INVESTIGATION OF CANNABIS EXTRACT AS A POSSIBLE CANDIDATE FOR THE PREVENTION AND TREATMENT OF INDUCE CANCER CELLS IN ALBINO MICE

A Ph.D. DISSERTATION

BY

MD. RASHED IMAM

Registration No. 1205084

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
FACULTY OF VETERINARY AND ANIMAL SCIENCE
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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Approved as to style and contents by

Prof. Dr. S.M. Harun-Ur-Rashid
Supervisor

Prof. Dr. Md. Abu Hadi Noor Ali Khan
Co-Supervisor

Prof. Dr. Begum Fatema Zohara

Chairman, Examination Committee

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR-5200
MAY, 2023

DEDICATED

TO

MY MOTHER

প্যাথলজি এন্ড প্যারাসাইটোলজি বিভাগ
ভেটেরিনারি এন্ড এনিমেল সায়েন্স অনুষদ
হাজী মোহাম্মদ দানেশ বিজ্ঞান ও প্রযুক্তি বিশ্ববিদ্যালয়
দিনাজপুর, বাংলাদেশ।

Department of Pathology and Parasitology
Faculty of Veterinary and Animal Science
Hajee Mohammad Danesh Science and
Technology University
Dinajpur-5200, Bangladesh.

CERTIFICATION

It is my pleasure to certify this thesis entitled **"INVESTIGATION OF CANNABIS EXTRACT AS A POSSIBLE CANDIDATE FOR THE PREVENTION AND TREATMENT OF INDUCE CANCER CELLS IN ALBINO MICE"** to the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200 for the degree of Doctor of Philosophy (Ph.D.).

I hereby certify that:

1) The candidate has fulfilled the residential requirement.
2) The research work embodied in the thesis was carried out by the candidate.
3) The data to the best of our knowledge, genuine and original.
4) Entire or no part of the research work has been submitted in substance for any degree to the best of our knowledge and belief.

(Prof. Dr. S.M. Harun-Ur-Rashid)

Supervisor

Department of Pathology and Parasitology

Hajee Mohammad Danesh Science and Technology

University, Dinajpur-5200

DECLARATION

I hereby honestly declare that the thesis titled **"INVESTIGATION OF CANNABIS EXTRACT AS A POSSIBLE CANDIDATE FOR THE PREVENTION AND TREATMENT OF INDUCE CANCER CELLS IN ALBINO MICE"** has been composed solely by myself on the basis of the results of the research work of my direct and original investigation to the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, University of Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200 under the supervision of **Prof. Dr. S.M. Harun-Ur-Rashid,** chairman, the same Department, co-supervisor **Prof. Dr. Md. Abu Hadi Noor Ali Khan,** Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2200. Fulfillment of the requirement of the degree of Doctor of Philosophy (Ph.D.).

I further declare that this research work has not been submitted, in whole or in part, in any previous application for any academic degree in this University or any University. Except where states otherwise by reference or acknowledgment, the work presented is entirely my own.

