PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 5 (6): 585-588, 2006
ISSN 1680-5194

Effect of *Cannabis sativa* L. Seed (Hempseed) on Serum Lipid and Protein Profiles of Rat

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Abstract: *Cannabis sativa* L. is considered as nutritional plant. Whole hempseed typically contains over 30% oil (3%saturated and 28%unsaturated fatty acids) and about 25% protein. This study was conducted to evaluate the effect of hempseed on lipid and protein profiles of rats. After the acclimatization, at the beginning of experiment (day 1) the feeding of animal stopped and after 12hr fasting the animal anesthetized by ketamine/xylazine combination and 2ml blood of heart was taken. The rats had free access to normal diet and hempseed for 20 days and at the end of experiment (day 20) blood was taken again. The blood parameters were measured by automated biochemical analyzer. Hempseed significantly (p=0.004) decreased the mean fasting serum LDL level and also significantly increased the mean fasting serum HDL and total protein levels (p=0.01). The mean fasting triglyceride, total cholesterol and albumin levels didn't significantly change. The significant level was 0.05 or less. Short term hempseed feeding has improved blood lipid and protein profile. In the light of this research, it is recommended that individuals who have high cholesterol and LDL levels or affected to coronary artery diseases (CAD) and liver diseases, they can use hempseed variety that cultivated in Khorasan province of Iran in their food preparation.

Key words: Hempseed, lipid, protein, cholesterol, albumin, rat

Introduction

Cannabis sativa L. is a dioecious, annual, green, and leafy plant (Adams and Martin, 1996; Amar, 2001). All of Iranian, Pakistanian, Tajikistani and Afghani varieties are landrace (pure) varieties that belong to *Cannabis sativa* strain (Green, 2005; Small and Cronquist, 1997). As it grows well at low temperature, *Cannabis* is well adopted to temperate climates. The most important *Cannabis sativa* products in the food and drug trade are whole hemp seed, hulled hemp seed, hemp seed oil, marijuana, and hashish (Adams and Martin, 1996; Amar, 2001). The different preparation of *Cannabis sativa* have been used in Asian traditional medicine for treatment of variety of diseases including: inflammation, nausea, headache, hematochesia, diarrhea, and alopecia (McPartland, 2004; Xiaozhai and Clarke, 1995; Zias *et al*., 1993). In ancient Iranian Avesta medicine, hashish(bhanga) was mixed with wine to deliver anesthesia (Avicenna, 1999; Najmabadi, 2000). In Iran, Pakistan and Turkey roasted salty hempseeds (Per: Shahdaneh) with wheat still sold by street vender and herbal stores and it is very popular especially among children as a nut. Hempseed also used in Iran as birdseed for canary during mating period to increase stamina of male bird. Whole hemp seeds contain approximately 25% protein, 31% fat (in the form of nutritious oil), and 34% carbohydrate, in addition to an interesting array of vitamins and minerals (Callaway *et al*., 2002; Darshan and Rudolph, 2000). Hempseeds are second only to soybean in protein content (25% versus 32%) (Amerio, 1998; Callaway *et al*., 2002). Hemp protein is free of the trypsin inhibitors which block protein absorption and free of oligosaccharides found in soy, which cause stomach upset and gas as used in traditional Iranian medicine for treatment of flatulence (Avicenna, 1999). Approximately 65% of the protein in hempseeds is made up of the legumin protein edestin and is found only in hempseed and the other third of hempseed is albumin, another high quality globulin protein similar to that found in egg whites (Callaway *et al*., 2002; Darshan and Rudolph, 2000). The oil contained in hempseed is 75-80% polyunsaturated fatty acids (PUFAs) and only 9-11% of the lesser desired saturated fatty acids, hempseed oil is reputed to be the most unsaturated oil derived from plant kingdom and has dubbed" Nature's most perfectly balanced oil", due to the fact that it contains the perfectly balanced 3:1 ratio of omega 6(Linoleic/LA)to omega 3 (alpha-Linolenic/LNA) essential fatty acids, determined to be the optimum requirement for long-term healthy human nutrition (Erasmus, 1999; Simopoulos, 2002). In addition, it also contains smaller amounts of three other polyunsaturated fatty acids in gamma-linolenic acid (GLA), oleic acid and stearidonic acid (Callaway *et al*., 1997). This essential fatty acid combination is unique among edible oil seeds. The intoxicating properties of *Cannabis sativa* L. reside in sticky resin produced most abundantly in the flowering tops of female plants before the seeds mature. The main psychoactive compound in this resin is tetrahydrocannabinol (THC) (Leson, 1999;

Small and Marcus, 2003). Varieties of hemp that grown illegally in Khorasan province of Iran have a low resin content to begin with, and by the time the seeds are ready for harvest, resin production has dropped even further, finally the seeds must be cleaned and washed before using. As a result no THC is found in cleaned hempseed. This work is carried out to investigate the effect of hempseed on serum lipid and protein profiles of rat.

Materials and Methods

Experimental animals: Wistar rats were obtained from Central Animal House, Veterinary College, Urmia University. The animals were thus housed under standard laboratory conditions and had free access to feed and clean water and stabilized for two week before the start of experiment. At the first day of study the overnight fasting animals were anesthetized with ketamine/xylazine combination and blood samples for sera preparation were collected by cardiac puncture into sterile plain tubes. Serum samples were separated from the clot by centrifugation at 3,000rpm for 15 min using bench top centrifuge (MSE Minor, England).Serum samples were separated into sterile plain tubes and stored in the refrigerator for analyses. All analyzed were completed within 24h of sample collection. The same procedure was repeated in the end day (20th day).*Serum samples and biochemical determinations*

Serum was used for total protein, albumin, triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations. Determinations were performed using an automated biochemical analyzer (Multianalyser Technicon RA-XT, Bayer do Bresil).

