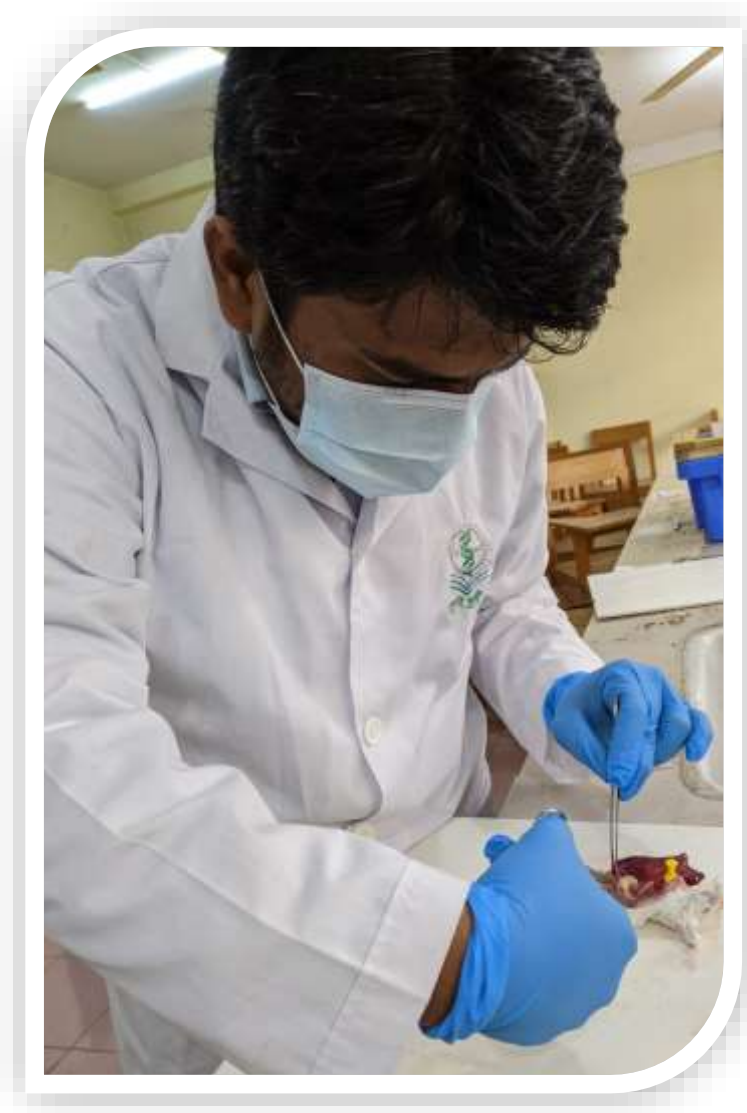


CHAPTER V

DISCUSSION



DISCUSSION

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

5.1.1 Body weight measurement of albino mice

The maximum body weight in male and female mice has been reported to be 27.3 ± 1.2 g and 23.3 ± 0.6 g, respectively (Goodrick *et al.*, 1980). The body weight of mice increased steadily from 27 ± 1.2 g to 33 ± 1.2 g in male mice and from 27 ± 1.2 g to 30 ± 1.0 g in female mice (Wang *et al.*, 2004). Similar changes were observed in the present study.

5.1.2 Biochemical profile

Type 2 diabetes, the glucose uptake of cells impaired due to insulin resistance (Scherer *et al.*, 1938). THC can increase insulin-induced glucose uptake, as demonstrated in a study in cultured adipocytes. Tumor necrosis factor (TNF) affects the effectiveness of insulin on glucose uptake by interfering with insulin signaling, so glucose levels decreased in the blood (Gallanta *et al.*, 2009). Similar findings were observed in the present study. In this present study, RBS test significant results show in the 1st week and 2nd week mice sacrificing groups versus the control group. In the 1st week mice sacrificing group gradually decreased RBS results according to an increase in the dose concentration. The control group's RBS results were 5.56 ± 0.78 mmolL⁻¹, and when 15mg/kg seven dose was injected into the mice RBS results were 3.20 ± 0.15 mmolL⁻¹ at the 3rd day of observation.

Higher rate of elevated creatinine kinase levels in hospitalized psychotic cannabinoid users, possibly due to cannabis activity in peripheral or brain tissues (Fruman *et al.*, 2020). The present study shows that a significant value ($p < 0.04$) was found in the 2nd week of the observation group. At the T1 dose injection 0.37 ± 0.07 mg/dL of the highest level that compares to other groups of treatment. Normally serum creatinine level in mice was 0.1-0.5mg/dL.

