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Hypercholesterolemic Effect of Drug-Type *Cannabis sativa* L. Seed (Marijuana Seed) in Guinea Pig

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Abstract: *Cannabis sativa* L. has two drug and nondrug varieties. Nondrug varieties of *Cannabis* are hemp but drug varieties commonly referred to as marijuana. Marijuana is considered nutritional and narcotic plant. Marijuana has not been studied extensively for its nutritional potential in recent years but whole hempseed typically contains over 30% oil (3%saturated, 28% unsaturated fatty acids) and about 25% protein. The objectives of the present study were to evaluate the effects of whole marijuana seed on lipid profile of guinea pig. This study was experienced on fourteen guinea pigs as case and control groups. In control group, guinea pigs were fed with normal diet while case group had free access to normal diet and marijuana seed for 60 days. At the end of experiment the feeding of animal stopped and after 12hr fasting, the animal anesthetized by ketamine/xylazine combination and 5 ml of blood of heart was taken. The blood parameters were measured by automated biochemical analyzer. Marijuana seed significantly increased total cholesterol and LDL-c levels (p=0.00) while HDL-c and triglyceride levels didn't significantly change. In spite of omega-3, omega-6 and omega-9 fatty acids that highly incorporated in hempseed, short term feeding of marijuana seed has not improved blood lipid profile. It may be due to hidden orexigenic phytocannabinoids that found in drug-type seed. In the light of this research, it is recommended that individuals who are affected to cardiovascular diseases and atherosclerosis, they should not use unclean marijuana seed that cultivated in Isfahan province of Iran in their food preparation on regular basis.

Key words: Marijuana, lipid profile, hypercholesterolemia, tetrahydrocannabinol, guinea pig

Introduction

Cardiovascular disease continues to be the leading cause of morbidity and mortality in the industrial world (Grundy *et al*., 2004). Reducing plasma low-density lipoprotein cholesterol (LDL-c) concentration is a primary strategy for reducing coronary heart diseases (CHD) risk, and the new guidelines for very high risk individuals indicate a LDL-c goal of <1.8 mmol/L (70 mg/dL) (O'Keefe *et al*., 2004; Tavazzi, 1999). It is unlikely, however, that maintaining such an extremely low LDL-c concentration can be achieved without therapeutic intervention. Statin drugs provide effective cholesterol-lowering therapy and are widely prescribed (Stein, 2003), but they are expensive and can cause side effects and even death (Evans and Rees, 2002; Farmer, 2003). Lowering plasma LDL cholesterol through nondrug strategies, such as ingesting specific food components, would therefore be most desirable. Several dietary constituents of plant origin are effective cholesterol-lowering agents, including plant sterols (Katan *et al*., 2003; Ostlund, 2004) and b-sitosterol (Callaway *et al*., 2002; Malini and Vanithakumari, 1990). Consequently, plant foods and manufactured products rich in these phytochemicals are being promoted to consumers as cardioprotective and beneficial to overall health but sometimes plant products are hazardous waste.

