INSULIN, THYROID HORMONE LEVELS AND METABOLIC CHANGES AFTER TREATED RATS WITH HEMP MILK

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ABSTRACT

The aim of study was to investigate the influence of hemp milk (emulsion of hemp seed grinded and mixed with water in 1:5 ratio) on body weight gain, internal organs weight, insulin and thyroid hormones serum concentration and blood lipid indices. The studies were carried out on 40 female Wistar rats and were divided into two experiments that differ the way of hemp milk administered to rats for 21 days. In Experiment I rats were given intragastically water (I), hemp milk (II) and hemp milk 1:1 (III). In Experiment II control animals had free access to the water and experimental rats had free access to the hemp milk instead of water. Results obtained provide the first demonstration that after hemp milk both intragastric and *ad libitum* treatment leads to the significant reduction of serum triglycerides and cholesterol content, including total, free and esterified forms of this sterol. Moreover, significant decrease of thyroid hormones was observed in experimental rats.

INTRODUCTION

Cannabis is probably one of the first plants to have been used (and later cultivated) by people. Throughout history hemp has often been an important plant for its useful for folk medicine for its stimulation of liver and kidney function, in industry as a source of fibre, and for the food provided by its seed [1]. The fruit of hemp are consumed whole or employed as a feed for birds and fishes [2]. Hemp seed shares with no other plant resource, both a high content of easily digestible complete protein and a rich source of oil providing a favorable ratio of the linoleic (C18:2, n-6, LA) and linolenic (C18:3, n-3, LNA) essential fatty acids required for proper human nutrition [3]; in addition to a significant contribution of stearidonic (C18:4, n-3, SDA) and gamma-linolenic(C18:3, n-6, GLA) acid of potential therapeutic efficiency [4, 5]. In the body GLA is normally derived from LA and serves as substrate for the formation of eicosanoids which perform numerous vital roles. The metabolic conversion of LA to GLA is slow in mammals. Further, it has been suggested that due to stress, aging or pathology (e.g., hypertension, diabetes, etc) formation of sufficient amount or balance of eicosanoids may be impaired, and this problem may be relieved by direct GLA supplementation [6].

Whole hemp seed contains also approximately 30% protein, 10% dietetic fibre, as well as a rich array of minerals, the latter of which is an important enzyme co-factor for human fatty acid metabolism [2, 7].

These unique nutritional qualities of hemp seeds provide possibility of improving both health and condition for humans and animals. However, influence of hemp seed on physiological and biochemical parameters has to be thoroughly examined.

Therefore, the aim of the present studies was to investigate the influence of hemp milk (emulsion of hemp seed grinded and mixed with water in 1:5 ratio) on body weight gain and internal organs weight in rats. Blood hormones concentration and some metabolic parameters reflecting insulin and thyroid action were also determined.

MATERIAL AND METHODS

The studies were carried out on 40 female Wistar rats (90±3 g) and were divided into two experiments that differ the way of hemp milk administration to rats for 21 days. Results of chemical analyses of hemp seed and hemp seed milk used in our experiments have been shown in table 1.

In Experiment I 24 rats were divided into three groups of 8 animals each: I control – water, II – hemp milk and III – hemp milk 1:1 (hemp milk mixed with water in 1:5 ratio). The water, hemp milk and hemp milk (1:1) were given intragastrically for 21 days (9 a.m.) at a volume 0.5 ml per 100 g of body weight/day, respectively. These all animals had free access to the water.

