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Effect of Alcoholic and Aqueous Extract of Cannabis Indica on Rate of Glucose, Na⁺, K⁺ and Ca Ion Uptake by T47D Cells: an In Vitro Study

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ABSTRACT: The present study is been undertaken to evaluate the rate of glucose and Na⁺, K⁺, Ca ions uptake by cancerous cells, when treated with alcoholic and aqueous extract of Cannabis Indica, which has a potential to increase the appetite level. Leaves of the Cannabis Indica collected from local growers. Two different extracts were prepared. T47D Cell line was purchased and cultured, dose optimization was done using IC-50 value and different groups of cellswith plant extract was prepared. Estimation of glucose and Na⁺, K⁺, Caions of bleached media was done for 6 days with GOD and Electrolyte analyzer method respectively.The observation shows that the alcoholic extract of Cannabis Indica enhances the uptake rate of Glucose by cancerous cells as compare to the aqueous extract and controls. Whereas there were no such changes in Na⁺, K⁺ and Ca ions uptake, when treated with plant extract.From the result, we have concluded that the active compound (THC) which is present in alcoholic extract of Cannabis Indica,increasing the uptake rate of glucose by T47D cells.Therefore we can say that, our body required more glucose after consumption of Cannabis Indica. Hence we can correlate that Cannabis stimulates the rate of appetite.

KEYWORDS: Cannabis Indica, appetite, glucose uptake.

I. INTRODUCTION

Cannabis sativa has a recorded history of medicinal use that dates back 5,000 years^[1].Very few reports are published on the pharmacologicalactivity of Cannabis Indica.Though there seems to be no consensus that Cannabis sativa and Cannabis indicabelong to two different species, they are definitely two different varieties or subspecies(Schultes: 1970). Cannabis is widely used as a recreational drug, because of the psychoactive effect of the major active constituent i.e. Δ9-tetrahydrocannabinol (THC), which is an FDA approved medication to treat nausea and emesis caused by cancer chemotherapeutic agents^[2].One of the most common effect of Cannabis(sativa /Indica) consumption is increase in appetite level, this is also because of the THC concentration ratio or due to the chemicals that are structurally related to THC and are collectively known as cannabinoids (CBD)^[3]these compounds possess THC-like effects. THC appeared to increase body weight in advanced cancer patients^[4]. And was found to increase appetite in anorexic Alzheimer's disease patients^[5] and patients with AIDS^[6], suggesting even greater therapeutic potential for these agents. Korte (1970) has conducted a comparative study of Cannabis sativa and Cannabis Indica in which he has shown certain differences especially in relation to THC content.Notably, In the 1970s some of the first experimental studies investigating the effects of THC on food intake in humans reported significant increases in consumption ofchocolate milk shakes and marshmallows^[7,8]. Mechanism states that both the type 1 cannabinoid receptor (CB1) and endocannabinoids have been associated with the brain regions that control food intake (Howlett et al., 2002), but the

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pharmacology is still unknown. In our study we have checked the rate of glucose uptake by cancerous cells (T47D) and correlated our result in terms of appetite stimulation.

II. MATERIAL AND METHODS

2.1. Plant material:

The leaves of Cannabis Indica were collected from local growers in a sterilized polythene bag.

2.2. Preparation of Extract:

The leaves of the plant were collected, surface sterilized with 0.1 % HgCl₂ and washed repeatedly with sterile distilled water for several times and kept for drying in hot air oven at 60°C temperature for 48 hours. After drying, the leaves were preserved separately in polythene bags at room temperature.

2.2.1. Aqueous extract:

The dried leaves of Cannabis Indica were crushed separately into fine powder with the help of mortar and pestle, then 0.5gm of the powdered extract was dissolved in 50 ml sterilized hot distilled water, so as to make 1% stock solution, this solution was kept overnight for stirring with the help of magnetic stirrer and filtered through Whatman No.1 filter paper. The solution is further filtered through syringe filter.

2.2.2. Alcoholic extract:

The dried medicinal plant material was blended with help of mortar and pestle, 0.5gm of powdered extract was dissolved in organic solvent i.e. Absolute alcohol and stirred for 3-4 hr with the help of magnetic stirrer. The resultant extract was filtered through a glass wool filter and then rinsed with a small quantity (about 30 ml) of 96% alcohol. The extract solution was further filtered through syringe filter.

2.3. Cell line material:

T47D cell line was purchased from National Centre of Cell Science (NCCS), Pune. Cells were cultured in culture flask (T-75) with RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS), 1% Penicillin and 0.5% gentamicin. The culture was incubated at 37°C in humidified 5% carbon dioxide. The cells were counted and viability was checked with Trypan blue exclusion test.