May, 2023
Dinajpur

(Md. Rashed Imam)
Candidate

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The Author
May, 2023

Abstract

Cancer is the second highest cause of mortality in the world, characterized by the uncontrolled growth and proliferation of cells resulting in death. Cannabinoids are naturally occurring compounds found in the *Cannabis sativa* plant. Cannabinoids inhibit angiogenesis and decrease metastasis in various tumors. The research work was conducted to evaluate the effects of cannabis extract on albino mice and the response of cannabis extract for the treatment and prevention of Ehrlich ascites carcinoma (EAC) cells induced cancer on albino mice. A total of 444 albino mice were collected from the animal house, at the Department of Biochemistry and Molecular Biology, University of Rajshahi. For the observation of the effects of cannabis extract on albino mice, 48 mice were randomly divided into four treatment groups i.e. T_0 (control), T_1 (5mg/Kg), T_2 (10mg/Kg) and T_3 (15mg/Kg). Every week 4 mice were sacrificed from all groups, blood was collected for biochemical and hematological analysis, and organs were collected for histopathological observation. Significantly ($P<0.05$) highest body weight was found in T3. No significant ($P>0.05$) effects of cannabis extract on hematological and biochemical parameters except in early-stage SGPT, SGOT, and alkaline phosphatase which were increased but with time it became normal and histopathology of different organs revealed no changes. For the treatment purpose of EAC-induced cancer, 204 mice were randomly divided into seven groups i.e group-1 (control, n=12) group-2 (EAC induced only, n=12), group-3 (Commercial Chemotherapeutic drug, n=36), group-4 (cannabis extract started injection 3 days after EAC induced, n=36), group-5 (cannabis extract started injection 7 days after EAC induced, n=36), group-6 (cannabis extract started injection 14 days after EAC induced, n=36) and group-7 (cannabis extract started injection 21 days after EAC induced, n=36). Mice of group-3, group-4, group-5 group-6 and group-7 were divided into four subgroups i.e T_0 (control), T_1 (5mg/Kg), T_2 (10mg/Kg) and T_3 (15mg/Kg) consisting of 12 mice in each. The results revealed that no tumor and ascitic fluid formation and no significant ($P>0.05$) effects on biochemical and hematological parameters in the early period of treatment (groups 4 and 5) whereas tumor and acetic fluid formation and had significant ($P>0.05$) effects on some biochemical and hematological parameters group at in the longer period of treatment (group 6 and 7), cytopathological study of ascitic fluids also revealed atypical cells under light microscope, histopathological study of tumor revealed sarcoma type cancer. For the prevention purpose of EAC-induced cancer, a total of 192 mice were randomly divided into four groups i.e group-1 (EAC induced after 3 doses of cannabis extract, n=48), group-2 (EAC induced after 7 doses of cannabis extract, n=48), group-3 (EAC induced after 14 doses of cannabis extract, n=48) and group-4 (EAC induced after 21 doses of cannabis extract, n=18). Mice of group-1, group-2, group-3 and group-4 were divided into four subgroups i.e T_0 (control), T_1 (5mg/Kg), T_2 (10mg/Kg) and T_3 (15mg/Kg) consisting of 12 mice in each. The effects of EAC cells induced cancer and preventive effects of cannabis extract on biochemical and hematological profiles were observed. In the biochemical profile, EAC significantly ($P<0.05$) increased SGPT, SGOT, alkaline phosphate and uric acid with an increased period of observation. EAC significantly ($P<0.05$) increased WBC and neutrophil but significantly ($P<0.05$) decreased lymphocyte. After 3^{rd} and 7^{th} doses of extract injection did not show cancer preventive effect. After the 14^{th} dose, 7.14% tumor and fluid formation, no significant effect ($p<0.05$) on biochemical and hematological parameters except SGPT, SGOT and alkaline phosphatase. EAC induced after 21^{st} doses of cannabis extract injection, no tumor and acetic fluid formation, no significant effect on biochemical and hematological parameters like as normal mice.

Abbreviations

%	: Percentage
&	: and
P<0.05	: Probability at 5% level
@	: At the rate of
<	: Is less than
>	: Is greater than
et al.,	: and others
$mmolL^{-1}$	: Millimoles per liter
mg/dl	: Milligrams per deciliter
cmm	: Cubic per centimeter
Δ 8 -THC	: Δ 8 -Tetrahydrocannabinol
2-AG	: 2-Arachidonylglycerol
AEA	: Anandamide
AED	: Atomic emission detector
AFD	: Alkali flame detector
Apaf-1	: Apoptotic protease-mediated factor
APC	: Antigen presenting cells
ATP	: Adenosine triphosphate
ALP	: Alkaline Phosphatase
ALT	: Alanine Aminotransferase
AST	: Aspartate Aminotransferase
BA	: Basophil
Bad	: Bcl2 associated death promoter
BAK	: Bcl2 homologous antagonist killer
BCSIR	: Bangladesh Council of Scientific and Industrial Research
BS	: Bone sarcomas
BCSIR	: Bangladesh Council of Scientific and Industrial Research
CB	: Cannabinoid
CB1	: Cannabinoid receptor 1

