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Effect of Cannabis Seeds on Some Biomedical Parameters in Male Rats

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ABSTRACT

Cannabis, sometimes known as hemp, is a plant that originated in Central Asia. Cannabis seeds nutritional profile is astounding. It is most digestible, balanced, natural, and complete supply of protein or amino acids. Cannabis seeds can be used in a variety of medical fields. Each 100 gm of the seeds used in the study contains 20, 26 and 37 gm of carbohydrate, protein and fat respectively. The present study used two groups of male rats (control and treatment groups). Rats were 11 weeks of average age. 10 mg/kg of body weight of powdered cannabis seeds were administrated orally to treatment group daily for 15 days. Alkaline Phosphatase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase (ALP, GOT, and GPT), creatinine and uric acid were performed for estimating the effect of cannabis seeds on renal and liver function. Enzymes Alkaline phosphatase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase (ALP, GPT, and GOT) as well as creatinine, uric acid and very low-density lipoprotein (VLDL) were not significantly ($p<0.05$) different in both groups. Level of Cholesterol, triglyceride and c-reactive protein were significantly ($p<0.05$) reduced. Serum total protein, albumin and globulin were significantly ($p<0.05$) elevated in treatment group in comparison to control group. In conclusion, this study found the effect of cannabis seeds on decreasing cardiovascular risk. It was found that

cannabis have good roles in protein metabolism, as well as albumin. The study has showed the role of cannabis in strengthen the immune system via elevation of globulin level. Moreover, cannabis seed has anti-inflammatory property through decreasing the level of CRP.

1. INTRODUCTION

Approximately 70000 unique plant species had been used in conventional medication. World health organization (WHO) has suggested that about 80% of the populace using the smallest amount one natural medication for their lifestyle's medical purposes. Herbal medicine and plants were used as remedy for many unique forms of sicknesses such as liver, kidney, coronary heart sicknesses, therefore, scientists around the world have studied unique sorts of herbal plant in phrases of chemical and physiological properties, indications, and their side effects. Cannabis species is among the herb plants that has been studied by many scientists in vitro round the world [1]. *Cannabis sativa* typically called hemp, its one in every of the oldest cultivated plants, and determinative its specific origin of its long history of cultivation. Cannabis is a green leafy plant with distinctive opposing, typically 7-fingered, lance-shaped leaves of dioecian (male and feminine flowers in numerous plants) [2]. *Cannabis sativa* L. is a popular herbaceous plant from central Asia that has been utilized in traditional medicine, it can be used as a drug (marijuana) or a non-drug (marijuana) (hemp). The former is often used for different purposes such as recreational and medical purposes, whilst the latter is critical to the fiber and food industry [1]. Different preparations of *Cannabis sativa* have been used as a traditional medicine in Asia to cure illnesses, including diarrhea, inflammation, headache, nausea, hematochezia and alopecia. *Cannabis sativa* has anti-inflammatory, analgesic, antipyretic, and antidiarrhea properties [1, 3, 4]. Due to a variety of factors, includes unlawful cultivation, diversity of active components, and low quantity of certain of them in plant [5]. Cannabis phytochemical study has been restricted, as it has broad medicinal usage of Cannabis products. Cannabinoids, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), are psychoactive components of cannabis plants that may be found in the flower, stems, stalks, and leaves, but they are not present in seeds until they are transferred from the flower to the seed's outer shell [6]. Liver and kidney are two active organs in the body. The functions of liver and kidneys functions can be evaluated while using medicine or herbal medicines. Estimation of normal kidney and liver can be performed via their activity and estimation of their enzymes. Damage or injury in the cells have direct effects on kidney and liver functions. Liver function can be estimated via the level of protein, albumin, globulin, cholesterol and triglyceride. As well as necrosis or injury of the hepatic cells can be determining by liver enzymes in serum such as GPT, ALP and GOT. It is well known that liver is a site for detoxification, clearing blood from poisoning and drugs. Kidney is another organ can be determining its functions using cholesterol and uric acid. Any medications that have direct effects on renal and the liver functions or lead to cells damage. So that any herbal drugs should estimate its toxicity and its effect on liver, renal cells and functions.