SGPT enzymes are to be the more sensitive measure for evaluating hepatocellular damage (Boutwell, 1961). Results obtained in mice groups in the present study showed significantly increased activity in the plasma ALT and AST. This can go in line with the fact reported by Poddar *et al.*, (1972) and Kew *et al.*, (1969) that high and its constituents are reported to affect certain enzymes causing certain degrees of liver

dysfunction. Significant ($p < 0.05$) results were obtained in the 1st week of observation. Gradually increased the SGPT results with increasing the time of cannabis extract dosing. This indicates that hepatocellular damage. SGPT values were at 1st week of observation 20.67 ± 1.86 U/L, 300.67 ± 86.61 U/L, 327.66 ± 61.49 U/L, and 674 ± 217.22 U/L. The normal SGPT value for mice is < 42 mg/dL. In this case-control group's SGPT value was normal, but when gradually increased the cannabis extract concentration as a dose that was injected into the mice then also SGPT values were increased. Cannabinoids are possible hepatotoxic substances associated with hepatic morphologic and enzymatic alterations (Borini *et al.*, 2004). The highest values of SGPT were seen in the albino mice with highest dose of cannabis extract injected into the mice. In the T2 treatment injected group, SGPT test results in were 327.66 ± 61.49 U/L, 243.66 ± 38.29 U/L, and 72.67 ± 30.03 U/L at 1st, 2nd, and 3rd week of dose injection respectively. When the cannabis extract was injected into the mice over a longer period, the results of the SGPT test gradually decreased. So here it can be said that with the increase of time, the concentration of this enzyme also decreases in the mice.

Increase in the ALP activity in both injected mice and human smokers and this will increase with the increase of dose and time but the ALT and the AST increase at the beginning of consumption then will decrease with time (Amna *et al.*, 2011). The same findings were observed in the present study, at 1st week of cannabis extract injected to the mice SGOT results increased, but increasing the dosing time the results become normal. SGOT test results were low in the T2 (10mg/kg body weight of mice) dose injection into the mice. Gradually increase SGOT levels with the time of observation. Trauma to skeletal muscle is among the doctors known to increase SGOT activity (Poddar *et al.*, 1972). The normal SGOT value for mice is < 42 mg/dL. In this case-control group, SGOT value was normal, but when gradually increased the cannabis extract concentration, SGOT values were also increased into the blood of albino mice. The highest values of SGOT were seen when the highest dose of cannabis extract injected into the mice.

ALP activity was increased in rats with the increase of dose and time and the difference between the level in the high and the low dose groups was significant ($P < 0.05$) during and after the experimental period (Amnah *et al.*, 2011).

In this research, the same findings were observed. In all groups of mice, increase the time of cannabis extract injection also increase the value of alkaline phosphatase in the blood of albino mice. The significant level for the 1st week treatment group was 3%, and the values were 100.67 ± 6.17 U/L, 310.00 ± 30.32 U/L, 495.67 ± 192.32 U/L, and 982.67 ± 276.83 U/L in the T0, T1, T2 and T3 respectively. The 2nd and 3rd-week results did not show any significant value. ALP is a “maker” enzyme for plasma membrane and endoplasmic reticulum (Malomo, 2000). The increase in the activity of serum alkaline phosphatase might be due to the leakage of the enzyme from the tissue into the serum. These suggest tissue damage such as muscle injury; cardiac infection and hepatic necrosis (Yakubu *et al.*, 2001). A 3% level of significance value was obtained when there were sacrificed on the 1st week of different doses injection on to the mice.

After cannabis use, total cholesterol concentration changed to 199.15 ± 23.3 mg/dL and 180.95 ± 19.3 mg/dL (Moya-Salazar *et al.*, 2020). The same findings observed in T3 dose injection in the 1st, the 2nd, and 3rd week were 130.33 ± 10.33 mg/dL, 134.00 ± 1.53 mg/dL, and 137.33 ± 3.38 mg/dL respectively. There were no significant results observed in any group of mice.

Some studies showed that cannabis users show higher BMI, total cholesterol, LDL-cholesterol, and triglycerides that were metabolic parameters, however, reached statistical significance although certainly demonstrated a trend for unfavorable cardiometabolic profile among cannabis smokers observed by Issa *et al.*, (2014). In the present study, there were no significant observations found. TG levels were normal in all treatment groups of mice. But in the 1st week of observation $P=0.02$ significant value and in the 2nd week of observation $P=0.03$ significant value was observed.

High uric acid level (above 6.8 mg/dL) known as hyperuricemia. This can lead to a disease called gout that causes painful joints that accumulate urate crystals (Barkas *et al.*, 2018). The same observation was found in this research, in the control group of mice uric acid levels were 5.93 ± 0.59 mg/dL, 7.00 ± 0.17 mg/dL, and 5.87 ± 1.39 mg/dL in 1st, 2nd, and 3rd week of observation respectively. Increasing the doses concentration also increase the uric acid value in blood of albino mice, such as 5.93 ± 0.59 mg/dL, 25.43 ± 7.54 mg/dL, 13.13 ± 3.40 mg/dL, and 30.37 ± 5.60 mg/dL in the 1st week of T0, T1, T2, and T3 mice observation group. Increasing the dose concentration of cannabis extract also increases the uric acid level of mice.

5.1.3 Hematological profile

Chronic administration of Crude Cannabis Resin (CCR), (20 mg/kg and 40 mg/kg) to albino rats for 21 days, the changes in various hematological indices such as Packed Cell Volume (PVC), total leukocyte count, differential leukocyte count, Red Blood Cell (RBC) count, absolute lymphocyte count, monocyte count, neutrophils and eosinophil counts were evaluated (Obonga *et al.*, 2005). The results showed that the hematological indicators such as erythrocytic and leucocytic counts were not significant ($p < 0.05$). Hb results of the T0 group were 14.13 ± 0.61 mg/dL, 14.26 ± 0.63 mg/dL, and 14.20 ± 0.15 mg/dL. In all the group's of mice, the test result comparisons there were no significant values observed.

