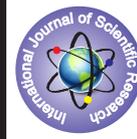


A STUDY ON SERUM PROFILE OF PEROXIDANTS (MDA) IN CANNABIS USER AND CANNABIDIOL ARE NEUROPROTECTIVE –ANTIOXIDANTS IN DIABETIC AND NON-DIABETIC SUBJECTES.



Biochemistry

KEYWORDS: Diabetes Mellitus, Cannabis sativa, Lipid peroxidation, Malondialdehyde (MDA).

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ABSTRACT

An imbalance between reactive oxygen species production and antioxidant scavenging has been implicated in diabetes. Reports indicate that several complications of diabetes mellitus are associated with increased activity of free radicals and accumulation of lipid peroxidation products.

The correlation of Cannabis user groups with duration of one-month, one-year and five year (Both diabetic and non-diabetic groups) with serum Fasting Blood Glucose level and serum Malondialdehyde (MDA) level. The present study was conducted on 105 male-subjects aged between 25 and 65 years. This study was conducted at Department of Biochemistry, Sardar Patel Medical College, Bikaner, India. Fasting blood samples were obtained from 55 diabetic male subjects and 50 healthy control subjects. Blood samples were withdrawn who were taking 5 to 10 gm of cannabis per day and analyzed for Fasting Blood Glucose and serum Malondialdehyde (MDA) by standard technique using UV-Spectrophotometer.

Fasting Blood Glucose and serum Malondialdehyde (MDA) were measured and the results were compared with those of controls. Fasting Blood Glucose and MDA levels were found to be significantly lower in diabetic and non-diabetic subjects using cannabis 5 to 10 gm per day for the last one month, one year and five years when compare with normal control and diabetic control subjects ($p < 0.01$).

Study shows a significant decreased in blood sugar and MDA level recorded diabetic patients and non diabetic groups using cannabis for the last one year and five year as well as in the whole group as compared to that of normal control. It might be possible that either cannabis may enhanced the insulin release or increase the penetration of sugar into the cells and might be due to antioxidant activity of cannabis.

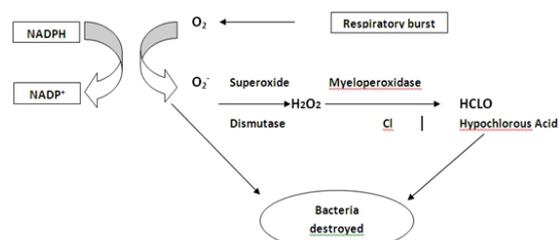
INTRODUCTION

Diabetes is a chronic disease with serious metabolic disturbances in carbohydrate, protein and fat metabolism arising due to insulin deficiency or insulin action. According to WHO reports, diabetes is among the most serious and widely spread disease. It is mostly spread in the low to middle income countries and has affected approximately 347 million people^[1]. A variety of symptoms are linked to this disease, with the most common being thirst, polyuria, blurring of vision and weight loss. On advanced stages, as a result of hyperosmolar state, serious symptoms

such as stupor and coma may develop, which if left untreated may lead to death^[2]. Beside these primary symptoms, some serious complications may be involved if the glucose level of the blood is not controlled in time. Some of the complications are cardiovascular disease including heart attack, severe neuropathy, retinopathy, nephropathy, osteoporosis and foot damage. Some rare cases of a linkage between diabetes and cancer have also been reported^[3,4].

Respiratory Burst and the Oxygen Free Radicals:-

Respiratory burst is a remarkable property of the neutrophils, macrophages, B cells and other phagocytic cells^[5,6]. These cells after activation increase their oxygen uptake, which may raise upto 50 folds^[7]. This increased oxygen uptake is then followed by the breakdown of this oxygen by the enzyme respiratory burst oxidase as shown below:



The superoxide radical formed from the above reaction is responsible for a variety of reactions, forming products such as oxidized halogens, oxidizing radicals and singlet oxygen^[8].

