mg/dl)	139.73 ± 28.41	140.375 ± 25.51	p = 0.478

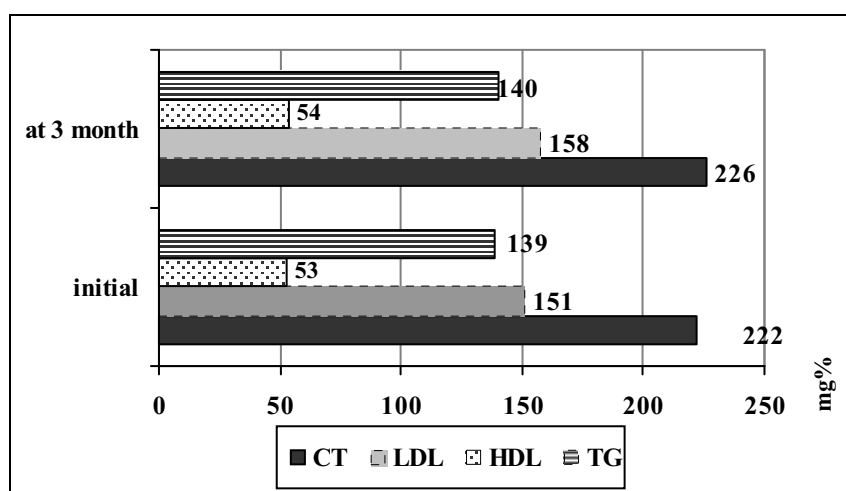


Figure 2. Effects of dietary recommendations at group B

The reduction of total cholesterol (7.1%) that it was gained in this study with 26 mg isoflavones from red clover/d is much closed with the results that have been obtained in other studies with isoflavones from soy protein. So James A. Anderson from the Clinical Nutrition University of Kentucky (1995) reported a significant decrease of serum total cholesterol and LDL cholesterol by a daily intake of 30-50 mg isoflavones from soy protein. He found that in individuals with normal cholesterol, the level of total cholesterol dropped of by 7.7%, while in those with severe hypercholesterolemia (>335 mg/dl) the reduction was over 24%. The average reduction percentage was 9.3%.

The fact is that the results obtained at the group with isoflavones from red clover are more important that the results that were obtained with diet. This can be partially explained by the observations that the diet is much more difficult to hold such a long period of time (3 month) and the study is very difficult to control. These difficulties didn't appear in the case of isoflavones supplements.

There was an excellent tolerability of red clover isoflavones and any abandonment at all the subjects.

## CONCLUSIONS

In this short term study, isoflavones from red clover (*Trifolium pratense*) had a significant influence on total cholesterol in the cases of slightly hypercholesterolemia, this influence being much important that in the case of the hypocholesterolemiant diet. Also there

was an important increase of HDL cholesterol (“good cholesterol”), very close to the statistical significance.

In the entire period, all subjects had an excellent tolerability at supplements from red clover. This pilot study can represent the start point to other medium or long term studies in order to demonstrate the influence of isoflavones from *Trifolium pratense* on lipid profile and the tolerance of the specific isoflavones.

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## REZUMAT

### STUDIUL PRIVIND EFECTUL HIPOLIPEMIANȚ AL SUPLIMENTELOR ALIMENTARE CU IZOFLAVONE OBTINUTE DIN *TRIFOLIUM PRATENSE*

În prezentul studiu s-a evaluat efectul unui supliment alimentar cu izoflavone obținut din *Trifolium pratense* (trifoi roșu) asupra colesterolului plasmatic. Studiul s-a desfășurat în cadrul Spitalului Clinic de Recuperare - Secția Cardiologie, Cluj-Napoca, România, în perioada ianuarie-martie, 2005, și a inclus un număr de 40 de subiecți cu hipercolesterolemie ușoară. Primul grup (*Grupul A-izoflavone*) a fost format din 15 persoane care au avut colesterol total =  $226,56 \pm 15,01$  mg/dl și LDL colesterol =  $153,06 \pm 6,98$  mg/dl; aceștia au primit o doză de 26 mg izoflavone pe zi din *Trifolium pratense* (trifoi roșu) sub forma unui supliment alimentar (produsul Flavosin). Cel de-al doilea grup (*Grupul control B-dietă*) a fost format din 25 de persoane care au avut colesterol total =  $222,21 \pm 26,36$  mg/dl și LDL colesterol =  $151,61 \pm 61,26$  mg/dl; aceștia au urmat exclusiv dietă hipolipidică; necesarul caloric a fost stabilit individualizat și a fost în medie  $1450 \pm 268$  kcalories/zi. La grupul A, colesterolul seric a înregistrat o reducere de 7,1% statistic semnificativă ( $p=0,02$ ). S-a înregistrat, de asemenea, o creștere semnificativă a HDL colesterolului ( $p=0,055$ ) care este un factor protector pentru ateroscleroză în caz de hipercolesterolemie. LDL colesterolul și trigliceridele nu au înregistrat modificări semnificative ( $p=0,135$ , respectiv  $p=0,055$ ). La grupul B s-a găsit o paradoxală, dar nesemnificativă creștere a tuturor parametrilor lipidici. În concluzie, rezultatele arată că suplimentul cu izoflavone din trifoi roșu a fost eficient în acest studiu pe termen scurt în reducerea colesterolului total și poate fi folosit, în consecință, pentru prevenirea bolilor cardiovasculare aterosclerotice.