

CHAPTER VI

SUMMARY AND CONCLUSIONS



SUMMARY AND CONCLUSIONS

To perform the research work on the title “**INVESTIGATION OF CANNABIS EXTRACT AS A POSSIBLE CANDIDATE FOR THE PREVENTION AND TREATMENT OF INDUCE CANCER CELLS IN ALBINO MICE**” three main objectives were conducted to accomplish the whole research.

Objective 1: To observe the effects of cannabis extract on albino mice

6.1.1 Summary

The body weight of albino mice was measured daily. The biochemical, hematological, and histopathological tests were done to observe the effects of cannabis extract in albino mice. The significant results of each test are presented below:

- 1) Significantly increases ($p < 0.01$) the body weight of mice at 1st week, 2nd week, and 3rd week respectively.
- 2) RBS test values significantly ($p < 0.01$) in the 2nd and 3rd week decreased.
- 3) Serum creatinine values show significant ($p < 0.05$) variation in the 2nd week of observation.
- 4) SGPT test values show significant ($p < 0.05$) variation in the 1st, 2nd, and 3rd week of observation. SGPT values increase significantly with increasing the time of cannabis extract injection.
- 5) SGOT values also ($p < 0.01$) significantly increased with the time of cannabis extract injection.
- 6) In the 1st week and 2nd week of observation, alkaline phosphatase shows significant ($p < 0.01$) variation. Alkaline phosphatase values increase significantly with the time of cannabis extract injection.
- 7) No significant variation was observed in serum cholesterol level. But serum triglyceride shows ($p < 0.05$) significant variations in the 1st week and 2nd week of observation.
- 8) Significantly ($p < 0.05$) increases the uric acid level in the 1st week of observation.
- 9) There were no significant variations observed in hematological test values.

6.1.2 Conclusions

- RBS test values decrease because a cannabis extract has insulin-inhibitory activity.
- Cannabinoids are nitrogenous compounds, so the serum creatinine and uric acid increases after increasing the intraperitoneal injection of cannabis extract.
- SGPT, SGOT, and alkaline phosphatase values increase due to hepatocellular damage to the mice. That also conforms to the histopathology of the liver.
- No significant difference in the CBC count because no kind of infection occurred in the mice due to the cannabis extract injection.

Objective 2: To evaluate the responses of cannabis extract for the treatment of EAC cells induced cancer

Group 1: Control group of mice

6.2.1 Summary

- 1) No significant ($p < 0.05$) variations were observed in body weight among the groups of mice.
- 2) There were no significant ($p < 0.05$) variations were observed in the biochemical profile among the groups of mice.
- 3) Also in the hematological profile no significant ($p < 0.05$) varied among the groups of mice.

6.2.2 Conclusions

- Due to the normal diet maintained, there was no significant body weight was gained in albino mice.
- Biochemical and hematological parameters were also normal.

Group 2: Inoculated only EAC cells no treatment was provided

6.3.1 Summary

- 1) Significantly ($p < 0.05$) body weight was gained in the EAC-induced mice.
- 2) SGPT, SGOT, and alkaline phosphatase values show significant ($p < 0.05$) variation. Significantly increased the values of this test values with time progresses.
- 3) A significant increase was observed in WBC count ($p < 0.001$) and neutrophil count. No significant variation was observed in Hb, RBC, and platelet count.
- 4) Cytology of fluid also shows the typical cells were found in the ascitic fluid of albino mice.
- 5) Histopathology observation also shows sarcoma type cancer cells were found in the tumor.
- 6) Tumor growth rates and viable cell count were also very high.

6.3.2 Conclusions

- Significant body weight gain due to ascitic fluid formation in the abdominal cavity.
- Hepatocellular damage occurs.
- A high WBC count indicates infection, inflammation, cancer, and immunological disorder.
- Typical cells form tumors with increasing time.
- Tumor histopathology shows sarcoma type cancer cells.

Group 3: Cytotoxic agent was used for treatment purposes

6.4.1 Summary

- 1) No significant variation ($p < 0.05$) was observed among the treatment groups.
- 2) Serum creatinine shows a significant variation ($p < 0.05$) observed on the 3rd day of observation.
- 3) SGPT and SGOT values also show ($p < 0.05$) variations on the 3rd day of observation.
- 4) Serum cholesterol shows a significant variation ($p < 0.004$) in the 2nd week, the highest value was observed when T2 treatment was provided.
- 5) WBC count significantly decreased ($p < 0.001$) among all observation groups.
- 6) Cytology of fluid also shows typical cells were found in the ascitic fluid of albino mice.
- 7) Histopathology observation also shows sarcoma-type cancer cells were found in the tumor.
- 8) Tumor growth rates and viable cell count were decreased.