The prevalence of diabetes is currently estimated to be about 6.4% worldwide and there has been a dramatic increase in the diagnosis of type 2 diabetes in the past two decades^[9]. Oxidative stress has been considered to play a central part in the progression of diabetes^[10]. Free radicals have important role in the pathogenesis of diabetes and a relationship between oxidative stress and diabetes complications^[11,12]. Excess production of free radicals, mainly due to hyperglycemia, causes oxidative stress, which further aggravates the development of diabetes and its complications^[13]. Oxidative stress may be increased in diabetic patients since persistent hyperglycemia causes an increased production of oxygen free radicals though auto oxidation of glucose and non-enzymatic glycation of proteins^[14]. Increased levels of the products of oxidative damage to lipids and protein have been detected in the plasma of diabetic patients^[15]. In healthy individuals, oxidative damage to tissue is prevented by a system of defenses which includes antioxidant enzymes and vitamins^[16].

Regular marijuana use is associated with favourable indices related to diabetic control, say investigators. They found that current marijuana users had significantly lower fasting insulin and were less likely to be insulin resistant, even after excluding patients with a diagnosis of diabetes mellitus. Marijuana (*Cannabis sativa*) has been used for centuries to relieve pain, improve mood, and increase appetite. Outlawed in the United States in 1937, its social use continues to increase and public opinion is swinging in favor of the medicinal use of marijuana. There are an estimated 17.4 million current users of marijuana in the United States. Approximately 4.6 million of these users smoke marijuana daily or almost daily^[17].

Medical cannabis (also referred to as Medical Marijuana):-

Cannabis or Cannabinoids may be useful in treating alcohol abuse,

attention-deficit hyperactivity disorder (ADHD or Ad/HD), collagen induced arthritis, asthma, atherosclerosis, colorectal cancer, depression,^[18] gliomas, skin tumors, Huntington's disease, zeukemia, Parkinson's disease,^[19] sickle-cell disease, anorexia nervosa and decreased blood glucose with MDA level case reports found that marijuana helped reduce.^[20]

Pharmacological Action of Cannabis sativa

Cannabis has been widely used for hundred of years as an intoxicant or an herbal remedy. Pure delta tetrahydrocannabinoid constituents of marijuana. It is now available by prescription as dronabinol. The use of crude marijuana as a medicine would entail smoking the drug or creating herbal preparation of it. Crude marijuana, an undefined herb containing approximately 480 substances.

The oral THC has generally been found to be as effective or more effective for nausea than pro-chloroperazine.^[21] According to the study by (Chang and Colleagues, 1979), THC level of at least 10 ng/ml were effective in preventing nausea. (Orr and Colleagues, 1980)^[22] Studied those patients who were refractory to pro-chloroperazine.

MATERIALS AND METHODS

The present study was conducted on 105 male-subjects aged between 25 and 65 years during the period of August 2011 to October 2013. They were randomly selected irrespective their caste and creed. Those individuals who were suffering from other inflammatory conditions like tuberculosis, leprosy, pregnancy, cancer, skin diseases, gout, liver and kidney diseases were excluded to rule out any increase in inflammatory markers due to causes other than DM. They were divided into the following groups (Table 1).

Only those subjects included in the present study, who were taking 5 to 10 gm of cannabis per day. Majority of these subjects were Brahmin by caste, belonging to lower middle class. After their small earning, they spent their rest of time in worshipping lord Shiva and residing in temple area called "Bagechi". There they used to take cannabis "bhang" routinely in-group of 5-8 persons in the form of semi solid mass or in solution form. They presented themselves voluntarily for the present study on the basis of personal request, relationship and their eternal eagerness to know about the effect of "Bhang" 5 ml of venous blood samples collected at the time of presentation of patients OPD in EDTA bottles and samples were centrifuged at 3000 rpm for 10 minutes. Plasma was collected taking care to avoid hemolysis. (Table 1).

The samples were analyzed for the following parameters by UV-Spectrophotometer using standardized glucose oxidase-peroxidase method,^[23] are blood Glucose and Serum malondialdehyde (MDA) was studied by the method proposed by Buege and Aust^[24].

STATISTICAL ANALYSIS: Statistical analysis was done, using the SPSS 20. mean SD were calculated. The control and study group was analyzed using unpaired "t"-test.