Table 1. Composition of hemp seed and hemp milk

Composition %	Hemp seed (uncovered)	Hemp milk
Ash	6.99	0.47
Protein (N x 6.25)	30.4	2.6
Total carbohydrates	19.91	0.3
Crude fiber	8. 3	0.4
Water	6. 0	91.6
Fat	28.40	4.61
Energic valueKcal/100 g product	456.84	53.09
Vitamins, mg/g		
E	30.1	0.42
C	14.4	2.1
B_1	9.3	1.1
B_2	10.7	0.8
B_3	25.1	2.4
B_6	3.1	0.03
Fatty acid composition (% fat)		
Palmitic (C16:0)	6.2	6.5
Stearic (C18:0)	2.5	2.4
Oleic (C 18:1)	9.9	10.2
Linoleic (C18:2)	55.2	57.1
Gammalinolenic (C 18: 3 n-6)	2.8	2.1
Alfalinolenic (C 18: 3 n-3)	21.3	19.8
Stearidonic (C18:4 n-3)	1.2	1.2
Arachidic (C 20:0)	0.6	0.4
Eicosenoic (C 20: 1)	0.3	0.25
Amino Acids (g/16g N or g/100 g protein)		
Alanine	4.26	5.20
Arginine	13.93	13.56
Cystine	6.15	nd
Glutamic Acid	14.68	16.49
Glycine	4.40	4.74
Histidine	3.89	3.62
Isoleucine	3.85	4.07
Leucine	6.46	6.78
Lysine	3.31	3.62
Methionine	6.70	5.42
Phenylalanine	3.96	4.07
Proline	3.96	4.97
Serine	3.65	3.84
Threonine	2.84	3.39
Tyrosine	2.94	3.62
Valine	4.43	5.65

Composition %	Hemp seed (uncovered)	Hemp milk
Heavy metals, mg/kg		
Cu	15.75	0.09
Cd	0.12	0.01
Pb	1.50	0.02
Zn	55.35	5.96
Hg	0.02	0.01
Cannabinoids, _g/g		
_9 tetrahydrocannabinol	0.1	nd
Cannabidiol	0.9	nd
Cannabinol	nd	nd

In Experiment II (16 rats, n=8) animals of Group 1 – control had free access to the water and rats of Group 2 - free access to the hemp milk instead of water.

All rats were housed in standard conditions (temp 23°C, photoperiod 12:12) and were fed *ad libitum* with the laboratory food. Three times a week body weight and daily hemp milk and water intake were noted. Twenty-four hours after the last treatment, all of the animals were decapitated then internal organs weighted and blood collected.

Hormones in serum were assayed radioimmunologically using kits: from Linco Research (USA) for insulin, from Orion Diagnostica (Finland) for triiodothyronine (T_3) and thyroxine (T_4) and from CIS Bio International (France) for free triiodothyronine (fT_3) and free thyroxine (fT_4). Glucose was determined using o-dianisidin, peroxidase and glucose oxydase. Serum triglycerides level were assayed by the method of Foster and Dunn [8], and different cholesterol forms – enzymatically using cholesterol oxidase [9].

All data are presented as mean ±SEM. Statistical comparison of Experiment I data was performed by ANOVA followed by the Multiple Range Test of Duncan. An unpaired Student's *t* test was used to assess differences between 2 groups of Experiment II.

RESULTS

The means of hemp milk drank *ad libitum* by rats was 28 ml/100g b.w./day, whereas the mean amount of water drank was 12.3 ml/100 g b.w./day. The good health and condition of all animals were noted during investigation.

Results in Table 2 showed non significant reduction of body weight only after intragastrically treatment (17%) and drinking *ad libitum* (5%) non-diluted hemp milk. Simultaneously, no changes were observed for kidney and liver. To the contrary, intragastrically administration hemp milk and hemp milk (1:1) enhanced no significantly thyroid gland and adrenals by 20% and 8% respectively, in comparison to control.

Our data showed that the factors containing in hemp milk influenced on endocrine system (Table 3). The changes concerning blood insulin were distinct and were expressed increase insulin level by 86% and 76% in group II and III of Experiment I respectively, compared with control. Free access to hemp milk for 21 days caused lesser elevation (18%) of blood insulin. Besides changed level of insulin, deep alterations were also noticed in blood thyroid hormones concentrations of hemp milk treated rats. These alterations depend on the way of hemp milk administration. The increase of thyroxine, fT_4 and fT3 ($p<0.05$) levels have been showed after intragastrically hemp milk provided. Contrary alteration, however deeper, were observed for fT_4 ($p<0.01$) and fT_3 ($p<0.05$) of animals which had free access to the hemp milk for 21 days.

