CANNABIS INDUCED TOXICITY AND CYTOPROTECTIVE ROLE OF VITAMINS C & E AS ANTI-OXIDENTS

Zahoor Ahmed[1], Hajira Isaq[2], Zahid Sarfaraz Khan[3], Jamil ur Rahman[4], Sikandar Hayat[5], Muhammad Ishaq[2], Mudassir Ahmad Khan[1]

ABSTRACT

Objective: To compare the protective role of vitamin C and E against Cannabis sativa induced toxicity in male mice.

Introduction:Cannabis is the most commonly used illegal drug in the Pakistan and other parts of the world. Cannabis plant extract comprises of more than 421 chemicals.Cannabis affects nearly every system of the body in humans. Cannabis has an effect on hepatic functions and study showed an increase in ALP&ALT levels, whereas the levels of AST decreases in men and rats after use of Canabbissativa extract.

Subjects & Methods: This comparative study was carried out at department of Biochemistry, Khyber Medical College, Peshawar, Department of Pharmacy &Institute of Chemical Sciences, University of Peshawar in the year 2015 on 150 apparently healthy male mice meeting the predetermined selection criteria.

Results: Results indicated that mice (Group B and C) which were given vitamin C (100mg/kg body weight) and vitamin E (200 mg/kg body weight) respectively and to see whether they have reversed these changes in the cardiac enzymes caused by Cannabis; encouraging results have been obtained and these change were significantly higher ($P<0.005$) for ALP, ALT and LDH in both the groups B and C respectively when compared with group A mice.

Conclusion: Results exposed that the administration of Cannabis sativa extract had acute toxic effects on mouse liver & cardiac enzymes which were significantly reversed by the concurrent administration of antioxidants i.e., vitamins C & E respectively.

Keywords: Cannabis, Liver enzymes, Cardiac enzymes, Vitamin C, Vitamin E, antioxidants, KMC, ICS.

INTRODUCTION

Cannabis is the most commonly used illegal drug in the Pakistan and other parts of the world. The drug is usually smoked, but is sometimes eaten. Today, there are more than 12 million Cannabis users in the Pakistan and more than 300 million regular users all over the world[1]. The cannabis plant comprises more than 421 chemicals of which 61 are cannabinoids[2]. Interestingly, more than 2000 compounds are formed by pyrolysis during smoking of cannabis[3] and they are symbolized by different classes of chemicals comprising nitrogenous compounds, simple fatty acids, hydrocarbons, amino acids, terpenes and sugar respectively[4]. Literature reveals that Cannabis affects nearly every system of the body in humans. It combines several properties of tranquillizers, alcohol, hallucinogens and opiates; it is psychedelic, anxiolytic, analgesic and sedative; it stimulates appetite and has various systemic effects. Furthermore, its severe poisonousness is tremendously low: no deaths directly due to serious cannabis use have ever been reported[5].

Studies in male rodents after administration of tetra-hydrocanabinnol (THC) show significant decrease in both testosterone and gonadotropins which is due to inhibition of the gonadotropic releasing hormone (GnRH) in the hypothalamus. The most potentially damaging effect of cannabis on spermatogenesis is the marked increase in abnormal forms of sperm cells resulting in various congenital anomalies of central nervous system, cardiovascular and genitourinary system in human and rodents. Cannabis use decreases the mean fasting serum LDL-c levels and also significantly increases the mean fasting serum HDL-c and total protein levels[6-7]. Cannabis has an effect on hepatic functions. Study shows an increase in Alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) levels, whereas the levels of Aspartate aminotransferase (AST) decreases in men and rats after use of Cannabissativa extract[8].

The present study was planned to assess the cytoprotective effect of the use of vitamin C & E on level

[1] Department of Biochemistry, Khyber Medical College Peshawar, KPK, Pakistan.
[2] Institute of Chemical Sciences, University of Peshawar, KPK, Pakistan.
[3] Department of Anatomy, Khyber Girls Medical College Peshawar, KPK, Pakistan.
[4] Department of Biochemistry, Rehman Medical College Peshawar, KPK, Pakistan.
[5] Institute Kidney Disease, Hayatabad Medical Complex, Peshawar, KPK, Pakistan.