Hematological parameters such as erythrocyte and leucocyte counts were not significantly ($p < 0.05$) affected by the treatment with the crude cannabinoid resin-treated animal groups for the first two weeks of treatment (Cruickshank, 1976). In this research, the similar findings were observed. In the T0 group, RBC counts were 7.2 ± 0.84 million/cmm, 6.50 ± 0.85 million/cmm, and 6.47 ± 0.79 million/cmm. No significant ($p < 0.05$) values were observed in the RBC counting.

Healthy white blood cell count is key to a properly functioning immune system. Causes of a low or high white blood cell count include conditions that affect the immune system, infections, and certain medications (Tashkin, 1972). Total leukocyte (WBCs) number decreased in the mice treated groups and reached a significant level ($p < 0.05$) for the group which was treated with a high dose (0.6 gm/g). In the control group, WBC counts were $3.48 \pm 0.95 \times 10^3$ /cmm, $4.10 \pm 0.61 \times 10^3$ /cmm, and $3.40 \pm 0.30 \times 10^3$ /cmm. But when injected cannabis extract into the mice the WBC value was decreased, compared to the normal mice. In the treatment group T2 the test values were $4.10 \pm 0.61 \times 10^3$ /cmm and $2.91 \pm 0.28 \times 10^3$ /cmm. WBC values decreased due to cannabis extract injected into the mice for the longer period of time.

In the control groups, the platelets count were $3.65 \pm 0.60 \times 10^5$ /cmm, $3.52 \pm 0.31 \times 10^5$ /cmm, and $6.17 \pm 1.64 \times 10^5$ /cmm. The general range for platelets has been counted $1.5\text{--}4.5 \times 10^5$ /mcL per mouse. There were no significant changes occurred due to different concentrations of cannabis extract injected into the mice.

5.1.4 Cannabis extract analysis by GC-MS

Mass spectrometry (GC-MS) to investigate three isomeric cannabinoids commonly found in cannabis products - cannabidiol, THC, and tetradecane (Liu *et al.*, 2011). In this research, the same cannabinoids were observed during mass spectrometry procedure. Tetradecane, cannabichromene, cannabinol, CBG, and THC these chemicals were identified when ethanolic extraction of cannabis was prepared.

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

In this case study, mice were stored in normal conditions. Where normal diet and water were provided for them. Mice were sacrificed every week for pathological observation. Pathological observation includes biochemical, hematological, histopathological, and cytopathological observation. No treatment was provided in any groups of mice. 4 groups of mice were observed in this group.

Group 1: Control group of mice

5.2.1.1 Body weight measurement of albino mice

Maximum body weight in male and female mice has been reported to be 27.3 ± 1.2 g and 23.3 ± 0.6 g, respectively (Goodrick *et al.*, 1980). The body weight of mice increased steadily from 27 ± 1.2 g to 33 ± 1.2 g in male mice and from 27 ± 1.2 g to 30 ± 1.0 g in female mice (Wang *et al.*, 2004). Body weight, age, and developmental stage of the animals used are characteristics that can affect the study's results (Kilkenny *et al.*, 2009; Jackson *et al.*, 2017). The age and the body weight of the animals can affect drug metabolism, gene expression, metabolic parameters, and other dependent variables measured in animal studies (Mccutcheon *et al.*, 2009; Ihedioha *et al.*, 2013; Jackson *et al.*, 2017).

In this research, the same investigation was observed. Mice's body weight was increased gradually with time of feeding normal food and water. There were no significant ($p < 0.05$) results observed in the control group of mice.

5.2.1.2 Biochemical profile

Serum enzymes play an important role as diagnostic markers in neoplasia as well as to know the disease condition. Biochemical test results in the control group of mice, no significant differences were observed (Kathriya *et al.*, 2010). Mice serum enzyme test values were not increased (Ghosh, 2011). Similar results were found in the present study of RBS, serum creatinine, SGPT, SGOT, ALP, and uric acid in control mice. No significant difference in observation occurs in the control group of mice.

5.2.1.3 Hematological profile

The RBC count for the control group was determined to be $4.03 \pm 0.208 \times 10^6/\text{cmm}$, in this research lowest RBC count was $3.50 \pm 0.22 \times 10^5/\text{cmm}$ on 3rd day, and highest RBC count $4.50 \pm 0.85 \times 10^5/\text{cmm}$ at 2nd week of observation (Banaja *et al.*, 2021). There were similar findings were observed in RBC count. Banaja *et. al.*, (2021) observed that the Hb count in the control group was observed to be $13.52 \pm 0.7\text{g/dl}$, in this research lowest Hb count was $13.89 \pm 0.33\text{g/dL}$ on 3rd day, and highest hemoglobin count $14.26 \pm 0.63\text{g/dL}$. In this research, hemoglobin value was found nearly similar. The differential count of the blood samples had been done, and the values for the control group had Neutrophils at $17 \pm 3\%$, Eosinophils at $0.2 \pm 0.1\%$, 0% Basophils, Lymphocytes at $80 \pm 3\%$ and no Monocytes (Siba *et. al.*, 2021). In this present study, the Neutrophil's lowest value was $45.12 \pm 0.42\%$ and the highest value was $57.03 \pm 0.84\%$, the lymphocyte's lowest value was $41.02 \pm 0.45\%$ and the highest value was $54.22 \pm 0.22\%$.