6.4.2 Conclusions

- Cytotoxic agent Ifodex is a nitrogenous alkaloid compound. So serum creatinine shows significant value during the early observation period.
- An early stage of Ifodex injection hepatocellular damage occurs.
- Chemotherapy drugs can affect rapidly dividing cells in the body, including WBC. So WBC count decreases due to the cytotoxic agent Ifodex injection.

Group 4: Treatment started after 3 days of EAC inoculation

6.5.1 Summary

- 1) Body weight shows a significant variation ($p < 0.05$) in the 3rd week of treatment among treatment groups.
- 2) RBS and serum creatinine did not show a significant variation ($p < 0.05$) among the treatment groups.
- 3) SGPT shows significant ($p < 0.05$) variations on the 3rd day of observation among the treatment groups.
- 4) SGOT shows significant ($p < 0.05$) variations in the 2nd week of observation among the treatment groups.
- 5) Alkaline phosphatase shows significant ($p < 0.001$) in the 2nd week of observation among the different treatment groups.
- 6) Uric acid shows significant ($p < 0.05$) in the 2nd and 3rd weeks of observation among the different treatment groups.
- 7) WBC count shows not any significant ($p < 0.05$) among the treatment groups.
- 8) The ascitic fluid of albino mice did not observed.
- 9) The tumor did not observed.

6.5.2 Conclusions

- At the early stage of cannabis extract injection for treatment purpose, it shows anticancerous properties.
- Several biochemical parameters were significantly increased due to EAC inoculation in the mice.

Group 5: Treatment started at seven days after EAC inoculation

6.6.1 Summary

- 1) Body weight shows a significant variation ($p < 0.05$) in the 2nd and 3rd weeks of observation among treatment groups.
- 2) Serum creatinine shows a significant variation ($p < 0.05$) among the treatment groups at the 3rd week of observation.
- 3) SGPT and SGOT did not show significant ($p < 0.05$) variations.
- 4) Alkaline phosphatase shows significant variation ($p < 0.001$) in the 3rd week of observation among the treatment groups.
- 5) Serum cholesterol, TG and uric acid did not show significant ($p < 0.05$) observation among the different treatment groups.
- 6) WBC count shows significant ($p < 0.05$) variation in the 2nd and 3rd week of observation among the treatment groups.
- 7) The ascitic fluid volume decreased compared to the EAC-inoculated mice.

6.6.2 Conclusions

- At the early stage of cannabis extract injection for treatment purpose, it shows anticancerous properties.
- Several biochemical parameters were significantly increased due to EAC inoculation in the mice.

Group 6: Treatment started after fourteen days of EAC inoculation

6.7.1 Summary

- 1) Significant variations ($p < 0.05$) were observed in the body weight at 2nd week of observation among the different treatment groups.
- 2) RBS shows significant variations ($p < 0.05$) on the 3rd day among the treatment groups.
- 3) No significant variation was observed on SGPT and SGOT tests.
- 4) Significant ($p < 0.05$) variations were observed at 1st week, 2nd week and 3rd week of observation compared to the different treatment groups of mice.
- 5) Serum cholesterol and TG did not show any significant ($p < 0.05$) variations among the different treatment groups of mice.
- 6) Uric acid shows significant ($p < 0.05$) variation in the 3rd week of observation among the different treatment groups.
- 7) WBC count shows important findings and significant ($p < 0.001$) variations were observed on the 3rd day, 1st week and 2nd week of observation. The highest WBC value was $(116.83 \pm 1.05 \times 10^3/\text{cmm})$ in the 3rd week T3 group.
- 8) Cytology of fluid also shows typical cells were found in the ascitic fluid of albino mice.
- 9) Histopathology observation also shows sarcoma-type cancer cells were found in the tumor.
- 10) Tumor growth rates and viable cell count were also very high.

6.7.2 Conclusions

- Significant body weight gain due to ascitic fluid formation in the abdominal cavity.
- Hepatocellular damage occurs.
- A high WBC count indicates infection, inflammation, cancer, and immune disorder.
- Typical cells form tumors with increasing time.
- Tumor histopathology shows sarcoma.

