EFFECT OF CANNABIS SATIVA EXTRACT ON THE LIVER AND CARDIC ENZYMES OF NORMAL HEALTHY MICE

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ABSTRACT

Objective: To evaluate the effect of *Cannabis sativa* on cardiac and liver enzymes of apparently normal healthy mice.

Material and Methods: This comparative study was carried out at Department of Pharmacy, Institute of Chemical Sciences, University of Peshawar and Khyber Medical College, Peshawar in the year 2015 on 100 apparently healthy mice meeting the predetermined selection criteria.

Results: Means $\pm$ SD cardiac enzymes of mice fed on Cannabis extract viz., CPK and LDH were 271.9 $\pm$ 12.07 IU/L & 275.9 $\pm$ 12.07 IU/L whereas liver enzymes like ALP, ALT & AST were 198.6 $\pm$ 4.63 IU/L, 49.9 $\pm$ 0.40 IU/L and AST 82.40 $\pm$ 5.83 IU/L and were significantly higher when than normal healthy mice (group A).

Conclusion: The results of instant study showed that administration of *Cannabis sativa* extract has acute toxic effect on liver and cardiac enzymes in mice.

Key words: *Cannabis sativa*, mice, cardiac enzymes, liver enzymes, NIH, ICS, KMC, Peshawar.

INTRODUCTION

One of the most important concerns throughout the world today is the problem of overpopulation. The population of the world is growing faster than the supplies of food, shelter and fuel; therefore most of the people have started using drugs like cannabis, heroin, cigarette and alcohol etc., to relieve their pain and tension[1-2]. Cannabis is dry, shredded mix of flowers, stems, leave and seeds of the hemp plant (*Cannabis sativa*). *Cannabis sativa* is considered as a nutritional green and leafy plant. It contains over 30% oil (3% saturated and 28% unsaturated fatty acids) and about 25% proteins. The main psychoactive ingredient in cannabis is delta-9-tetrahydrocannabinol (THC), but more than 400 other chemicals are also found in the plant. The concentration of THC in the cannabis determines how strong its effects will be. Cannabis smoking disrupts the hormonal balance of both male and female[3]. Chronic smokers of cannabis have shown various endocrine changes, including decreased testosterone levels and reduced sperm counts in males, and decreased luteinizing hormone (LH) and prolactin levels in the luteal phase of the menstrual cycle in females, resulting in shorter periods and more anovulatory cycles[4].

It is recommended that individual who has high cholesterol and LDL-c levels are expected to develop coronary artery disease and liver diseases. HDL-c and LDL-c level are important for the assessment of lipids profiles[5]. Previous research has also indicated that cannabis effect the hepatic functions. Alkaline phosphatase (ALP), alanine aminotrasferase (ALT) and aspartate aminotransferase (AST) levels measurement shows that plasma ALP activity for men and rat increases after using *Cannabis sativa*. Plasma ALT activity for men and rat also increases after using cannabis sativa however plasma AST activity for men and rat decreases with the use of *Cannabis sativa*[6]. The present study was designed to evaluate the effect of *Cannabis sativa* extract on liver and cardiac enzyme in apparently normal healthy mice.

MATERIALS AND METHODS

Experimental Animals: Adult male mice weighing 25-35 gm were bought from the National Institute of Health, (NIH) Islamabad, Pakistan. The animals were accustomed for one week in the animal house facility at the Department of Pharmacy, University of Peshawar, Khyber Pakhtunkhwa (KPK), Pakistan. They were housed in groups of six in rectangular plastic polycarbonate cages (1500U; Tecniplast, Italy) with wire mesh tops containing a feeding area and water bottle. The contact bedding and nesting material were dust free wood chips disseminated evenly all over the solid floor and were altered regularly every day. The animals were preserved at 12/12 hours light/dark cycle at a temperature of 20-24°C (68-75.2°F) with relative dampness of 40-70%. The environmental variables were patterned regularly at the cage level with all aseptic conditions.

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Water and food were provided regularly. Experiments on mice were completed according to the rules and regulations of the Ethical Committee of the Department of Pharmacy, University of Peshawar following the United Kingdom Animal (Scientific Procedure) Act 1986. The study was conducted in the following places animal house and Histology Laboratory of Department Pharmacy, Institute of Chemical Sciences, University of Peshawar and Khyber Medical College, Peshawar, KPK, Pakistan.

Details of Grouping: A total of 100 apparently normal healthy male BALB/C mice weighing 25-35 gm were used in the instant study. All animals were kept in the animal house under standard conditions at a room temperature for 45 days duration. Animals were randomly divided into two groups viz group A group B respectively. Fifty (n=50) apparently normal healthy mice were given plain water and commercial standard mash feed orally for 45 days served as control group (Group A), whereas the same number (n=50) of mice were fed on *Cannabis cannabis* extract 14 mg/kg orally once daily for period of 45 days (test group).