Group 2: Inoculated only EAC cells no treatment was provided

5.2.2.1 Body weight measurement of albino mice

In EAC-bearing mice, a regular rapid increase in ascites volume was noted. Ascites fluid was direct nutritional source of tumor cells and a rapid increase in ascites fluid with tumor growth would be a means to meet the nutritional requirements of tumor cells (Prasad *et al.*, 1994). When only EAC inoculated 1×10^6 cells/mouse, no treatment was provided in the group of mice, significantly changes observed in the body weight.

5.2.2.2 Biochemical profile

Serum biochemical parameters such as SGOT, SGPT, and ALP were significantly ($p < 0.001$) increased in the EAC control group compared to the normal control group, (Sagnik *et al.*, 2015). In this present study, the SGPT value shows 3% significant value in the first week and 4% significant value in the 2nd week of mice. Similar outcomes were seen after the EAC inoculation of mice without injection of cannabis extract. The blood's level of the SGPT enzyme grew with time. SGPT is more specific to the liver and is thus a better parameter for detecting liver injury (Williamson *et al.*, 1996). Well-known (Muhammad *et al.*, 2011) that there were significant elevations in the levels of SGPT, SGOT, and ALP in liver diseases and disorders and hepatocellular damage caused by several agents.

5.2.2.3 Hematological profile

Total white blood cell (WBC) count which is often elevated during infections, one of the nonspecific markers of inflammation and can be linked with some particular kinds of cancers (Hussain *et al.*, 2007). (Su *et al.*, 2014) WBC can be divided into five types, including lymphocyte (LY), monocyte (MO), neutrophil (NE), eosinophil (EO), and basophil (BA). The total WBC count was significantly ($p < 0.001$) increased and the level of RBCs and Hb was reduced in EAC inoculated mice. Similarly in this study also WBC count increased, $p < 0.001$, $p = 0.001$, and $p = 0.002$ level of significance in 1st week, 2nd week, and 3rd week EAC inoculating mice group respectively. The highest value of WBC was 96.47 ± 3.29 (T2) in the 2nd-week mice sacrificing group.

Group 3: Cytotoxic agent was used for treatment purposes

5.2.3.1 Body weight measurement of albino mice

Body weight, age, and developmental stage of the animals used are characteristics that can affect the study's results (Kilkenny *et al.*, 2009; Jackson *et al.*, 2017). The age and the body weight of the animals can affect drug metabolism, gene expression, metabolic parameters, and other dependent variables measured in animal studies (Mccutcheon *et al.*, 2009; Ihedioha *et al.*, 2013; Jackson *et al.*, 2017). The animal's age plays an important role in modeling human diseases; examples across different fields are presented here. Growth duration in male and female albino mice has been reported to be 23.5 ± 0.4 g months and 29.3 ± 0.5 g months, respectively (Goodrick 1980). It has been suggested that during the growth phase, body weights should be measured every week and during the maintenance phase every two weeks (Hoffman *et al.*, 2002). In this present study mice weight gradually increased with time. Normal diet maintained and adequate amount of water supplied.

5.2.3.2 Biochemical profile

Our study showed a non-significant decrement in creatinine levels in post-chemotherapy to prechemotherapy ($P > 0.05$). The non-significant decrement in creatinine levels might be due to low muscle mass or malnutrition. It has been shown that chemotherapy can induce necrosis of kidney cells. This damage is clinically manifested as increased levels of creatinine (Cho *et al.*, 2016). On the other hand, previous studies showed that creatinine levels significantly increased in post-chemotherapy than in pre-chemotherapy due to impaired formation or impaired excretion of urine (Siena *et al.*, 2013). The current study showed non-significant increments of alanine aminotransferase levels in postchemotherapy compared to pre-chemotherapy ($P > 0.05$). The non-significant increments of ALT levels might be possibly due to an inflamed condition of the liver, severe scarring of the liver, or death of liver tissue caused due to chemotherapy. Therefore, close liver function test monitoring is advised for patients starting a new chemotherapy regimen. On the contrary, previous studies reported that the serum levels of ALT significantly increased

in postchemotherapy than pre-chemotherapy. The results of the present study showed non-significant increments of AST levels in post-chemotherapy compared to prechemotherapy ($P > 0.05$) (Hyogo, 2013). This result can be due to the damage of the tissues and cells of the liver, skeletal muscle, heart, and brain, which resulted due to chemotherapy. On the other hand, a study reported that significant increment of AST levels in post-chemotherapy compared to prechemotherapy because of the toxic effect of chemotherapy on liver cells and other organs that leads to necrosis (Kaplan, 2013).