Cannabinoids and the endocannabinoid system appear to be essential in the treatment of liver disorders and normal liver function. Cirrhosis (liver scarring) and fatty liver disease are examples of this (FLD)[7]. People with viral liver disease may benefit from cannabinoids to help them cope with traditional therapies like chemotherapy [8]. Following the legalization of cannabis in certain countries, such as Canada and the United States, there is likely to be a surge in interest in medicinal cannabis, particularly for chronic refractory symptoms and palliative illnesses like those seen in chronic kidney disease (CKD) patients [9]. Hempseed is usually referred to be one of the most nutritionally complete food sources due to its high nutritional characteristics. Hempseed may be fed on whole (hulled seed) or deshelled (hempseed kernel), in addition to in processed meals like as oil, flour, and protein powder

[10]. Despite the fact that genotypes and environmental variables have revealed a wide range of hempseed compositions, it generally contains 25–35 percent lipids with a unique and precisely balanced fatty acids (FAs) composition; 20–25 percent easily digestible proteins rich in important amino acids; and 20–30 percent carbohydrates, the majority of which is dietary fiber, mostly in the form of dietary fiber. Hemp protein is made up mostly of globulin and albumin, which are distinguished by their high levels of arginine and glutaminic acid. The modulation of organ function and human metabolism was shown to be positive with these two types of hemp protein. Hemp seed protein also contains a number of antioxidative bioactive peptides and phytic acid and trypsin inhibitors [11]. Oil makes around 30 to 35 percent of hempseed, with unsaturated fatty acids accounting for 90 percent of the total essential fatty acids, linolic acid, omega-3 fatty acid, and monounsaturated fatty acid dominate hempseed oil. Linolic acid is necessary as a precursor for the formation of dihomo-linolenic acid (DGLA) and arachidonic acid, whereas -linolenic acid is required for omega-3 fatty acid assembly. These fatty acids have been studied extensively for their ability to protect against cardiovascular disorders, Hempseed's outer shell contains the majority of the carbs, while dehulling eliminates three-quarters of the fiber content. Statistics on the characteristics of hempseed fiber are currently lacking. Hempseed is also high in polyphenols, which include hydroxycinnamic acid in particular [2]. Few researches have looked at the impact of cannabis seeds in the diets of animals and humans on physiological, immunological, and oxidative state and health, so that the study aimed to find the effects of cannabis seeds on liver and renal function and also the possible of using cannabis seeds as herbal medicine for health care. The aim of this study is to examine physiological impact of cannabis whole seeds. Identifying the relationship(s) between liver and renal functions and cannabis seeds, as well as the anti-inflammatory effect of cannabis seeds and possible of using cannabis seeds as herbal medicine for health care in male rats.

2. METHODS AND MATERIALS

Two groups of male rats were designed including control group and the treatment group with their average weight was 245 ± 10 gm and each group were contained 5 male rats. The animals were uncovered for period 12 hours under light-darkish cycle and treated constant with popular protocols. The animals were kept in plastic cages and allowed adaptation duration of seven days in a good ventilated room with a temperature of 28±2C^{o}. Cannabis seeds were obtained from PK cannabel company in Russia-Moscow. The seed of cannabis were crushed into a powder form using special grinder. 10 mg/kg body weight of powdered cannabis seeds were suspended in 1 ml of distal water and drenched by gavage tube to the treatment group orally for 15 days. The control group were received only distilled water for 15 days. The animals were anesthetized using special jar containing cotton saturated with chloroform and diethyl-ether in ratio 1:1. The blood were collected via cardiac puncture, centrifuged at 3000 rpm, the serum were collected and stored at -20 C^{o}[12]. Biochemical tests were performed using cobas c 311 (Roche diagnostics, Reagent were used originally from Roche company, Germany-2018) for both groups. Tests were included liver function test: Serum GOT, serum GPT and serum ALP. Renal function test including creatinine (SCr) and uric acid (SUA). Serum globulin, albumin, total protein, cholesterol, very low-density lipoprotein (VLDL) and triglyceride (TG) levels were also estimated. C- reactive protein (CRP) levels were determined using titration method by using cobas c 311 (Roche diagnostics, Reagent were used originally from Roche company, Germany-2018). The reagents used for GPT contained TRIS buffer, L-alanine, albumin (bovine), LDH (microorganisms), stabilizers; preservative, 2-Oxoglutarate, NADH in different concentrations according to the manufactory. ALP reagent composted of 2-amino-2-methyl-1-propanol, magnesium acetate, zinc sulfate, N-(2-hydroxyethyl) -ethylenediamine tri-acetic acid, p-nitrophenyl phosphate. Materials used in GOT kits were: TRIS buffer, L-aspartate, MDH (microorganism), LDH (microorganisms), albumin (bovine), NADH and 2-oxoglutarate. Serum total protein kits contained sodium hydroxide, potassium sodium tartrate, potassium iodide, copper sulfate. Reagent used for