Statistical analysis: The results were expressed as mean ± SD, differences between means analyzed using paired sample-t-test. P values of 0.05 or less were taken as being statistically significant.

Results

Table 1 shows the lipid parameters of rat at the first day and end day (20th day) of study. The data expressed as mean ± SD. The HDL of rats treated with hemp seed were significantly (P= 0.01) increased 20 days post treatment. HDL increased from 41±2.44 at the first day to 51.9 ± 1.32 at the end day. LDL of rats treated with hemp seed were significantly (P=0.00) reduced from 117.7 ± 4.66 to 82.9 ± 6.82 at the end day. The total cholesterol of rats treated with hemp seed also were non-significantly (P=0.387) increased from 91.7 ± 4.22 at the first day to 96.6 ± 3.81 at the end day. Triglyceride also was affected falling non-significantly (P=0.165) from 106±5.60 at the first day to 78.6±18.86 at the end day. Table 2 shows the protein profile and weight change of rat at the first day and end day of study. The total protein of rats treated with hempseed were significantly (P=0.01) increased from 7.65±0.49 at the first day to 9.15±0.19 at the end day but the albumin increased non-significantly (P=0.21) from 3.60±0.11 at the first day to 3.87±0.33 at the end day. The weight of rats treated with hempseed were significantly (P=0.00) increased from 165.8±5.9 at the first day to 200.1±7.2 at the end day of study.

Discussion

In the present study, a significant increase in levels of HDL and total protein and also a significant decrease in level of LDL were observed upon treatment with hempseed during 20 days. Serum concentration of total cholesterol, triglyceride and albumin showed non-significant changes during the study. According to other studies HDL and LDL are important for assessment of lipid profile. It is well known that hyperlipidemia is one of the major risk factors for atherosclerosis (Fan *et al.,* 1999). An increase in the concentration of lipids results in liberation of lysosome and trigger cell's degeneration. Major component of total cholesterol is LDL which is directly related to coronary artery disease (CAD) (Brousseau and Schaefer, 2000). It is recognized as major atherogenic lipoprotein and primary target of lipid lowering therapy. Recently emphasis is given that elevated level of LDL is important factor for CAD and lowering its level through diet and medication has been shown to reduce the progression of CAD more. The serum LDL decreased significantly after treatment with hempseed. HDL has preventive role in CAD, it has been shown to reduce the endothelial incorporation of lysophosphatidyl cholines (lyso-PC). The HDL level was significantly raised after treatment with hempseed. The presence of soluble fibres and polyunsaturated fatty acids (PUFAs) could partly explain these effects of hempseed. Dietary PUFAs and their biologic metabolites can favorably influence the fatty acid profile in LDL when compared to saturated fats in the diet, which are strongly associated with coronary heart disease (Brousseau and Schaefer, 2000; Callaway, 2004). The high content of omega-6 and omega-3 fatty acids, and the relatively high phytosterol content of hemp foods, makes them beneficial to cardiovascular health. Additionally, phytosterols (e.g., b-sitosterol), of which hemp seed contains 438 mg/100g, have been shown to reduce total serum cholesterol by an average of 10% and LDL by an average of 13% (Deferne and Pate, 1996; Fenstrom, 1999; Malini and Vanithakumari, 1990). Besides the importance of a proper dietary ratio of linoleic to alpha-linolenic acid in maintaining the polyunsaturated fatty acid composition of neuronal and glial membranes, membrane loss of polyunsaturated fatty acids has been found in such neurodegenerative disorders as Alzheimer's and Parkinson's diseases, and it has been suggested that a diet with a proper

Table 1: Serum lipid profile of normal adult Wistar rats at the first day and end day of study

HDL-cholesterol*	LDL-cholesterol*	Triglyceride	Total cholesterol	Parameter(g/dl)
41±2.44	117.7±4.66	106±5.60	91.7±4.22	First day
51.9±1.32	82.9±6.82	78.6±18.86	96.6±3.81	End day

Note: Values are expressed as mean ± SD. The significant level was 0.05 or less.*P<0.05.

Table 2: Serum protein profile and weight change of normal adult Wistar rats at the first day and end day of study

Weight*(g)	Albumin (g/dl)	Total protein* (g/dl)	Parameter
165.8±5.9	3.60±0.11	7.65±0.49	First day
200.1±7.2	3.875±0.33	9.15±0.19	End day

Note: Values are expressed as mean ± SD. The significant level was 0.05 or less. *P<0.05.

balance of omega-6 to omega-3 fatty acids may help delay or reduce the neurologic effects of these diseases and increases immunity (Darshan and Rudolph, 2000; Leson, 1999). A fatty acid preparation with a ratio of omega-6 to omega-3 fatty acids of 4, which is practically identical to that in hemp oil, has been shown to improve the quality of life of Alzheimer's disease patients (Yehuda *et al*., 1996; Youdim *et al*., 2000). Hempseed mainly contains tyrosine, histidine, tryptophan, cysteine, b-caryophyllene, myrcene and high level of vitamin E (α and *Y*-tocopherols), which have been reported to act as antioxidants which may be having protective role in hyperlipidemia and hepatoprotective properties in several liver diseases (Kriese *et al*., 2004; Odani, 1998). The serum total protein was significantly raised after treatment with hempseed. We suggest that using of very cleaned hempseed can decrease the risk of cardiovascular disease and has hepatoprotective activity but according to other unpublished studies that have been done on guinea pig and rat in our laboratory, unclean hempseeds that contaminated with tetrahydrocannabinols (THCs), the main psychoactive substances of *Cannabis sativa* L., lead to dyslipidemia. Actually THC is an orexigenic compound and increases appetite whereas it is antioxidant (Mechoulam and Hanu, 2001).

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