Address for correspondence:
Dr. Zahoor Ahmed
Associate Professor
Department of Biochemistry, Khyber Medical College, Peshawar, KPK, Pakistan
E-mail: zakhan70@yahoo.co.uk

of liver function enzymes such as ALP, ALT and AST and cardiac enzymes such as CPK and LDH.

SUBJECTS AND METHODS

Animals: Adult male mice weighing 30-40 gm were procured from the National Institute of Health (NIH), Islamabad, Pakistan. The animals were kept for one week in the animal house facility provided by 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 changed regularly on daily basis. 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 40-65%. Adequate aeration was delivered to avoid accretion of allergen, odor, metabolic gases and particulate wreckage. 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, Department of Pharmacy, Institute of Chemical Sciences, University of Peshawar and Khyber Medical College, Peshawar, KPK, Pakistan during the year 2015.

Assay Kits: The kits for cardiac and liver enzymes were supplied by Merck Diagnostics, USA[9-11]. Cardiac and liver enzymes were determined by enzymatic colorimetric methods. Assay was performed at Department of Biochemistry, Khyber Medical College Peshawar, KPK, Pakistan.

Grouping Details: A total of 150 apparently healthy male mice of same size, age and weight were used in the study. All the animals were kept in the animal house under the standard condition of room temperature in the solid bottom polypropylene cages for period of 45 days. They were randomly divided into three groups. Group A (n=50) comprised of normal healthy mice and were fed orally on Cannabis extract (14 mg/kg body weight) and plain water for 45 days. Group B mice (n=50) were fed orally on Cannabis extract (14 mg/kg) and vitamin C (100 mg/kg body weight) for 45 days. Whereas group C (n=50) were given Cannabis extract (14 mg/kg) and vitamin E (200 mg/kg body weight) for 45 days orally.

Preparation of Drug Solutions: Solutions of Cannabis sativa, vitamin C and vitamin E were prepared according to the literature method[12-14].

RESULTS

Table–I depicts comparison of liver enzymes between group A and B mice. A highly significant change ($p<0.005$) for serum ALP and ALT in group B mice were recorded when compared with group A. However this change was insignificant for AST and serum bilirubin. A highly significant change in the levels of Alkaline phosphatase and Alanine transaminase enzymes was observed, however a non-significant change was noticed in Aspartate transaminase and bilirubin levels when group A and group C were compared (Table–II) . Comparison of cardiac enzymes in group C with group A male mice is depicted in table–III. Insignificant change ($p>0.005$) was observed for CPK in group C mice when compared with group A. However in case of LDH a highly significant change ($p<0.005$) was noted when

Table I: Comparison of mean SD Liver enzymes in mice fed on Cannabis extract 14 mg/kg and Vitamin C 100 mg/kg (Group B) with Group A.

Liver Enzymes	Group A	Group B	T. test	p value
Alkaline phosphatase (IU/L)	198.60±4.63	127.0±0.91*	87.1168	<0.0001
Alanine transaminase (IU/L)	49.90±0.40	24.6±2.39*	9.56287	<0.0001
Aspartate transaminase (IU/L)	82.40±5.83	77.2±2.37	1.53566	0.127
Billirubin (mg/dL)	0.49±0.02	0.45±0.03	-0.520579	0.6038

* P<0.005 when compared with Cannabis extract alone fed mice (Group A).

Table II: Comparison of mean SD Liver enzymes in mice fed on Cannabis extract 14 mg/kg & Vitamin E 200 mg/kg (Group C) with Group A.

Liver Enzymes	Group A	Group C	T. test	p value
Alkaline phosphatase (IU/L)	198.60±4.63	118.6±1.58*	-197.382	<0.00001
Alanine transaminase (IU/L)	49.90±0.40	25.6±1.58*	96.3118	<0.00001
Aspartate transaminase (IU/L)	82.40±5.83	78.5±1.71	-1.07204	0.2869
Billirubin (mg/dL)	0.49±0.02	0.44±0.03		>0.99999

* P<0.005 when compared with Cannabis extract alone fed mice (Group A).

Table III: Compression of mean SD Cardiac enzymes in mice fed on Cannabis extract14 mg/kg and Vitamin C 100 mg/kg (Group B) with Group A.

Cardiac Enzymes (IU/L)	Group A	Group B	T. test	p Value
Creatine phosphokinase (CPK)	271.9±12.07	270.0±8.53	0.772073	0.4420
Lactate Dehydrogenate (LDH)	275.9±12.07	355.9±8.83*	3.82953	0.0002

* P<0.005 when compared with Cannabis extract alone fed mice (Group A).