Table 2.
Effect of hemp milk adhibited rats intragastrically (Experiment Table 2. I, 0.5ml/100 g b.w.) and *ad libitum* (Experiment II) for 21 days on body weight gain and internal organs weight

Parameter	ExperimentI hemp milk intragastrically			ExperimentII hemp milk *ad libitum*	
	I control	II hemp milk	III hemp milk (1:1)	1 control	2 hemp milk
Daily body gain, [g]	2.89±0.39	2.57±0.13	2.95±016	3.10±0.16	2.94±0.09
Liver [g/100g b.w.]	3.91±0.04	3.88±0.10	3.89±0.09	4.10±0.10	4.08±0.11
Kidneys [g/100g b.w.]	0.66± 0.02	0.68±0.02	0.69±0.01	0.67±0.01	0.73±0.01
Thyroid [mg/100g b.w.]	6.19±0.60	7.41±0.51	6.18±0.46	6.20±0.43	6.10±0.55
Adrenals [mg/100g b.w.]	26.90±2.86	25.92±2.05	29.19±1.24	30.29±1.54	31.08±1.39
Ovaries [mg/100g b.w.]	59.23±3.41	58.47±3.26	56.55±2.88	53.35±2.17	61.20±1.44
Uterus [g/100g b.w.]	26.90±2.86	25.92±2.05	29.19±0.4	30.29±1.54	31.08±139

Table 3.
Effect of hemp milk adhibited intragastrically (Experiment I, 0.5 ml/100g b.w.) and *ad libitum* (Experiment II) for 21 days on hormone levels in serum rat

Parameter	Experiment I hemp milk intragastrically			Experiment II hemp milk *ad libitum*	
	I control	II hemp milk	II hemp milk (1:1)	1 control	2 *ad libitum*
Insulin [ng/ml]	0.55±0.13	1.02± 0.29	0.96± 0.34	0.72± 0.17	0.85±0.35
T_4 [nmol/L]	43.68±3.71	54.92± 6.13	45.44± 3.46	51.36±3.55	44.86± 2.83
fT_4 [pg/ml]	17.65±3.05	23.31± 2.89	20.48± 2.17	21.81±1.97 A	12.61± 0.99 A
T_3 [nmol/ml]	1.47±0.06	1.58± 0.12	1.43± 0.07	1.65± 0.07	1.52± 0.10
fT_3 [pg/ml]	4.48± 0.29 a	5.61± 0.42 a	4.84± 0.23	5.58± 0.28 a	4.62± 0.17 a

a, b – values in rows with the same letters differ significantly ($p<0.05$)
A, B – values in rows with the same letters differ significantly ($p<0.01$)

Simultaneously, differences in insulin and thyroid hormone levels were accompanied by changes in carbohydrates and lipid metabolism (Table 4).

Serum glucose level was increased in hemp milk drinking and intragastric treated animals. This increase was significantly only after intragastrically administered of non-diluted hemp milk. This way of animals treatment also evoked augmentation of triglycerides and all forms of cholesterol in rats serum. In contrary, hemp milk mixed with water in 1:1 ratio, after the same way of administration, significantly decreased all lipid parameters.

It has to be emphasized that hemp milk adhibited *ad libitum* evoked a markedly reduction of all lipid serum parameters. This decrease was due to significant depression of free cholesterol ($p<0.01$) and triglycerides ($p<0.01$) and drop of total and esterified cholesterol.