estimation of serum albumin was contain Buffer; preservatives; surfactants, BCP, buffer; preservatives; surfactant and the materials used in kits for serum globulin materials were TRIS/HCl buffer, NaCl, EDTA; preservative, latex particles coated with polyclonal anti-human β2-microglobulin antibody (rabbit). PIPES buffer, Mg2+, sodium cholate, 4-aminophenazone, phenol, fatty alcohol polyglycol ether, cholesterol esterase (Pseudomonas spec.), cholesterol oxidase (E. coli), peroxidase (horseradish) were used in serum cholesterol reagent. Materials used to estimate serum TG concentration were PIPES buffer, Mg2, sodium cholate, ATP, 4-aminophenazone, 4-chlorophenol, lipoprotein lipase (Pseudomonas spec.), glycerol kinase (Bacillus stearothermophilus), glycerol phosphate oxidase (E. coli) and peroxidase (horseradish). For VLDL the Dilution buffer: potassium dihydrogen-phosphate, dipotassium hydrogen-phosphate, potassium chloride, sodium aside, magnesium sulfate heptahydrate, sodium phosphor-tungstate n-hydrate Lipoprotein-lipase, cholesterol esterase, diaphorase, nicotinamide adenine dinucleotide, tetrazolium salt, glycerol dehydrogenase, cholesterol dehydrogenase used in reagent. CRP reagent was contained HEPES buffer, Anti-human CRP antibody (goat) and Latex-conjugate. The statistically analyzed data was given as the mean± SD of two groups, with the means compared using IBM SPSS 26 software. At $P<0.05$, the differences were statistically significant [13].

3. RESULTS

Using male albino rats as test subjects, the results demonstrated the effect of cannabis seeds on a range of biochemical or medical markers. Table 1 showed the effect of Cannabis seeds on serum total protein, serum albumin and serum globulin in the treatment group were showed significant alteration in the serum biomarkers ($P<0.05$). The serum GPT, GOT and ALT in control and the treatment group were showed no significant difference ($P<0.05$) as showed in table 2. Effect of cannabis seeds on the level of serum creatinine and serum uric acid in control and treatment group was not significantly ($P<0.05$) different (table 3). The cholesterol and triglyceride levels showed significant ($P<0.05$) decrease in treatment group when compared with the control group while VLDL showed no significantly ($P<0.05$) changed in treatment and control groups (table 4). The influence of Cannabis seeds for C- reactive protein in male rats showed reduction in treated group significantly ($P<0.05$) (table 5).

Table 1: Effects of cannabis seeds on the level of serum total protein, serum albumin and serum globulin in (gm/dL) in control and treatment group (mean ± SD).

Groups	Total protein	Albumin	Globulin
Control	6.56 ±0.16	3.52 ±0.14	2.66 ±0.57
Treatment	7.47 ±0.33*	4.49 ±0.40*	4.25 ±0.28 *

* The levels are significantly higher ($P<0.05$) in treatment group compare to control group.