Table IV: Comparison of mean SD Cardiac enzymes of mice fed on Cannabis extract 14 mg/kg and Vitamin E 200 mg/kg (Group C) with Group A.

Cardiac Enzymes (IU/L)	Group A	Group C	T. test	p value
Creatine phosphokinase (CPK)	271.9±12.07	267.4±17.07	0.537342	0.5929
Lactate Dehydrogenase (LDH)	275.9±12.07	258.0±17.09*	40.4393	0.00001

* P<0.005 when compared with Cannabis extract alone fed mice (Group A).

compared with Cannabis alone fed mice (Group A). Table–IV represents the comparison of cardiac enzymes in mice fed on cannabis and vitamin E (200 mg/kg); group C with group A. The data revealed a non-significant change in group C mice when compared with only Cannabis fed mice group A. LDH of group C mice was observed to have a highly significant change (p<0.005) was observed as compared with group A mice.

DISCUSSION

The liver is the main organ responsible for metabolic, secretary and detoxification functions in the body. Hence it regulates various important metabolic functions in mammalian systems. Liver damage is associated with the distortion of the metabolic functions. The levels of enzymes such as ALP, ALT and AST are established marker enzymes for assessment of the functional integrity of hepatocytes[15-16]. We were the first to carry out this type of study in this part of the world i.e., to analyze the protective role of vitamin C and E which are known to be potent antioxidants. 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[17], assaults on the organ and to a reasonable extent the toxicity of the extract[18]. Alkaline phosphatase (ALP)is a "marker" enzyme for the plasma membrane and endoplasmic reticulum[19]. The elevated activity of serum ALP might be due to the leakage of enzymes from the tissue into the serum. These suggest tissue damage such as cardiac infection; hepatic necrosis and muscle injury. Aspartate transaminase (AST) is localized within the cells of heart, liver, gills, kidneys, muscles and some other organs. The enzyme is very important in monitoring and assessing liver cytolysis. The significant increase in both liver & serum AST following of the leaves could be due to denovo synthesis of the enzymes leading to increased activity than the normal control[20]. The elevated activity in serum AST may suggest a pathological condition on the liver.

Vitamin C is a strong antioxidant capable of scavenging a wide variety of reactive species[21]. Ascorbate is the most water soluble antioxidant in human plasma against lipid peroxiadation induced by aqueous peroxyl radicals, activated neutrophils or gas-phase of cigarette smoke[22-23]. In the instant study when these mice (Group B and C) which were fed on vitamin C (100 mg/kg body weight; group B) and vitamin E (200 mg/kg body weight; group C) in combination with Cannabis (14 mg/Kg body weight) and to see wither they have reversed these changes in the cardiac enzymes caused by Cannabis; encouraging results were obtained (p<0.0005) for LDH in both groups C and D respectively. Similar trend in results were observed for CPK and AST respectively in which both vitamin C and vitamin E have markedly decreased the changes caused by Cannabis in group B and C respectively and are consonance with the results observed by Tabrizi and coworkers (2012) and Al-Shaibaniet.al., (2013)[24-25].

CONCLUSION

The administration of Cannabis had acute toxic effect on mice liver & cardiac enzymes which were significantly reversed by the concurrent administration of antioxidants i.e., ascorbic acid (vitamin C) and vitamin E respectively.

REFERENCES

1. Mandal, T. K., and Das, N. S. Testicular toxicity in cannabis extract treated mice: association with oxidative stress and role of antioxidant enzyme systems. Toxicology and Industrial Health. 2010; 26(1):11-23.
2. Mechoulam, R. Br. J. Pharmacol. 2005; 146:913-915.
3. Appendino, G; Chianese, G; Taglialatela-Scafati O. Curr. Med. Chem. 2011; 18:1085-1099.
4. Perez-Reyes, M; White, W.R.; McDonald, S. A.; Hicks, R. E. Pharmacol. Biochem. Behav.1991; 40:691-694.
5. Johns A. Psychiatric effects of Cannabis. British J

Psych. 2001; 178:116-122.