5.2.3.3 Hematological profile

The WBCs count decrement in post-treatment might be due to the suppressed hematopoietic stem cells by chemotherapy which are important for WBC proliferation. This result is consistent with a recent study that reported that the WBC count significantly decreased post-chemotherapy compared to prechemotherapy ($P < 0.001$) due to the side effect of chemotherapy on WBC production. In the present study, WBC counts also decreased on the 3rd day, 1st week, 2nd week, and 3rd week of observation. Cancer patients develop an infection after taking chemotherapy, because of the reduction of white blood cells that are important for fighting against infection (Kaplan, 2013).

Group-4: Treatment started after three days of EAC inoculation

5.2.4.1 Body weight measurement of albino mice

The maximum body weight in male and female mice has been reported to be 27.3 ± 1.2 g and 23.3 ± 0.6 g, respectively. The body weight of mice increased steadily from 27 ± 1.2 g to 33 ± 1.2 g in male mice and from 27 ± 1.2 g to 30 ± 1.0 g in female mice (Wang *et al.*, 2004 and Abhinav *et al.*, 2022) observed that a significant body weight gain of EAC-inoculated control mice was observed at day 15 compared with day 0, with a maximum gain (41.81 ± 0.44 g). Compared with the control group, the body weight (32.55 ± 0.65 g, 30.69 ± 0.95 g, and 29.79 ± 1.07 g) had significantly ($p < 0.05$) reduced with cannabis extract injection of 25, 50, and 100 mg/kg respectively. In this study, the body weight at 3rd day, 1st, 2nd and 3rd weeks of observation groups were 24.11 ± 0.62 g, 31.56 ± 2.05 g, 37.80 ± 2.02 g, and 44.46 ± 2.56 g respectively.

5.2.4.2 Biochemical profile

The liver enzymes AST, ALT, and ALP are widespread in the tissues but are more concentrated in the liver, bone, intestine, and placenta. In a healthy liver, these enzymes are usually low. However, following tissue damage, changes occur in both function and membrane permeability leading to the escape of enzymes from the cells (Ezeonwu *et al.*, 2013). In this case, SGPT shows $p < 0.05$ significant value observed at 3rd day T2 groups. The highest SGPT value 99.67 ± 1.63 U/L compare to the control.

5.2.4.3 Hematological profile

Pre-treatment and post-treatment hematological parameters. There were no significant differences in the pretreatment hematological parameters between the no-recurrence group and the recurrence group at the early stage of diagnosis (Hyun *et al.*, 2020). In the current study, there were similar results were found. No significant values were observed at the early stage of EAC induction.

Group 5: Treatment started after seven days of EAC inoculation

5.2.5.1 Body weight measurement of albino mice

Significant body weight gain of EAC-inoculated control mice was observed at day 15 compared with day 0, with a maximum gain (41.81 ± 0.44 g). Compared with the control group, the body weight (32.55 ± 0.65 g, 30.69 ± 0.95 g, and 29.79 ± 1.07 g) had significantly ($p < 0.05$) reduced with cannabis extract treatment of 25, 50, and 100 mg/kg, respectively (Abhinav *et al.*, 2022). In this study, the body weight on 3rd day, 1st, 2nd and 3rd weeks of observation groups were 22.03 ± 2.08 g, 26.30 ± 1.61 g, 33.10 ± 1.31 g, and 41.63 ± 1.89 g respectively. A significant value ($p < 0.05$) was observed in the 2nd and 3rd week of body weight observation.

5.2.5.2 Biochemical profile

It is well known that there are significant elevations in the levels of serum SGPT, SGOT, and ALP in liver diseases and disorders and hepatocellular damage caused by several agents. An increase in these enzyme levels is also observed with cardiac damage due to myocardial infarction and liver disorders (Sonnenwirth *et al.*, 1980). In this research SGPT increased highest in 3rd week of T3 (317.67 ± 45.02 U/L), SGOT increased highest in 2nd week of T3 (653.6 ± 61.44 U/L) ALP increased highest in 3rd week of T3 (1626.7 ± 181.26 U/L). Due to this liver enzyme elevation, it indicate that hepatocellular damage occurs.

5.2.5.3 Hematological profile

WBCs, produced in the bone marrow, are indicators of inflammation, trauma, allergies, leukemia, or infections; WBC infiltration is frequently observed in numerous types of cancer (Wang *et al.*, 2015; Hussain *et al.*, 2007) observed that total white blood cell (WBC) count which is often elevated during infections, is one of the nonspecific markers of inflammation and can be linked with some particular kinds of cancers. Total white blood cell (WBC) count which is often elevated during infections, is one of the nonspecific markers of inflammation and can be linked with some particular kinds of cancers (Hussain *et al.*, 2007).