CB2	: Cannabinoid receptor 2
CBC	: Cannabichromene
CBD	: Cannabidiol
CBDA	: Cannabidiolic acid
CBDV	: Cannabidivarin
CBG	: Cannabigerol
CBN	: Cannabichromene
CCR	: Crude Cannabis Resin
Cdk	: Cyclin dependant kinases
CE	: Cannabis extract
CRC	: Colorectal cancer
CRPC	: Castration-resistant prostate cancer
CTL	: Cytotoxic T lymphocytes
CY	: Cyclophosphamide
DC	: Dendritic Cell
DELCD	: Dry electrolytic conductivity detector
DNA	: Deoxyribonucleic acid
EAC	: Ehrlich ascites carcinoma
EBV	: Epstein–Barr virus positive
EBV	: Epstein-Barr virus
ECS	: Endocannabinoid systems
EDTA	: Ethylenediamine tetraacetic acid
EMT	: Epithelial-mesenchymal transition
EO	: Eosinophil
ER	: Endoplasmic reticulum
FDA	: Food and Drug Administration
FID	: Flame ionization detector
FNAC	: Fine Needle Aspiration Cytology
GABA	: Glutamatergic neurotransmission
GC	: Gas chromatography

GC-MS : Gas chromatography-mass spectroscopy

GM-CSF : Granulocyte-macrophage colony-stimulating factor

GC-FID : Gas chromatography with flame-ionization detection

GVAX : Gene-transduced tumor vaccine

HB : Hemoglobin

HLA : Human leukocyte antigen

HER2 : Human epidermal growth factor 2

HGSC : High-grade serous cancer

HNSCC : Head and neck squamous cell carcinomas

HID : Helium ionization detector

HPLC : High-performance liquid chromatography

HSV-1 : Herpes simplex virus type 1

IC50 : Inhibitory concentration at 50 %

ICAM : Intercellular adhesion molecule

ID50 : lethal dose 50

IL : Interleukins

IL-2 : Interleukin-2

I.P. : Intraperitonium

INF : Interferon-alpha

LNCaP : Lymph Node Carcinoma of the Prostate

LP : Long primer

LY : Lymphocyte

MDSC : Myeloid-derived suppressor cells

MDA-MB : M.D. Anderson - Metastatic Breast

MMP-2 : Matrix metalloproteinase-2

MO : Monocyte

MH : D-Mannoheptulose

MHC : Major histocompatibility complex

MUC1 : Mucin glycoprotein 1

MVA : Modified vaccinia virus Ankara

MW	: Molecular weight
NCI	: National Cancer Institute
NE	: Neutrophil
NPD	: Nitrogen-phosphorus detector
NDV	: Newcastle disease virus
PAP	: Prostatic acid phosphatase
PARP	: Poly (ADP- robose) polymerase
PBS	: Phosphate buffered saline
PCV	: Packed Cell volume
pRB	: Retinoblastoma protein
PROSTVAC	: Prostate-specific antigen (PSA)-based pox-viral vaccine
PDT	: Photodynamic therapy
PDD	: Pulsed discharge ionization detector
PFS	: Progression-free survival
PlGF	: Placental growth factor
PID	: Photoionization detector
PVC	: Packed Cell Volume
OC	: Ovarian Cancer
OS	: Overall survival
OS	: Osteosarcoma
OSE	: Ovarian surface Epithelium
PSA	: Prostate-specific antigen
PFS	: Progression-free survival
RBC	: Red Blood Cell
RBS	: Random blood sugar
RNA	: Ribonucleic Acid
ROS	: Reactive Oxygen species
RR	: Response rate
RT	: Radiation therapy
Smac	: Second mitochondrial activator of caspases

SGPT	: Serum glutamic pyruvic transaminase
SGOT	: Serum glutamic-oxaloacetic transaminase
STS	: Soft tissue sarcomas
TAAs	: Tumor-associated antigens
TAK1	: Transforming growth factor beta-activated kinase 1
TCD	: Thermal conductivity detector
TME	: Total mesorectal excision
TG	: Triglyceride
TORC1	: Target of Rapamycin Complex 1
TP53	: Tumour protein p53
TRIB3	: Tribbles Pseudokinase 3
TRICOM	: Immune-enhancing cost stimulatory molecules
TRPV1	: Transient receptor potential vanilloid receptor 1
TRPV2	: Transient receptor potential vanilloid receptor 2
TSTA	: Tumor specific transplantation antigen
THC	: Tetrahydrocannabinol
THCA	: Tetrahydrocannabinol acid
TID	: Thermionic ionization detector
Tregs	: Regulatory T cells
VEGF	: Vascular endothelial growth factor
USA	: United States of America
UV	: Ultra violet
VEGF	: Vascular endothelial growth factor
VEGFR 1	: Vascular endothelial growth factor receptor 1
VEGFR 2	: Vascular endothelial growth factor receptor 2
WBC	: White Blood Cell
WHO	: World Health Organization

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