6. Sapra, M. M., Sharma, P. P., & Kothari, L. K. Effect of vitamin C deficiency on testicular structure in the guinea pig. Journal of Postgraduate Medicine. 1987; 33(2): 69.

7. Dixit, V. P. Effects of Cannabissativa extract on testicular function of presbytisentellus entellus. Plantamedica. 1981; 41(03):288-294.

8. Borini, P., Guimarães, R. C., &Borini, S. B. Possible hepatotoxicity of chronic Cannabis usage. Sao Paulo Medical Journal. 2004; 122(3): 110-116.

9. Young DS. Effects of pre-analytical variables on clinical laboratory tests. 2nd Ed., AACC Press, 1997. P. 195.

10. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed. AACC 2001.

11. Sanhai WR, Christenson RH, Cardiac and muscle disease. Clinical Chemistry. Theory, Analysis, Correlation, 4th Ed., Kaplan, LA, Pesce, AJ., Kazmierczak, S.C., (Mosby Inc. eds St Louis (USA) 2003. P. 566 and Appendix.

12. Hsu P. C., Guo Y. L., Antioxidant nutrients and lead toxicity. Toxicol.,2002; 180:33-44.

13. Uzunhisarcikli M., Kalender Y., Dirican K., Kalender S. Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. Pesticide Biochem Physiol. 2007; 87:115-122.

14. Ullah I., Subhan F., Rauf K., Badshah A., Ali G. Role of gastrointestinal motility/gastric emptying incisplatin-induced vomiting in pigeon. African J Pharmacol. 2012; 6: 2592-2599.

15. Jaeger J. J., Hedegaard H. A Review on Liver Function Test: The Danish Hepatities C. 2002.

16. Adaramoye O. A., Osaimoje D. O., Akinsanya M. A. Changes in antioxidant status and biochemical indices after acute administration of artemether-lumefantrine and halofantrine in rats. Basic ClinPharmacolToxicol., 2008; 102:412-418.

17. Malomo, S. O. Toxicological implication of ceftriaxon administration in Rats.Nis J Biochem. Mol. Biol. 2000; 15 (1):33-38.

18. Yakubu, M. T., Bilbis, L. S., Lawal, M., Akanji, M.A. Evaluation of selected parameters of rats liver and kidney function following repeated administration of yohimbin. Biochemistry. 2003; 15 (2):50-56.

19. Wright P.J., and Plummer D.T., the use of urinary enzyme measurement to detect renal damage caused by nephrotoxic compound. Biochem. Pharmacl. 1974; 12:65.

20. Yakubu, M.T., Akanji, M.A., Salu, I.O. Protective effect of ascorbic acid on some selected tissues of ranitidine treated rats. Nig. J. Biochem. Mol. Biol. 2001; 16 (2):177-182.

21. Halliwell, B. vitamin C: antioxidant or prooxidant in vivo? Free Radical Res. 1996; 25:439-454.

22. Frie, B., Forte, TM., Ames, BN and Cross, CE., Gas phase oxidants of cigarette smoke induced lipid peroxidation & changes in lipoprotein properties in human blood plasma: protective effects of ascorbic acid.. Biochem. J. 1991; 277:133-138.

23. Frie, B., England, L. and Ames BN., Ascorbate is an outstanding antioxidant in human blood plasma. Proc. Natl. Acad. SciUSA. 1989; 86:6377-6381.

24. Tabrizi, B. A., Kararoudi, M. N., &Mahmoudian, B., Evaluation of serum levels of AST, ALT, total bilirubin, glucose, urea and creatinine in mice after administration of Tc-99m MIBI. International Journal of Animal and Veterinary Advances, 2012; 4(1):68-70.

25. Al-Shaibani, E. A., Alarami, A. M., Al-Awar, M. S., Salih, E. M., & Al-Eryani, M. A. Antioxidant protective effect of vitamin E in penicillin and streptomycin-induced hepatotoxicity in guinea pigs. J Agricol Biol. Sci, 2013; 8(7):546-554.

ONLINE SUBMISSION OF MANUSCRIPT

It is mandatory to submit the manuscripts at the following website of KJMS. It is quick, convenient, cheap, requirement of HEC and Paperless.

Website: **www.kjms.com.pk**

The intending writers are expected to first register themselves on the website and follow the instructions on the website. Author agreement can be easily downloaded from our website. A duly signed author agreement must accompany initial submission of the manuscript.