Group 6: Treatment started after fourteen days of EAC inoculation

5.2.6.1 Body weight measurement of albino mice

Ehrlich ascites carcinoma (EAC) is a rapidly growing carcinoma with very aggressive behavior and can grow in almost all strains of mice. EAC is used as ascites or a solid form due to these purposes, if the ascites fluid contains the tumor cells that are injected intraperitoneally (i.p), the ascites form is obtained, but if it is injected subcutaneously (s.c), a solid form is obtained (Zeybek, 1996). In ascitic form, it has been used as a transplantable murine tumor model to investigate the antitumor effect of several natural and synthetic chemical substances (Segurd *et al.*, 2000). Inoculation of EAC cells

resulted in the appearance of the ascitic fluid. The ascitic fluid is the direct nutritional source of the tumor cells (Gupta *et al.*, 2004). So, a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirements of growing tumor cells (Bhattacharya *et al.*, 2012). This hypothesis was evident in the present study since inoculation of EAC cells into mice caused a significant increase in the body weight of the EAC – bearing mice. Such an increase was due to the accumulation of the ascitic fluid volume in the peritoneal cavity (Altun *et al.*, 2004).

5.2.6.2 Biochemical profile

For the past several years, serum enzymes play an important role as diagnostic markers in neoplasia as well as to know the disease condition. (Kathriya *et al.*, 2010) Several reports reveal that tumor cell causes liver damage and disturbances in hepatic cell metabolism, which lead to changes in serum enzyme activity (Dolai *et al.*, 2012). Similar results were found in the present study in elevated levels of SGPT, SGOT, ALP, and uric acid in the EAC control group. The increased levels of above mentioned biochemical parameters may be interpreted as a result of hepatocellular damage by EAC. CE treatment at both doses restored the elevated biochemical parameters to the normal range, indicating protection against tumor cell-induced hepatotoxicity.

5.2.6.3 Hematological profile

Hb parameters of tumor-bearing mice on the 14th day showed significant changes compared to the normal mice. The total WBC count, protein, and PCV were found to increase with a reduction in the hemoglobin content of RBC (Raju *et al.*, 2009). Differential count of WBC showed that the percentage of neutrophils increased while that of lymphocytes decreased (Raju *et al.*, 2009). Similar findings were observed in the present study WBC count increased, neutrophil increased but lymphocyte decreased.

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

5.2.7.1 Body weight measurement of albino mice

For the growth of EAC cells ascitic fluid is an essential nutritional requirement and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (Haldar *et al.*, 2010). The body weight of tumor-bearing mice was increased due to an increase in ascitic fluid volume. In the present experiment increase in body weight, ascitic tumor volume, tumor weight, and tumor cell count were observed in EAC control. Treatment of EAC tumor-bearing mice with plant extracts at the doses of 10 and 15mg/kg significantly decreased the body weight, tumor volume, tumor weight, and viable tumor cell count and increased the nonviable tumor cell count. These results indicate either a direct cytotoxic effect of plant extracts on tumor cells or an indirect local effect.

5.2.7.2 Biochemical profile

Hepatocytes are severely damaged in animals with EAC cells (Ramadori *et al.*, 1983). This can lead to an increase in the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum of EAC-bearing mice (Pizzuti *et al.*, 1993). Reduced level of these hepatic enzymes in serum is one of the indications of the antitumor potential (Chakraborty *et al.*, 2006 & Sundaram *et al.*, 2012). Dolai *et al.*, (2012) reported that the EAC control group exhibited raised activities of liver enzymes such as SGOT, and SGPT. Results of the present study demonstrated that the liver activities of AST and ALT were increased in EAC – bearing mice as compared with that of normal mice. It was found that EAC led to some liver dysfunction. These results are in harmony with the results of (Halder *et al.*, 2010). The elevation of liver enzymes is an index of impaired liver functions due to cancer as observed in the EAC group. It was found that Ehrlich carcinoma induced a rise in MDA level which was associated with impaired hepatic function, as evidenced by increased serum activities of AST and ALT. The level of liver enzymes increased in the serum of EAC – bearing mice indicating general toxicity (Mansour *et al.*, 2010). Dolai *et al.*, (2012) found that EAC mice

exhibited raised activities of liver enzymes such as SGOT, and SGPT, due to hepatocellular damage. These results were supported by the results of histopathological examination of liver tissues since a liver section of EAC-bearing mice showed various histopathological alternations including an increased number of necrotic hepatocytes with deeply pyknotic nuclei, congestion associated with brown pigment deposition and thickening of wall on the central vein by increased collagen content. It was also reported that the presence of tumors in the human body or experimental animals is known to affect many functions of the vital organs, especially the liver, even when the site of the tumor doesn't interfere directly with organ functions (Deways, 1982).

5.2.7.3 Hematological profile

Evaluation of Hb parameters is one of the reliable paraclinical methods to diagnose evaluate and monitor diseases. Hematological parameters can predict the severity, mortality, and follow-up treatment of cancer (Guo *et al.*, 2019; Mouabbi *et al.*, 2017). Routinely assessed hematological parameters provide an overview of health status, particularly concerning anemia, systemic immunological response as well as hemostatic involvement. There was also a finding of significantly lower values of the total white blood cell count as well as absolute neutrophil and lymphocyte counts compare to the EAC-induced cancer. Our finding is consistent with a previous report. Total white blood cell count and its sub-populations are known to be cellular markers of inflammation; however, lower values have been associated with immunosuppression and cancer treatment (Steele 2012).