RESULTS AND DISCUSSION:

In this study, table II shows that the increase in blood parameters for normal healthy subjects that is normal control group and Diabetic control subjects and parameters represent Mean and SD along with p value which show highly significant (p <.001) when compared to Diabetic control subjects. This indicates that lipid peroxidation is significantly increased in diabetes.

The mean blood sugar level was reduced to 98.48 ± 9.06 with a range of 85.4 to 108.4 mg percent in normal control subjects using 5 to 10 gm cannabis per day for the last one month (Table-V). Although the decrease in blood sugar in cannabis user was not statistically

significant as compared to that of normal subjects as evident by P-value (P<0.20) as shown in table III. However slight decrease in blood sugar in cannabis user might be due to that cannabis may enhance the insulin release activity resulting decrease the blood sugar level.

The blood sugar level in normal healthy subjects using cannabis for the last one year ranged from 79.8 to 94.6 with an average of 88.2 ± 5.5 mg percent (Table III); whereas it ranged from 76.2 to 94.1 with a mean of 86.24 ± 6.48 mg percent in those normal subjects using 5 to 10 gm cannabis per day the last 5 year (Table III).

The blood sugar level was found to be reduced (251.8 ± 31.9 mg%) in diabetic subjects using 5 to 10 gm cannabis per day for the last one month as compared to that of diabetic control (262.1 ± 35.3 mg%) as shown in table IV. The reduction in blood sugar diabetic patients using cannabis for the last one month was not highly significant as revealed by P-value (P<0.05; Table IV).

The mean blood sugar level in diabetic subjects using cannabis for the last one year was found to be 201.1 ± 9.7 with a range by 190.5 to 214.0 mg percent; while it ranged from 178.4 to 209.4 with an average of 194.6 ± 9.7 mg percent in diabetic subjects using cannabis for the last five years (Table IV). The decrease in blood sugar level in both the groups of diabetic cannabis user as compared to diabetic control was statistically significant as evident by P-value, which was less than 0.001 in both the groups (Table IV).

The MDA level was to be reduced to 1.09 ± 0.21 with a range of 0.82 to 1.32 nmol/ml in normal control subjects using 5 to 10 gm cannabis per day for the last one month (Table III). However, slight decrease in MDA concentration in cannabis user normal subject as compared to that non-cannabis users control group was not statistically significant as evident by P-value (P<0.20; Table III).

The MDA level in normal healthy control subjects using cannabis for the last one year ranged from 0.31 to 1.01 with an average of 0.61 ± 0.28 nmol/ml (Table III); whereas it ranged from 0.10 to 0.41 with a mean of 0.22 ± 0.10 nmol/ml in the subjects using cannabis 5 to 10 gm per day for the last five year (Table III).

The MDA concentration was found to be reduced to 3.06 ± 0.44 with a range of 2.56 to 3.61 nmol/ml in diabetic subjects using 5 to 10 gm cannabis per day for the last one month (Table IV). The decrease in MDA level in cannabis user was not significant as compared to that of diabetic control (3.24 ± 0.80 nmol/ml) as evident by P-value (P<0.90) as shown in table IV.

The mean MDA level in diabetic subjects using cannabis for the last one year was found to be 2.23 ± 0.40 with a range of 1.80 to 2.85 nmol/ml whereas it ranged from 0.87 to 2.17 with an average of 1.61 ± 0.44 nmol/ml in diabetic subjects using cannabis 5 to 10 gm per day for the last five year. (Table IV). The decrease in MDA levels in both the groups of diabetic cannabis user was statistically significant as compared to that of diabetic control as evident by P-value, which is less than 0.05 and 0.001 respectively. The decrease was directly proportional to the duration of cannabis intake. The decrease was highly significant in those diabetic cannabis users who used to take cannabis for the last five years.

CONCLUSION : The values of MDA and serum blood glucose were found to be decreased in study groups. The value of MDA and serum blood glucose in study group showed highly significant changes when compared to control group and having cannabis 5 to 10 gm per day for the last one year and last five years. It might be possible that either cannabis may enhanced the insulin release or increase the penetration of sugar into the cells and might be due to antioxidant activity of cannabis.