2.4. Chemicals:

Glucose oxidase peroxidase kit was purchased from Pathozone Diagnostics, Kagal, India. All the chemicals used in the study were of extra pure analytical grade.

2.5. Dose optimization of plant extract:

This is done by calculating IC₅₀ value of both the extract respectively. T47D cells were seeded into a 96-well plate at a density of 1.2×10^4 cells/well. After achieving confluence, cells were incubated in medium containing different concentrations of leaves extract that were 0.1%, 0.1%, 0.01%, 0.001% for both the extract i.e. alcoholic and aqueous. The plate was incubated for 24hrs. After that 100µL of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (5 mg/mL) (MTT) was added to each well and incubated for a further 4 h. Next, 100 µL dimethyl sulfoxide (DMSO) was added to cells in each well, incubated for 30min, and optical density measured at 575 nm.

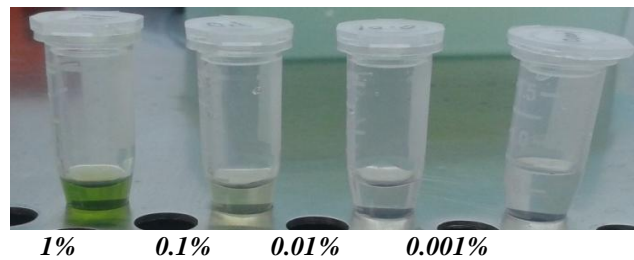
A cytotoxicity assay is performed to calculate the level of toxicity of aqueous and alcoholic plant extract on T47D cell line through an MTT assay. Four different dilutions were prepared of both the plant extract- 1%, 0.1%, 0.01%, 0.001%.

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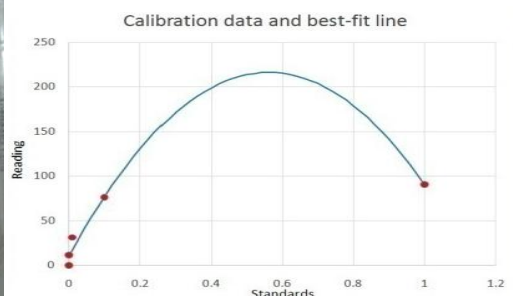
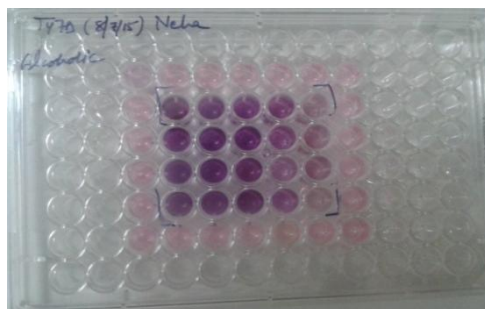
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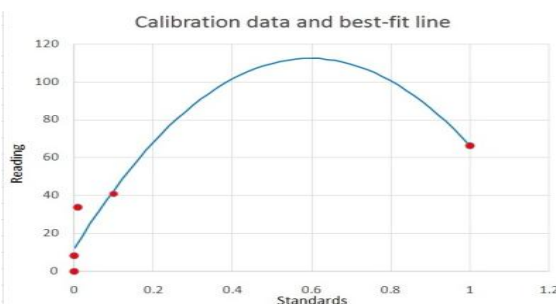
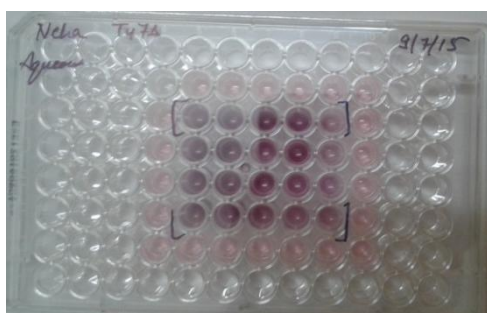


Calculated the cytotoxicity level of both plant extract by plotting the graph- cytotoxicity value vs. number of cells/ml and calculated the IC-50 value. The results are as follows:

Alcoholic:



Aqueous:



The IC50 value of Alcoholic extract was coming approx 0.06% and for Aqueous 0.13%.