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

5.3.1.1 Body weight measurement of albino mice

The growth of EAC cells ascitic fluid is an essential nutritional requirement, and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (Halder *et al.*, 2010). The body weight of tumor-bearing mice was increased due to an increase in ascitic fluid volume. In the present experiment increase in body weight, ascitic tumor volume, tumor weight, and tumor cell count were observed in EAC-inoculated mice.

5.3.1.2 Biochemical profile

Serum enzymes play an important role as diagnostic markers in neoplasia as well as to know the disease condition (Kathryn *et al.*, 2010). Dolai *et al.*, (2012) and Ghosh *et al.*, (2011) reveal that tumor cell causes liver damage and disturbances in hepatic cell metabolism, which lead to changes in serum enzyme activity. Similar results were found in the present study that elevated levels of SGPT, SGOT, ALP, TG, and uric acid in cancer preventive effects at three doses of injection. Since the discovery of Braunstein and Kritzmann in 1937, transaminases (SGPT and SGOT) have been used to indicate the severity of many diseases including liver disease, hepatocyte necrosis, and even hepatitis. (Karmen *et al.*, 1955), (Wroblewski *et al.*, 1956) Variations in the serum transaminase levels and other related enzymes were observed in the affected muscle cells of the heart, hepatocytes, and also in other cancers. The increased levels of above mentioned biochemical parameters may be interpreted as a result of hepatocellular damage by EAC. No changes occur in RBS and creatinine kinase enzyme activity.

5.3.1.3 Hematological profile

WBCs, produced in the bone marrow, are indicators of inflammation, trauma, allergies, leukemia, or infections; WBC infiltration is frequently observed in numerous types of cancer (Wang *et al.*, 2015). Hussain *et al.*, (2007) observed that total white blood cell (WBC) count which is often elevated during infections, is one of the nonspecific markers of inflammation and can be linked with some particular kinds of cancers. Su *et al.*, (2014) WBC can be divided into five types, including lymphocyte, monocyte, neutrophil, eosinophil, and basophil. In this present study, WBC count also increased, cannabis extract injected at this time as a preventive purpose did not show anticancerous properties.

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

5.3.2.1 Body weight measurement of albino mice

EAC is a transplantable, poorly differentiated, malignant tumor that appeared initially as a spontaneous murine mammary adenocarcinoma (Ozaslan *et al.*, 2011). It can induce both solid and ascitic forms of tumor in almost all strains of mice, due to these concerns it has been widely used for several years to study the antitumor properties of several natural and synthetic compounds (Halder *et al.*, 2010, Manojkumar *et al.*, 2009; Dolai *et al.*, 2012). Treatment of EAC tumor-bearing mice with cannabis plant extracts significantly decreased their body weight (Dolai *et al.*, 2012). In this present study, the same observation was found. Cannabis extract decreases the body weight of albino mice compared to the control mice.

5.3.2.2 Biochemical profile

AST and ALT are found in serum and various bodily tissues but are most commonly associated with the liver. Aspartate transaminase also called as serum glutamic oxaloacetic transaminase and glutamic pyruvate transaminase called as alanine transaminase are associated with liver parenchymal cells. It is raised in acute liver

damage (Gaze, 2007). ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis, or infiltrative diseases of the liver (Harada *et al.*, 1986). Alkaline phosphatase (ALP) activity was increased in rats with the increase of dose and time, and the difference between the level in the high and the low dose groups was significant during and after the experimental period (Irfan *et al.*, 2017). Alanine aminotransferase (ALT) activity showed an incredible increase after the second dose in the high-dose group compared to the control group but returned to a lower level after the last dose, and Aspartate aminotransferase (AST) activity revealed also significant elevation after the second dose, but after the last dose, the activity decreased significantly compared to the control group. Similarly, the present study indicated that SGPT, SGOT, and ALP results are possible for hepatocellular damage of the liver. But the other biochemical test values were not significant compared to the control group.

5.3.2.3 Hematological profile

Hematological parameters of EAC cell-bearing mice on the 14th day showed significant changes when compared with normal mice ($P < 0.05$). The total WBC count was found to increase with a reduction in the hemoglobin content and total RBC count. The differential count of WBC showed that the percentage of neutrophils was increased while that of lymphocytes was decreased when compared with normal mice (Osman *et al.*, 2011). In the present study, the same observation happened. RBC test values of the different groups were studied. RBC data were presented by (Santos *et al.*, 2016) ($8.90 \pm 0.90 \times 10^6/\text{cmm}$) for collections by heart puncture of mice, and similar results were observed in the present study. RBC count ($6.27 \pm 0.42 \times 10^6/\text{cmm}$) in the 2nd week of observation.

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

5.3.3.1 Body weight measurement of albino mice

The body weight of tumor-bearing mice was increased due to an increase in ascitic fluid volume (Haldar *et al.*, 2010). In the present experiment increase in body weight, ascitic tumor volume, tumor weight, and tumor cell count were observed in EAC control. Treatment of EAC tumor-bearing mice with plant extracts at the doses of 10 and 20 mg/kg significantly decreased the body weight, tumor volume, and tumor weight for cannabis extract injection. These results indicate either a direct cytotoxic effect of plant extracts on tumor cells or an indirect local effect, which may involve macrophage activation and vascular permeability inhibition (Dolai *et al.*, 2012).