Table 2: Effects of cannabis seeds on the level of serum GPT, GOT and ALT in (U/L) in control and treatment group (mean ± SD).

Groups	SGPT	SGOT	SALT
Control	37.03 ± 3.32	117.73 ±11.57	244.33 ±60.01
Treatment	39.9 ± 8.73^{NS}	120.05 ± 30.55^{NS}	237.5 ± 44.20^{NS}

NS: No significant different between control and treatment groups.

Table 3: Effects of cannabis seeds on the level of serum creatinine and serum uric acid in (mg/dL) in control and treatment group (mean ± SD).

Groups	Creatinine	Uric acid
Control	0.33 ± 0.005	2.36 ± 0.25
Treatment	0.37 ± 0.031^{NS}	2.38 ± 0.35^{NS}

NS: No significant different between control and treatment groups.

Table 4: Effects of cannabis seeds on the level of serum creatinine and serum uric acid in (mg/dL) in control and treatment group (mean ± SD).

Groups	Cholesterol	Triglyceride	VLDL
Control	73.03 ± 3.20	123.8 ± 14.89	25 ± 3
Treatment	56.92 ± 5.74*	71.85 ± 10.40*	26 ± 10.55^{NS}

* The concentrations are significantly lower (P<0.05) in treatment group compared to control group.
NS: No significant different between control and treatment groups.

Table 5: Effects of cannabis seeds on the level of serum C- Reactive Protein (U/L) in control and treatment group (mean ± SD).

Groups	C-Reactive protein
Control	0.93 ± 0.13
Treatment	0.17 ± 0.08*

* The concentrations are significantly lower (P<0.05) in treated group when compare to the control group.

4. DISCUSSION

The liver has important effect on the function of various organs with inside the frame system. due to its key characteristic in metabolism (xenobiotic) and its gateway function withinside the frame system, it's miles vulnerable to xenobiotic-brought on damage [14]. The liver is essential for metabolism, detoxification, and biotransformation. In order to measure the degree of injury or destruction caused by ingesting cannabis seeds prior to biopsy, changes in liver biomarkers and function tests may be used [15]. Albumin and globulin combine to make up total protein in serum; therefore, changes in the concentration of either of these fractions will affect total protein levels. A significant change in albumin, globulin, and total protein concentrations suggested that the powdered cannabis seeds stimulated protein synthesis and/or mobilization. Masses of serum proteins, as well as provider proteins, enzymes, complement, and immunoglobulins, make up the globulin fraction. The majority of them are made by the liver, with the exception of immunoglobulins, which are produced by the plasma cells. More specifically, the observed rise in globulin level might be attributable to the cannabis seeds ability to generate antibodies [16]. The greatest concentration of protein in the plasma is albumin which accounts more than half of the total protein in serum. About 30 to 40 percent of the total body's albumin is pooled within the intravascular tissue and it carries a large number of molecules throughout the body. The distribution of bilirubin, hormones, metals, vitamins, and medicines are aided by the albumin. It plays a vital role in metabolism of fat through fatty acids binding and keeping them in a soluble state within the plasma. It prevents the blood fluid from seeping into tissues [17]. Chronic liver illness, such as cirrhosis or nephrotic syndrome, causes decrease in the level of the albumin [18]. As a result, the remarkable rise of albumin may suggested that the seeds (cannabis) might assist to control the flow of water from the bloodstream into human tissue and may have a hepatoprotective impact [19]. This is also confirmed by the ratio of albumin to globulin which results in substantial rise in protein content [20]. Some of tests assess how well the liver performs its daily functions of making protein and removing bilirubin, a blood waste. Other tests measure the amount of enzymes released by liver cells in response to injury or disease. Enzyme serum glutamic pyruvic transaminase (SGPT) is known to rise in the presence of liver illness and has been used to assess the effect of cannabis on hepatocytes [21, 22]. Serum glutamic oxaloacetic transaminase (SGOT) is mostly found in gill cells, kidney cells, muscle cells, and liver parenchyma cells [23]. SGOT is most prevalent in the coronary heart diseases as compared to other body tissues such as the liver, striated muscle, and kidney. Elevated mitochondrial SGOT noticeable in widespread tissue necrosis throughout cardiac infarction and likewise in