2.6. Experimental design:

The cultivated T47D cells was grouped in three different groups having cell density 1.5×10^5 /mL in each well of 6 well plate with 2mL of RPMI 1640, whereas the test groups contained IC50 concentration of plant extract. The groups are as follows:

- **Group 1:** Control, cells cultured with only normal growth medium
- **Group 2:** Test Cells cultured with normal growth medium + Alcoholic extract of Cannabis Indica
- **Group 3:** Test Cells cultured with normal growth medium + Aqueous extract of Cannabis Indica

Each group was kept in Co2 incubator and been analyzed after 24 hr every day for 1 week.

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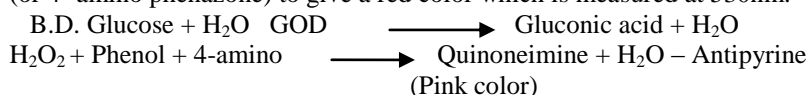
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2.7. Estimation of glucose:

For estimation of glucose we collected the supernatant i.e. bleached media every day for 1 week, from each group, and calculated the glucose concentration difference with the control as well as with the original media, from glucose oxidase (GOD) method.

Glucose oxidase (GOD) acts on glucose to produce gluconic acid and hydrogen peroxide. Breakdown of hydrogen peroxide by peroxidase is coupled with oxidation of phenol. The oxidized phenol complexes with 4-amino antipyrine (or 4- amino phenazone) to give a red color which is measured at 530nm.



2.8. Estimation of Na⁺, K⁺ and Ca ions Uptake:

For estimation, we collected the supernatant i.e. bleached media every day for 1 week, from each groups, and calculated the uptake level of glucose, Na⁺, K⁺, Ca²⁺ ions concentration from glucose oxidase (GOD) method, with Electron Analyzer instrument. Glucose oxidase (GOD) acts on glucose to produce gluconic acid and hydrogen peroxide. Breakdown of hydrogen peroxide by peroxidase is coupled with oxidation of phenol, oxidized phenol complexed with 4-amino antipyrine (or 4- amino phenazone) to give a red color which is measured at 530nm[11].

Reagents Used in GOD: Phosphate buffer (pH-7), Enzyme reagent which contains GOD, POD, 4-amino antipyrine and phenol and Glucose standard i.e. Media (RPMI-1640).

III. OBSERVATION

Time(hrs)	Groups		Glucose (mg%)		Na ⁺ (m.eq/L)		K ⁺ (m.eq/L)		Ca ²⁺ (mg%)	
0	Only media		194		145		6.2		3.2	
24	Alcoholic	C1	176	186	142	141	6.0	6.0	3.3	3.3
	Aqueous	C2	177	184	135	142	6.1	6.0	3.7	3.2
48	Alcoholic	C1	160	171	142	145	6.1	6.2	3.5	3.3
	Aqueous	C2	175	178	136	144	6.1	6.2	3.9	3.3
72	Alcoholic	C1	124	149	135	142	6.2	6.0	3.9	3.2
	Aqueous	C2	153	161	142	147	6.2	6.3	3.4	3.2
96	Alcoholic	C1	110	129	144	141	6.1	6.0	3.7	3.3
	Aqueous	C2	131	148	138	149	6.2	6.0	3.3	3.2
120	Alcoholic	C1	87	106	145	147	6.3	6.2	3.5	3.4
	Aqueous	C2	117	120	135	145	6.0	6.2	3.8	3.4
144	Alcoholic	C1	55	94	149	149	6.4	6.3	3.5	3.5
	Aqueous	C2	63	80	144	150	6.3	6.3	3.9	3.5

C1 is the control for Alcoholic and C2 is the control for Aqueous.