5.3.3.2 Biochemical profile

In the current study serum ALT and AST activity showed a major increase in EAC-bearing mice compared with the normal group because inoculation of Ehrlich cells induces organ dysfunction and metabolic disturbance (Abu-Sinna *et al.*, 2003). The hepatotoxic effect of arsenic is mainly the cause of the increment of ALT and AST activities through the leakage of these enzymes into the bloodstream (Navarro *et al.*, 1993). In this study, SGPT, SGOT, and ALP increased due to hepatocellular damage occurring. But other biochemical parameters were normal such as RBS, serum creatinine, serum cholesterol, serum triglyceride, and uric acid.

5.3.3.3 Hematological profile

Most studies indicate that the EAC induced mice, the white blood cells are mainly changed mainly neutrophils and lymphocytes in peripheral blood (Park *et al.*, 2019). Increase in the number of neutrophils can cause an increase in the total number of white blood cells (Lohman *et al.*, 2018). Under normal conditions, peripheral blood leukocytes and neutrophils are dynamically changing within 24 hrs, lower in the morning and higher in the afternoon. In pathological conditions, neutrophils are

elevated in acute infection, severe trauma, blood cell destruction, hemorrhage, acute poisoning, malignant tumors, etc. Neutrophils are reduced by Gram-negative bacilli infection, blood system diseases, and biophysics. Chemical factors such as damage and autoimmune diseases are common. Studies have shown that tumor-associated neutrophils can promote tumor development. Xue *et al.*, (2011) found preoperative neutrophil elevation, in patients with poor prognosis, and short survival time. In Li Sen's study, they found that neutrophils enhance the migration, invasiveness, and epithelial-mesenchymal transition (EMT) of EAC-induced cancer.

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

5.3.4.1 Body weight measurement of albino mice

Significant body weight gain of EAC-inoculated control mice was observed at day 15 compared with day 0, with a maximum gain ($41.81 \pm 0.44\text{g}$) (Abhinav *et al.*, 2022). Compared with the control group, the body weight ($32.55 \pm 0.65\text{g}$, $30.69 \pm 0.95\text{g}$, and $29.79 \pm 1.07\text{g}$) had significantly ($p < 0.05$) reduced with cannabis extract treatment of 15, 30, and 45 mg/kg, respectively. The cisplatin at 3.5 mg/kg reduced body weight significantly ($p < 0.05$) when compared with the control ($24.43 \pm 0.51\text{g}$). The percentage increase in body weight was also calculated and there was a significant increase in the control group, which was around 98.81% on the 15th day of tumor inoculation. In this study, cannabis extract was used as a preventive purpose for EAC-induced cancer. Body weight changes did not occur vigorously as EAC inoculated mice, this indicates that cannabis extract shows preventive properties against cancer cell proliferation.

5.3.4.2 Biochemical profile

For the past several years, serum enzymes play an important role as diagnostic markers in neoplasia as well as to know the disease condition (Kathriya *et al.*, 2010). Several reports revealed that tumor cell causes liver damage and disturbances in hepatic cell metabolism, which lead to changes in serum enzyme activity (Ghosh *et al.*, 2011). Similar results were found in the present study is elevated levels of SGPT, SGOT, and ALP in the EAC control group. The increased levels of above mentioned biochemical parameters may be interpreted as a result of hepatocellular damage by EAC. Cannabis extract used as a treatment at both doses restored the elevated biochemical parameters to normal range, indicating protection against tumor cell-induced hepatotoxicity.

5.3.4.3 Hematological profile

According to work that the reference values for mice hematology, the Haemoglobin value (12.52 ± 0.7 mg/dl) of the present study was less than the one obtained in their experiment (13.63 ± 1.4 mg/dl), the similar could be said about the Neutrophils percentage, which was ($22.64 \pm 6.4\%$) (Delwatta *et al.*, 2018). The Haemoglobin value was nearly similar to the control mice's hemoglobin value in the research conducted (Brahmi *et al.*, 2019). The lymphocyte percentage was (75.17 ± 6.6), which was in agreement with the values obtained in the control group of the present study. The lymphocyte count of the control mice was ($78.7 \pm 6.7\%$), nearly similar to the control of the present study, and the same goes for the hemoglobin control value, which was also nearly similar to the control value of the mentioned experiment, 11.5 ± 0.40 mg/dl. The RBC value elevated along with the cannabis extract treatment, and an identical course was followed by the hemoglobin value. The WBC experienced the opposite effect; the WBC values were decreasing with the injection of the cannabis extract. In the differential count of WBC, the Neutrophils percentage was similar in the control and it increased with the treatment. The eosinophil percentage was negligibly low and in control, and there were no eosinophil observed in the treated mice. The lymphocyte had a small elevation with the treatment values. The basophil count was not available, and the monocyte count was also zero, indicating the absence of those cells in the blood of the test subjects. This was in agreement with the findings of Santos *et al.*, (2016).