Assay Kits: The kits for Cardiac and liver enzymes were determined by colorimetric method using kit supplied by Merck (America)[8-10]. All other reagents used were of analytical grade and were prepared in glass distilled water. Assay were performed at Department of Biochemistry, Khyber Medical College Peshawar, KPK, Pakistan.

RESULTS

The results of present study is based on one hundred (n=100) apparently normal healthy mice and were included on the basis of predetermined selection criteria. The prevalence of means + SD cardiac enzymes in control mice (Group A) and test group (Group B) is shown in Table-1. The data showed that creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were observed to be 268.8±6.93 IU/L and 3±61.76.06 IU/L and in group B mice it was observed to be 271.9±12.07 IU/L and 275.9±11.07 respectively. Upon comparison it was found that serum LDH was significantly higher ($p<0.005$) when compared with control group. Similarly table-2 revealed prevalence of liver enzymes for group A and B. It is evident from table that serum ALP, ALT, and AST in group B were found to be 198.6±4.63 IU/L, 49.9±0.40 IU/L and 78.0+2.82 IU/L respectively were significantly higher ($p<0.005$) when compared with control group mice (Group A). However non-significant results were obtained for serum bilirubin.

DISCUSSION

The measurement of biochemical parameters in mice can be used to assist the extent of deleterious effects of *Cannabis* extract on animal. blood[11]. These biochemical parameters provide information on general state of blood chemistry[12]. It is inferred from the present study that marijuana extract may have a toxic effect on hematological parameters by altering the blood chemistry and may induces anaemia by causing bone marrow depression through inadequate production of red blood cell[13] and ultimately cells death[14]. This may also be attributed to drastic loss in weight of mice. Liver function indices usually assess liver damage rather than liver function. The liver is the major organ responsible for metabolism, secretary function and detoxification in the body. Liver damage is associated with the distortion of these metabolic functions. The enzymes are usually elevated in acute hepototoxicity or mild hapatocellular injury, but tend to decrease with prolonged intoxication due to damage to the liver[15]. The significant change in cardiac enzymes and liver enzymes for mice administered with marijuana (*Cannabis sativa*) is an indication of a pathological condition on liver and cardiac parameters[16]. The results of present study show that exposures of mice to cannabis caused significant alteration in bio-

Table-1: Comparison of means ± SD Cardiac Enzymes of mice fed on Cannabis extract 14 mg/kg (Group B) with control mice (Group A)

Cardiac Enzymes	Group A	Group B	t . test	p value
Creatine phosphokinase. (CPK) U/L	268.8 6.93	271.9 12.07	-1.574	0.119
Lactate Dehydrogenase (LDH) U/L	361.7 6.06	275.9 12.07*	44.921	0.0001

* p< 0.005 when compared with normal group (Group A).

Table-2: Comparison means ± SD Liver Enzymes in mice fed on Cannabis extract 14 mg/kg (Group B) with control mice group (Group A).

Liver Enzymes	Group A	Group B	t . test	p value
ALP (IU/L)	113.9 0.55	198.6 4.63*	-83.041	<0.00001
ALT (IU/L)	28.0 0.78	49.9 0.40*	776.659	<0.00001
AST (IU/L)	78.0 2.82	82.4 5.83*	-4.80415	<0.00083
Bilirubin (mg/dL)	0.43 0.027	0.49 0.02	-	>0.99999

* p< 0.005 when compared with normal group (Group A).

chemical parameters of both liver and cardiac function and are consistent with the above stated studies.

Alkaline phosphatase (ALP) is a "maker" enzyme for plasma membrane and endoplasmic reticulum[17]. The increase in activity of serum alkaline phosphatase might be due to the leakage of enzyme from the tissue into serum. These suggest tissue damage such as muscle injury; cardiac infection and hepatic necrosis[18]. Alanine transaminase (ALT) and aspartate aminotranspharase (AST) is localized within the cells of liver, heart, gill, muscles, kidney and some organs. The enzymes are important in assessing and monitoring liver cytolysis. The significant increase ($p<0.005$) in both enzymes following the administration of marijuana extract could be due to denovo synthesis of the enzymes molecules leading to concentration higher than control and is consonant with the study carried out by Yakubu and colleagues (2001), Uboh FF and colleagues (2008) and Uboh *et* al., (2012)[18-20]. The biochemical parameters monitored in the heart the useful "markers" for the tissue damage. The analysis of activities of various enzymes in the tissues and body fluids plays a vital role in the diagnosis and investigation[20], assaults on the organ and to a reasonable extent the toxicity of the extract[21]. Tissue enzymes can indicate tissue cellular damage caused by chemical compounds, long before structural damage that can be detected by conventional histological techniques. The present study reveals a highly significant change ($p<0.005$) for serum LDH in group B when compared with group A is in agreement with the above cited studies.

CONCLUSION

Administration of marijuana (*Cannabis*) extract has an acute toxic effect on mice cardiac and liver enzymes respectively.

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