Cannabis sativa L. is a dioecious and annual plant (Adams and Martin, 1996a).The most important *Cannabis sativa* L. products in the food and drug trade are whole hempseed, hulled hempseed, hempseed oil, hempseed milk, marijuana seed, marijuana leaf (grass), and hashish (Adams and Martin, 1996a). In China, Iran, Pakistan and Turkey roasted salty *Cannabis* seeds (Per: Shahdaneh that means king of seeds) with wheat still sold by street vender and herbal stores and it is very popular especially among children as a nut (Callaway, 2004; Karimi and Hayatghaibi, 2006). Hemp seed has been a valuable industrial crop for both food and fiber in Canada and European countries during the last decade (Callaway, 2004). In Iran *Cannabis* plant (both drug and nondrug types) is annihilated by government to avoid narcotic abusing. Whole hempseed contain approximately 25% protein, 31% fat, and 34% carbohydrate, in addition to an interesting array of vitamins and minerals (Callaway, 2004; Darshan and Rudolph, 2000; Leizer *et al*., 2000). The oil contained in hempseed is 75-80% polyunsaturated fatty acids (PUFAs) and only 9-11% of the lesser desired saturated fatty acids (Callaway, 2004; Darshan and Rudolph, 2000). *Cannabis* seed is a rich source of phytochemicals that include terpenoid compounds such as phytocannabinoids, plant sterols, and high content of polyunsaturated fatty acids, yet this abundant crop has been largely overlooked for its benefits to human abusing. 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 Δ^{9}-tetrahydrocannabinol (Δ^{9}- THC) (Elliot and Mechoulam, 2002). The concentration of THC in the seeds depends on the type of plant (fibre or drug hemp) as well as on the degree of contamination at the harvest (Ross *et al*., 2000). As a consequence only very low THC concentrations are found in the inside of the seeds (less than 2 mg/kg with drug hemp(marijuana) and less than 0.5 mg/kg with fibre hemp) (Ross *et al*., 2000; Small and Marcus, 2003). Therefore, the tidiness of the seeds plays the most decisive part in the concentration of THC in the seeds. According to the claim of marijuana abusers, drug-type variety of *Cannabis* that grown illegally in Isfahan province of Iran has a high intoxicating (euphoric) potency, albeit the increased rate of psychosis among the young people proves this claim. We were, therefore, motivated to critically evaluate the possible effect of hempseed on lipid profile of guinea pig. In this study we used unclean, resin contaminated marijuana seed. As a result THC is found in unclean marijuana seed. THC is an appetite promoting agent and may be cause to overeating and metabolic syndrome in normal human and animals.

Materials and Methods

Experimental animal: All procedures that involved animals were approved by the Animal Care and Use Committee of Western Azerbaijan Veterinary College. The animals were housed individually in polycarbonate cages with sawdust bedding. Guinea pigs were kept in a $25^{o}C$ room with a 12h light: dark cycle and had free access to feed and clean water and stabilized for two week before the start of experiment. The fourteen healthy guinea pigs were divided into two equal groups. In control group, guinea pigs were fed with normal diet (fresh vegetables) while case group had free access to normal diet and marijuana seed for 60 days. At the end 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 3000rpm 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. Determinations of lipid parameters were performed using an automated biochemical analyzer (Multianalyzer Technicon RA-XT, Bayer do Brazil).

Statistical analysis: The results were expressed as mean ± SEM. Differences between means analyzed using independent sample-t-test. P values of 0.05 or less were taken as being statistically significant. Data were analyzed using version 13 of SPSS software.

Results

Table 1 shows the lipid parameters of control and case groups respectively at the end day (60^{th} day) of study. The data expressed as mean± SEM. Plasma high-density lipoprotein cholesterol (HDL-c) was not affected by the consumption of marijuana seed during the 2-month study (Table 1), although there was a trend for HDL-c to increase with marijuana seed intake (P = 0.07). In contrast, the plasma non-HDL-c fraction, containing mainly low- density lipoprotein cholesterol (LDL-c), increased significantly (P = 0.00). LDL-c is the predominant fraction in humans. The majority of plasma cholesterol in guinea pig fed purified diets is typically carried in the LDL-c fraction. Therefore, guinea pig is the best model for studying the effect of diet on LDL-c level (Fernandez and McNamara, 1991). The total Plasma cholesterol in guinea pigs fed marijuana seed increased significantly (P = 0.00). Triglyceride also was affected falling non-significantly (p = 0.86).