Group 7: Treatment started after twenty one days of EAC inoculation

6.8.1 Summary

- 1) Significant variations ($p < 0.05$) were observed in the body weight at 2nd week and 3rd weeks of observation among the different treatment groups.
- 2) RBS and serum creatinine did not show significant variations ($p < 0.05$) among the treatment groups.
- 3) Significantly increasing the values of SGPT, SGOT and alkaline phosphatase with time in all groups. The highest value of SGPT ($1277.00 \pm 78.04 \text{ U/L}$), SGOT ($1223.00 \pm 228.36 \text{ U/L}$) and alkaline phosphatase ($1354.67 \pm 100.07 \text{ U/L}$)
- 4) Serum cholesterol and TG did not show any significant ($p < 0.05$) variations among the different treatment groups of mice.
- 5) Uric acid shows significant ($p < 0.05$) variation on the 3rd day and 2nd week of observation among the different treatment groups.
- 6) WBC count shows important findings and significant ($p < 0.001$) variations were observed on the 3rd day, 1st week, and 2nd week of observation. WBC count increases significantly high with time.
- 7) Cytology of fluid also shows the typical cells were founded in the ascitic fluid of albino mice.
- 8) Histopathology observation also shows sarcoma type cancer cells were founded in the tumor.
- 9) Tumor growth rates and viable cell count were also very high.

6.8.2 Conclusions

- Significant body weight gain due to ascitic fluid formation in the abdominal cavity of albino mice.
- Hepatocellular damage occurs.
- The abdominal fluid formation also increases the uric acid level of biochemical observation.
- A high WBC count indicates infection, inflammation, cancer, and immunological disorder.
- Tumor histopathology shows sarcoma.

Objective 3: To determine the preventive efficacy of cannabis extract on EAC cells induced cancer.

Group 1: Effectiveness of three doses of cannabis extract in preventing cancer

6.9.1 Summary

- 1) No significant ($p < 0.05$) variations were observed in the body weight among the treatment groups of albino mice.
- 2) RBS and serum creatinine did not show significant ($p < 0.05$) variations among the treatment groups.
- 3) SGPT shows significant ($p < 0.05$) in the 1st, 2nd, and 3rd week of observations among the treatment groups.
- 4) SGOT shows significant ($p < 0.05$) in the 1st, 2nd, and 3rd week of observations among the treatment groups.
- 5) Alkaline phosphatase shows significant ($p < 0.05$) in the 1st, 2nd, and 3rd weeks of observations among the treatment groups.
- 6) All the biochemical parameters increase with the time of observation.
- 7) Serum cholesterol and TG did not show any significant ($p < 0.05$) variations among the different treatment groups of mice.
- 8) No significant ($p < 0.05$) variation was observed among the different treatment groups.
- 9) WBC count shows important findings and significant ($p < 0.001$) variations were observed in all the parameters among the treatment groups. WBC count increases highly with time.
- 10) Cytology of ascitic fluid also shows typical cells.
- 11) Histopathology observation also shows sarcoma-type cancer cells were found in the tumor.
- 12) Tumor growth rates and viable cell count were also very high.

6.9.2 Conclusions

- Significant body weight gain due to ascitic fluid formation in the abdominal cavity.
- Hepatocellular damage occurs.
- A high WBC count indicates infection, inflammation, cancer, and immune disorder.
- Typical cells form tumors with increasing time.
- Tumor histopathology shows sarcoma.

Group 2: Effectiveness of seven doses of cannabis extract in preventing cancer

6.10.1 Summary

- 1) No significant ($p < 0.05$) variations were observed in the body weight among the treatment groups.
- 2) RBS and serum creatinine did not show significant ($p < 0.05$) variations among the treatment groups.
- 3) SGPT shows significant ($p < 0.001$) on the 3rd day, 1st, 2nd, and 3rd week of observations among the treatment groups.
- 4) SGOT shows significant ($p < 0.001$) on the 3rd day, 1st, 2nd, and 3rd week of observations among the treatment groups.
- 5) Alkaline phosphatase shows significant ($p < 0.001$) in the 1st, 2nd, and 3rd weeks of observations among the treatment groups. This biochemical parameter increases with the time of observation.
- 6) Serum cholesterol and TG did not show any significant ($p < 0.05$) variations among the different treatment groups of mice.
- 7) No significant ($p < 0.05$) variation was observed in uric acid among the different treatment groups.
- 8) WBC count shows important findings and significant ($p < 0.001$) variations were observed in all the parameters among the treatment groups. WBC count increases highly with time.
- 9) Cytology of ascitic fluid also shows typical cells.
- 10) Histopathology observation also shows sarcoma type cancer cells were found in the tumor.
- 11) Tumor growth rates and viable cell count were also very high.

6.10.2 Conclusions

- Significant body weight gain due to ascitic fluid formation in the abdominal cavity.
- Hepatocellular damage occurs.
- A high WBC count indicates infection, inflammation, cancer, and immune disorder.
- Typical cells form tumors with increasing time. The total viable cell count decreased compared to the 3 doses of extract injection.