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Table No. (1)

S.No.	GROUP	NUMBER OF SUBJECT
1.	I st normal control	20
2.	II nd Diabetic subjects	30
3.	III rd Normal Subject cannabis user	30
4.	IV th Diabetic subjects cannabis user	25

TABLE II - MEAN VALUES OF BLOOD PARAMETERS IN NORMAL CONTROL SUBJECTS AND DIABETIC SUBJECTS.

Blood Parameters	Normal Control subjects(n=20)Subjects.(n=30)			Diabetic control Subjects. (n=30)			Significant	
	Mean + S.D	Range	S.E.	Mean + S.D	Range	S.E.	t	p
Fasting Blood Sugar (mg%)	104.48 + 6.46	94.6-117.2	1.45	262.13+ 35.39	192.0 - 316.4	7.07	21.86	<0.01 (HS**)
Malondialdehyde(MDA) nmol/ml	1.42+ 0.56	0.84 - 2.85	0.13	3.24 + 0.80	1.56 - 4.85	0.14	8.62	<0.01 (HS**)

HS** = Highly Significant.

TABLE III - COMPARISON OF MEAN VALUES OF BLOOD PARAMETERS IN NORMAL CONTROL SUBJECTS WITH NORMAL SUBJECTS USING CANNBIS 5 to 10 gm PER DAY FOR THE LAST ONE MONTH, ONE YEAR AND FIVE YEARS.

Blood Parameters	Normal Control subjects.(n=20)		Normal Subjects using Cannabis (for the last one month).(n=7)		Significant		Significant Normal Subjects using Cannabis (for the last one year).(n=8)ant		Significant		Normal Subject s using Cannabis (for the last five years).(n=15)		Significant	
	Mean + S.D.	S.E.	Mean + S.D.	S.E.	t	p	Mean + S.D.	S.E.	t	p	Mean + S.D.	S.E.	t	p
Blood Sugar (mg%)	104.49+ 6.46 (94.6-117.2)	1.45	98.48+9.06 (85.4-108.4)	4.04	1.40	<0.2015	88.2+5.50 (79.8-94.6)	2.46	5.62	<0.001 HS**	86.24+6.48 (76.2-94.1)	2.05	7.27	<0.001 HS**
Malondialdehyde (MDA) nmol/ml	1.43+ 0.57 (0.84-2.85)	0.13	1.09+0.21 (0.82-1.32)	0.09	1.78	<0.20 IS	0.61+0.28 (0.31-1.01)	0.12	4.82	<0.001 HS**	0.22+0.10 (0.10-0.41)	0.03	9.31	<0.001 HS**

Figures in parenthesis indicate the range.

TABLE IV - COMPARISON OF MEAN VALUES OF BLOOD PARAMETERS IN DIABETIC CONTROL SUBJECTS WITH DIABETIC SUBJECTS USING CANNBIS 5 to 10 gm PER DAY FOR THE LAST ONE MONTH, ONE YEAR AND FIVE YEARS.

Blood Parameters	Diabetic Control subjects. (n=30)		Diabetic Subjects using Cannabis (for the last one month).(n=5)		Significant		Diabetic Subjects using Cannabis (for the last one year).(n=5)		Significant		Diabetic Subjects using Cannabis (for the last five years).(n=15)		Significant	
	Mean + S.D.	S.E.	Mean + S.D.	S.E.	t	p	Mean + S.D.	S.E.	t	p	Mean + S.D.	S.E.	t	P
Blood Sugar (mg%)	262.13+ 35.38 (192.0-316.4)	7.07	251.80+ 31.94 (215.7-284.8)	14.23	2.44	<0.05 IS	201.10+ 9.74 (190.5-214.0)	4.36	10.34	<0.001 HS**	194.65+ 9.74 (178.4-209.4)	3.08	13.41	<0.001 HS**
Malondialdehyde (MDA) nmol/ml	3.24+ 0.80 (1.56-4.84)	0.16	3.06+0.44 (2.56-3.61)	0.19	0.20	<0.90 IS	2.23+0.40 (1.80-2.85)	0.18	1.31	<0.5	1.61+0.44 (0.87-2.17)	0.11	8.52	<0.001 HS**

Figures in parenthesis indicate the range.

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