Discussion

In the 1960s, Prof. Rafael Mechoulam isolated and identified the cannabinoids and the chief cannabinoid chemical in the marijuana plant: delta-9-tertrahydrocannabinol (Δ^{9}-THC) (Adams and Martin, 1996a). Approximately 20 years later, in the late 1980s, Allyn Howlett, identified a receptor (CB1) for THC that is a G-protein coupled receptor (GPCR) (Devane and Mechoulam, 1992).CB1 is a ubiquitous receptor found in the central nervous system and periphery, and in both neural as well as non-neural tissues (Breivogel and Childers, 1998; Di Marzo *et al.*, 2001). The CB2 receptor has a more limited distribution, principally in cells associated with the immune system, such as leukocytes, spleen, thymus, and tonsils (Goutopoulos and Makriyannis, 2002). Thirty years after the identification of THC, William Devane, identified a brain chemical, anandamide which binds to the cannabinoid receptor and causes changes which are qualitatively similar to those provoked by THC (Devane and Mechoulam, 1992). In the present study, a significant increase in levels of LDL-c and total cholesterol and also a non-significant change in levels of HDL-c and triglyceride were observed upon treatment with marijuana seed during 60 days. Major component of total cholesterol is LDL-c 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 (Kawahiri *et al*., 2000). The serum LDL-c increased significantly after treatment with marijuana seed. HDL-c has preventive role in CAD and epidemiological studies, as well as, studies in animal models of atherosclerosis,

Table 1: Serum lipid profile of normal adult guinea pigs in two groups

Group	Total cholesterol*(mg/dl)	Triglyceride(mg/dl)	LDL-C*(mg/dl)	HDL-C(mg/dl)
Control	25.28±.746	56.71±2.98	10.28±0.42	12.42±1.17
Case	52.57±2.66	55.85±3.78	30.85±2.58	15.28±0.918

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

support the cardioprotective role of HDL-c (Gordon *et al*., 1997; Kawahiri *et al*., 2000). The HDL level was non-significantly raised after treatment with marijuana seed. In spite of high content of omega-6, omega-3, omega-9 fatty acids and the relatively high phytosterol content of hempseed(Callaway, 2004), that makes it beneficial to cardiovascular health, short term of marijuana seed feeding didn't improve lipid profile (Table 1). Although there are many causes for the increased prevalence of cardiovascular diseases, it appears that nutritional factors, notably increased saturated fat consumption, play important role in promoting premature atherosclerosis (Keys, 1997) however, one of the apparent paradoxes has been the observation that the low saturated fat consumption recommended by several organization including the American Heart Association, the American Diabetes Association, and the American Dietetics Association, often results in decreasing HDL-c levels (Mensink *et al*., 2003). These data may be related to fatty acid profile of marijuana seed or some unidentified factors present in natural plant products but present evidences support subjective concerning of)^{9}-THC, as an appetizer. Although)^{9}-THC-fed mice continued to have elevated serum lipid levels (Paul *et al*., 2006). A growing body of evidence has also established that appetite is modulated by the cannabinoid system of the brain (Kirkham and Williams, 2001). The administration of the marijuana constituent)^{9} - THC stimulates appetite (Adams and Martin, 1996b; Koch, 2001) whereas the cannabinoid receptor antagonist SR 141716 reduces food intake (Arnone *et al*., 1997; Colombo *et al*., 1998; Simiand *et al*., 1998). Furthermore, recent studies have indicated that appetite is increased by the endogenous cannabinoid ligands 2-arachidonoylglycerol and anandamide (Verty *et al*., 2004; Williams and Kirkham, 2002). This provides additional evidence that the cannabinoid system is a positive modulator of food intake. Today, rimonabant (CB1 receptor antagonist) shows promise as a new approach to address cardiovascular risk factor management, specifically in the areas of obesity and metabolic syndrome (Van Gaal, 2004). According to other studies that have been done in our laboratory, using of very clean hempseed of nondrug *Cannabis sativa* L. cultivar that grown in Khorasan province of Iran improved lipid profile of rat and has cardioprotective and hepatoprotective effects (Karimi and Hayatghaibi, 2006). However this study shows that drug cultivar of *Cannabis sativa* L. that grown illegally in Isfahan province of Iran lead to hypercholesterolemia. We expect development of plurimetabolic syndrome after long term feeding of unclean, THC-contaminated marijuana seed or its byproducts including of marijuana seed oil, marijuana seed milk and hashish syrup in normal human and animals. Additional studies are underway in our laboratory to isolate the effects of lipid-soluble *Cannabis* seed phytochemicals such as THC, THC oil and hashish on lipoprotein metabolism and biomarkers of atherogenesis.

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