Group 3: Effectiveness of fourteen doses of cannabis extract in preventing cancer

6.11.1 Summary

- 1) No significant ($p < 0.05$) variations were observed in the body weight among the treatment groups.
- 2) RBS and serum creatinine did not show significant ($p < 0.05$) variations among the treatment groups.
- 3) SGPT shows significant ($p < 0.05$) in the 3rd week of observations among the treatment groups.
- 4) SGOT shows significant ($p < 0.001$) in the 1st, 2nd, and 3rd week of observations among the treatment groups.
- 5) Alkaline phosphatase shows significant ($p < 0.001$) in the 1st, 2nd, and 3rd weeks of observations among the treatment groups. This biochemical parameter increases with the time of observation.
- 6) Serum cholesterol and TG did not show any significant ($p < 0.05$) variations among the different treatment groups of mice.
- 7) significant ($p < 0.05$) variation observed in uric acid on the 3rd day and 2nd week among the different treatment groups.
- 8) No significant ($p < 0.05$) variations were observed among the different treatment groups.
- 9) Cytology of fluid also shows typical cells were found in the ascitic fluid of albino mice
- 10) Tumor growth rates and viable cell count also very decreased.

6.11.2 Conclusions

- No Significant changes occur in body weight gain due to a poor amount of ascitic fluid formation in the abdominal cavity.
- WBC count normal due to no infections and inflammation occurring.
- The total viable cell count decreased.
- The small number of mice shows tumors.

Group 4: Effectiveness of twenty one doses of cannabis extract in preventing cancer

6.12.1 Summary

- 1) No significant ($p < 0.05$) variations were observed in the body weight among the treatment groups.
- 2) RBS and serum creatinine did not show significant ($p < 0.05$) variations among the treatment groups.
- 3) No significant ($p < 0.05$) variations were observed on SGPT, SGOT and alkaline phosphatase among the treatment groups.
- 4) Serum cholesterol and TG did not show any significant ($p < 0.05$) variations among the different treatment groups of mice.
- 5) No significant ($p < 0.05$) variation was observed in uric acid among the different treatment groups.
- 6) No significant ($p < 0.05$) variations were observed in WBC count among the different treatment groups.
- 7) No ascitic fluid found in albino mice.
- 8) No tumor formation occur .

6.12.2 Conclusions

- 21 doses of cannabis extract show preventive properties against cancer.
- No hepatocellular damage occurs.
- No kidney damage occurs.
- No infection and inflammation occur.

6.13 Overall Summary and Conclusions

- 1) The cannabis extract doses that were used in this research (5mg/kg, 10mg/kg and 15 mg/kg) did not show any effects on hematological and histopathological observation. But in the biochemical test, early-stage SGPT, SGOT, and alkaline phosphate levels increased but with time it becomes normal.
- 2) After inoculating EAC, when cannabis does inject at an early period (3rd and 1st week) it prevents tumor and acetic fluid formation. Biochemical and hematological data also become normal. But in the late stage of treatment start (2nd and 3rd week) to the EAC inoculated mice, cannabis extract did not show anticancer properties.
- 3) The 14th and 21st doses of cannabis extract injection to albino mice prevent tumor and acetic fluid formation. Biochemical and hematological levels also become normal. But at 3rd, and 7th doses of extract injection did not show cancer preventive properties.

6.14 Limitations of this research

- 1) Three different concentration of cannabis extract (5mg/kg, 10mg/kg and 15mg/kg) doses were used in this study. High concentration (20mg/kg, 40mg/kg and 60mg/kg) doses should be used on albino mice to observe its effects.
- 2) For the anticancerous and preventative activities of cannabis extract, in vitro monitoring should be implemented.
- 3) Through molecular analysis, cancer of the sarcoma type must be examined for gene expression.
- 4) To confirm sarcoma type cancer, an immunohistochemistry test should be done.
- 5) How long the mice won't be effective as a preventative measure is yet unknown.

6.15 Recommendations

- 1) At the early stage (after 3rd days of EAC inoculation) of cannabis extract injection after inoculation of EAC cells into the albino mice, the cannabis extract shows anticancerous activity. No tumor and ascitic fluid formation occur.
- 2) Biochemical, hematological and histopathological parameters were seen to be normal at the early stage of cannabis extract injection after EAC inoculation.
- 3) Cannabis extract can prevent cancer when it used 14 to 21 doses on albino mice.
- 4) Cannabis extract can use as anticancerous drug at the early stage of cancer diagnosis.
- 5) Higher concentration of cannabis extract doses (such as 20mg/kg, 40mg/kg and 60mg/kg) should be used to observe the anticancerous and preventive properties on albino mice.
- 6) Human trial should be implemented for further research.