Table 4.
Effect of hemp milk adhibited intragastrically (Experiment I, 0.5-ml/100g b.w.) and *ad libitum* (Experiment II) for 21 days on metabolic indices in rats serum

Parameter	**Experiment I hemp milk intragastrically**			**Experiment II hemp milk *ad libitum***	
	group I control	**group II hemp milk**	**group III hemp milk (1:1)**	**group 1 control**	**group 2 *ad libitum***
Glucose [mmol/L]	4.91±0.16 a	5.14 ±0.19 a	5.55± 0.23	5.65± 0.21	5.95±0.21
Cholesterol total [mmol/L]	1.59± 0.06 A	1.74± 0.07 B	1.29± 0.05 AB	1.43± 0.12	1.28± 0.10
Cholesterol free [mmol/L]	0.24± 0.03	0.30± 0.03 a	0.21± 0.02 a	0.28± 0.03 A	0.19± 0.02 A
Cholesterol ester [mmol/L]	1.35 ±0.07 A	1.43 ±0.06 B	1.08± 0.04 AB	1.15± 0.10	1.09± 0.09
Triglycerides [mmol/L]	3.52± 0.26 a	4.29±0.21 aB	3.43± 0.21 B	4.03± 0.32 A	2.75± 0.14 A

a, b – values in rows with the same letters differ significantly ($p<0.05$)
A, B – values in rows with the same letters differ significantly ($p<0.01$)

Intragastric treatment by non-diluted hemp milk also evoked augmentation of triglycerides and all forms of cholesterol in rats' serum. In contrary, hemp milk mixed with water in 1:1 ratio, after the same way of administration, significantly decreased all lipid parameters.

It has to be emphasized that hemp milk adhibited *ad libitum* evoked a markedly reduction of all lipid serum parameters. This decrease was due to significant depression of free cholesterol ($p<0.01$) and triglycerides ($p<0.01$) and drop of total and esterified cholesterol.

DISCUSSION

Many substances that occur naturally in food plant may modify, in a significant way, the metabolic processes in their consumers. The evidence in the literature shows that this occurs through changes in hormones concentration [10].

Results of the presented studies provide the first demonstration that hemp milk leads to the reduction of the serum triglycerides and serum cholesterol content, including total, free and esterified form of the cholesterol.

The nutritional program and the food were the same for both control and experimental animals and so this decrease of blood lipid indices was presumably the results of an alteration in lipid metabolism in the liver after hemp milk consumption. Our results prove that rats treated by *ad libitum* of hemp milk showed the greatest improvement in blood lipids. Particularly, it can be seen in drop of triglycerides level.

Many studies with laboratory animals and humans have shown that mammalian thyroid hormones are integral to hepatic lipogenesis and inducers of hepatic fatty acid and triglyceride synthesis [11]. Moreover, the hypocholesterolemic activity of thyroid hormones has been well known [12]. Our results do not agree with those suggestions. The presented experiments prove hypocholesterolemic effects with lowered serum triglycerides and with simultaneously decrease of thyroid gland activity. In our opinion, changes of hormone levels and lipid metabolism after hemp milk are the elements of mechanism which connects both dietary specific essential fatty acids and composition of proteins, especially their sulfur amino acid content which exerts a hypocholesterolemic effects [13]. This suggestion is in agreement also with Cheema and Clandinin [14] who noted that PUFA the n-6 and n-3 families inhibit the rate of gene transcription for a number of hepatic lipogenic and glycolytic genes.

Epidemiological studies have suggested that higher concentration of circulating cholesterol and triglycerides is believed to be a major risk factor for atherosclerosis. New strategies could be developed for the prevention or treatment of coronary heart diseases. Hemp milk, which in our studies significantly lowered serum triglycerides and all form of serum cholesterol, seems to be one of that kind of a new proposition.

CONCLUSIONS

- Hemp milk adhibited *ad libitum* evoked significant reduction of free cholesterol ($p<0.01$) and triglycerides ($p<0.01$) and decreased total and esterified cholesterol in serum rats.
- Hemp milk mixed with the water in 1:1 ratio also significantly decreased triglycerides and cholesterol blood content of intragastric treated rats.
- The increase of thyroid hormone levels was observed after intragastric hemp milk provided. Contrary, the concentration of thyroid hormones of animal which had free access to hemp milk were significant decreased.

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