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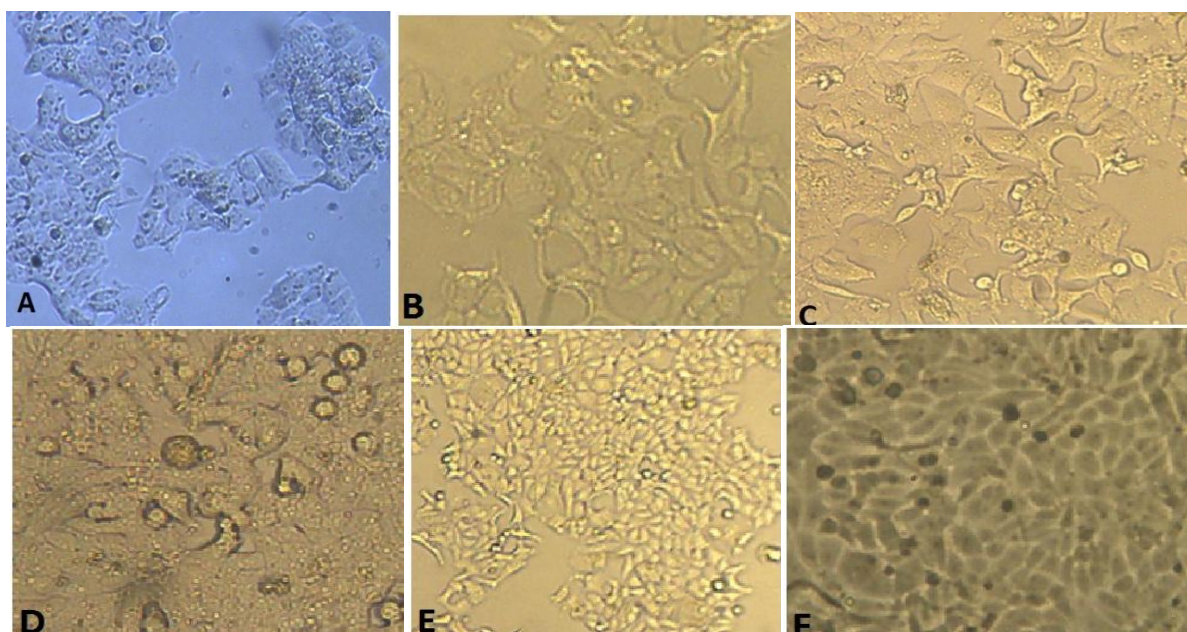
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3.1 Cell line Observation:

The cells was observed under inverted microscope, at different magnifications.



A is after 24hr(4X), B=48hrs(4X), C= 72hr (10X), D=96hr(10X), E=120hrs(4X) and F=144hrs(100X)

IV. RESULT

Two comparison studies were done on the basis of glucose uptake rate by the T47D cells, the one between both the test groups and another between tests and control respectively. The effect of leaf extract of Cannabis Indica on glucose uptake rate by the T47D cells at different interval of time was observed to be in increase concentration. It has been observed that the alcoholic extract enhance more glucose uptakeas compare to the aqueous one. It is also observed that the rate of glucose uptake in aqueous extract group was slow as compare to the alcoholic one. As we can see in (Table 1.0) there is around 10-20mg% increased glucose uptake in alcoholic as compare to the control, whereas there is only 5-10mg% uptake in aqueous solution. Whereas there were no such changes in Na⁺, K⁺ and Ca ions uptake, when treated with plant extract.

C1 is the control for Alcoholic and C2 is the control for Aqueous.

Conclusions: Active compound (THC) which is proven to stimulate appetite level assumes to be present in alcoholic extract of Cannabis Indica and because of this the alcoholic compound increasing the uptake rate of glucose by cancerous cells. Therefore we can correlate that yes there is an increase in appetite level after the consumption of Cannabis Indica.

REFERENCES

1. Mechoulam, R. Cannabinoids as therapeutics. Basel, Boston: Birkhäuser; 2005. p. xp. 272.
2. Thomas F. Gamage, BS and Aron H. Lichtman, PhD. The Endocannabinoid System: Role in Energy Regulation. *Pediatr Blood Cancer*. 2012 January.

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(A High Impact Factor, Monthly, Peer Reviewed Journal)

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3. Elsohly MA, Slade D. Chemical constituents of marijuana: The complex mixture of naturalcannabinoids. Life Sci. 2005. 78:539–548. [PubMed: 16199061]
4. Regelson, W.; Bulter, J.; Schulz, J. A9-tetrahydrocannabinol as an effective antidepressant and appetite-stimulating agent in advanced cancer patients. In: Braude, MC.; Szara, S., editors. The pharmacology of marijuana. New York: Raven Press; 1976. p. 763-776.
5. Volicer L, Stelly M, Morris J, et al. Effects of dronabinol on anorexia and disturbed behavior in patients with Alzheimer's disease. Int J Geriatr Psychiatry. 1997; 12:913–919. [PubMed: 9309469]
6. Plasse T, Gorter R, Krasnow S, et al. Recent clinical experience with dronabinol. Pharmacol Biochem Behav. 1991; 40:695–700. [PubMed: 1666930]
7. Hollister LE. Hunger and appetite after single doses of marijuana, alcohol, and dextroamphetamine. Clin Pharmacol Ther. 1971; 12:44–49. [PubMed: 5541134]
8. Abel E. Effects of marijuana on the solution of anagrams, memory and appetite. Nature. 1971; 231:260–261. [PubMed: 4930692]