chronic liver illnesses like hepatic tissue degeneration and necrosis [14]. Alkaline phosphatase is found in the cell membrane and endoplasmic reticulum, or the liver biliary ducts cell lining, bone and placental tissue [24]. Based on the tests on liver enzymes, no significant differences were found at a dose of 10 mg/kg body weight between the treatment and control group, this could indicate that using cannabis seeds at a dose of 10mg/kg body weight for 15 days are safe for liver. Serum creatinine and uric acid levels are traditional indicator for renal function [25]. Normally serum creatinine levels can also be used to gauge how fast the kidneys filter blood (glomerular filtration rate). Because blood creatinine levels vary so much from person to person, the glomerular filtration rate may offer a more realistic picture of renal function [26]. Serum creatinine is a popular measure for identifying changes in glomerular filtration rate (GFR) and chronic kidney disease (CKD) stage, with increased serum creatinine indicating renal disease and reduced renal function [27].

In the same way, patients with CKD frequently have increased serum uric acid levels. It's a straightforward biochemical diagnostic for kidney function that's either impaired or pathological [27]. Because renal clearance of serum uric acid is generally decreased after kidney injury or failure therefore renal function is the major confounder in studies investigating the connection between the serum uric acid and CKD progression [28].

The glomerular filtration rate is calculated using a specific formula that takes into consideration the serum creatinine counts as well as other variables including age and gender into consideration [29, 30]. For that reason, in the current study the same gender and age of rats were used. The result shows that there are no significant increase in the level of creatinine and uric acid, this indicating no significant effect were found on renal functions. On the other hand result showed decreasing in the level of serum cholesterol and triglyceride (TG) significantly, but VLDL was not changed significantly ($p<0.05$) in treatment group (table 4). Both cholesterol and triglycerides (TG) serve important physiological functions in keeping the healthy status of the body [31]. Cholesterol and TG are two indicators that may be used to assess cardiovascular risks since high cholesterol and TG levels can cause health problems, particularly those connected to the heart [32]. Many researches reveal that elevation of triglycerides are diagnosed as a hazard issue for cardiovascular disorder, emphasizing the pressing want for scientific trials to decide whether reducing blood triglyceride could decreases disorder hazard. [33, 34]. VLDL is produced in the liver and is in charge of transporting triglycerides to body cells, which are required for cellular activities. VLDL is made up less of fat and more of protein when triglycerides are transported to cells, leaving cholesterol on the molecule. VLDL's protein composition varies from that of chylomicrons in case the main protein structure is full-duration apo B (apo B100) rather than the truncated apo B48 version. VLDLs, like chylomicrons, are triglyceride-removing substrates for lipoprotein lipase. According to our findings, there is no influence on VLDL's regular functioning [35]. Decreasing cholesterol and triglycerides level were provided an indicative clue on using cannabis to decrease cardiovascular risks, however no significant change in the level of VLDL were recorded on using cannabis seed powdered for a duration of 15 days. C-reactive protein (CRP) in the treatment group was significantly decreased ($p<0.05$). This could indicate that cannabis seeds act as anti-inflammation agent in the body. Liver produces CRP in response to a number of inflammatory cytokines [36]. CRP levels rise quickly in reaction to trauma, inflammation, and infection, and then lowered quickly after solving the problem [37]. As a result, the reduction in CRP levels in the treatment group indicates that the cannabis seeds' anti-inflammatory properties are effective.

5. CONCLUSION

Using cannabis whole seeds at dose of 10 mg/kg body weight orally for 15 days are safe for the liver and kidney. Cannabis seeds have incredible effects on decreasing cardiovascular risk. Also have good roles in protein metabolism and synthesize as well as albumin and have role in strengthen the immune system via elevation of globulin level. Moreover, cannabis seed has anti-inflammatory property through decreasing the level of CRP.

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